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Decision-making under uncertainty and chronic distress : a translational and transdiagnostic approach

Lidia Cabeza Álvarez

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Préparée à la faculté des Sciences et Techniques de Besançon

Ecole doctorale n°554

Environnements-Santé

Doctorat de spécialité : Sciences de la Vie et de la Santé, Neurosciences

Par

Lidia CABEZA ALVAREZ

La prise de décision dans le cas d'incertitude et le stress chronique : une approche translationnelle et transdiagnostique

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UR 481 : Laboratoire de Recherches Intégratives en Neurosciences et Psychologie Cognitive

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Title : Decision-making under uncertainty and chronic distress: a translational and transdiagnostic approach

Keywords : decision-making – chronic distress – uncertainty – motivation – reward - flexibility

Abstract: Decision-making (DM) is integral to everyday-life. This integrative process of exploring options and exploiting solutions is vital and evolutionarily conserved. Individuals faced with complex choices must learn to ignore immediate gratifications in favour of long-term benefits. Several factors, such as chronic distress, modulate DM processing and could therefore impact its motivational dimensions. Animal models allow studying the influence of distress on DM in dynamic environments, which is relevant for public health concerns in a translational setting. This doctoral project aimed at characterizing the determinants of DM in a mouse model that reproduce many aspects of human symptomatology related to chronic distress. In order to better understand the pathophysiological and neurobiological mechanisms involved in DM and chronic distress, this work implemented behavioural, experimental psychology, neuroanatomical, biochemical and pharmacogenetic techniques within reinforcement learning paradigms in order to assess goal-directed behaviour. First, the Iowa Gambling Task (IGT), developed for humans, was adapted for mice to assess DM under uncertainty. The behavioural performances of these two species were directly compared under physiological conditions, illustrating similar behavioural patterns and interindividual variabilities. Secondly, this project studied the influence of prolonged distress on DM, reproducing the convergent activation of the HPA axis by chronic administration of corticosterone (CORT model). The results show how distress-induced impairment of instrumental learning and negatively valenced perceptions contribute to maladaptive directed behaviours and suboptimal uncertainty management. Finally, an anatomico-functional study (by cFOS/FOSB immunohistochemistry) of the brain regions underlying motivation, learning and DM and sensitive to stress was conducted. The results specify the involvement of the basolateral amygdala (BLA) and the anterior insular cortex (aIC) in distress-induced motivational deficits. This doctoral investigation also described the plasticity of corticotropin-releasing factor (CRF) neuropeptide signalling in the medial prefrontal cortex (mPFC). The results led to the hypothesis of high mPFC CRF levels as endophenotype of vulnerability to suboptimal DM under chronic distress. Thus, CRF signalling in the mPFC might modulate the activity of frontocortical-striatal loops required for adaptive directed behaviours. Finally, the last data show the behavioural and neurobiological consequences of modulating mPFC CRF signalling by DREADD technology in relation to motivation and behavioural flexibility. This study illustrates how reward and effort processing contribute to dynamically adjusting the motivational threshold for triggering directed behaviours in dynamic environments. Motivational deficits might be the consequence of aberrant BLA and aIC activity, which in turn would impact DM through disruption of reward and effort processing. In conclusion, the integrative and translational approach of this collaborative PhD project contributes, in a transdiagnostic perspective, to a better understanding of the many interacting dimensions involved in adaptive DM under uncertainty. It also illustrates the continuum of interindividual variabilities and the influence of distress adaptations on oriented behaviours in versatile environments, and towards a better therapeutic management of patients for a precision medicine in biological psychiatry.

Titre : La prise de décision dans le cas d'incertitude et le stress chronique : une approche translationnelle et transdiagnostique

Mots clés : prise de décision – stress chronique – incertitude – motivation – récompense – flexibilité

Résumé : La prise de décision (PD) est intégrale de la vie quotidienne. Ce processus intégratif d'exploration des options et d'exploitation des solutions est conservé et vital. Les individus confrontés à des choix complexes doivent apprendre à ignorer les gratifications immédiates au profit d'avantages à long-terme. Plusieurs facteurs modulent les processus de PD, comme le stress chronique qui, pourrait avoir un impact sur la motivation. Les modèles animaux permettent d'étudier l'influence du stress sur la PD dans des environnements dynamiques, ce qui est pertinent pour les questions de santé publique dans un cadre translationnel. Ce projet doctoral visait à caractériser les déterminants de la PD dans un modèle murin reproduisant de nombreux aspects de la symptomatologie humaine liée au stress chronique. Afin de mieux comprendre les mécanismes physiopathologiques et neurobiologiques impliqués, ce travail a mis en œuvre des techniques comportementales, de psychologie expérimentale, neuroanatomiques, biochimiques et pharmacogénétiques avec des paradigmes d'apprentissage par renforcement évaluant les comportements dirigés vers un but. Tout d'abord, la tâche de pari de l'Iowa (IGT), développée chez l'homme, a été adaptée chez la souris, afin d'évaluer la PD en condition d'incertitude. Les performances comportementales de ces 2 espèces ont été directement comparées en conditions physiologiques, illustrent des modèles et variabilités interindividuelles similaires. Ensuite, ce projet a étudié l'influence sur la PD d'un stress prolongé, reproduisant l'activation convergente de l'axe HPA par administration chronique de corticostérone (modèle CORT). Les résultats montrent comment l'altération de l'apprentissage instrumental et les perceptions à valence négative induites par le stress contribuent à des comportements dirigés inadaptés et à une gestion sous-optimale de l'incertitude. Enfin, une étude anatomo-fonctionnelle (par immunohistochimie cFOS / FOSB) des régions cérébrales sous-tendant motivation, apprentissage et PD et sensibles au stress, a été menée. Les résultats précisent l'implication de l'amygdale basolatérale (BLA) et du cortex insulaire antérieur (aIC) dans les déficits motivationnels induits par le stress. Cette étude décrit également les adaptations de la signalisation du neuropeptide corticolibérine (CRF) dans le cortex préfrontal médian (CPFm). Ces résultats ont conduit à proposer comme un endophénotype de vulnérabilité à une PD sous-optimale induite par stress chronique les niveaux élevés de CRF dans le CPFm. Ainsi, la signalisation du CRF dans le CPFm modulerait l'activité des boucles frontocorticale-striatale requise pour les comportements dirigés adaptatifs. Enfin, les dernières données illustrent les conséquences comportementales et neurobiologiques de la modulation de la signalisation CRFergique du CPFm par la technologie DREADD en relation avec la motivation et la flexibilité comportementale. Cette étude illustre comment les processus de la récompense et de l'effort contribuent à ajuster dynamiquement le seuil de motivation déclencheur des comportements dirigés dans des environnements dynamiques. Les déficits de motivation pourraient être la conséquence d'une activité aberrante de la BLA et de l'aIC, ayant un impact sur le traitement de la récompense et de l'effort, et donc sur la PD. En conclusion, l'approche intégrative et translationnelle de ce projet doctoral collaboratif contribue à une meilleure compréhension des nombreuses dimensions interactives impliquées dans la PD adaptative en situation d'incertitude. Elle illustre également le continuum de variabilités interindividuelles et l'influence des nombreuses adaptations au stress chronique sur les comportements orientés dans des environnements versatiles, avec pour perspective une meilleure prise en charge thérapeutique des patients pour une médecine de précision en psychiatrie biologique.

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“Il faut cultiver notre jardin”

Candide, Voltaire

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Figure 12. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour and physiology. This section aims at adapting a behavioural paradigm to evaluate DM in a murine model of human chronic distress-related disorders. Gambling tasks for mice rely on food rewards to motivate goal-directed behaviours, however, scientific literature reports aberrant feeding behaviour in the CORT model. The modifications of the gambling task aim at facilitating appetitive and consummatory behaviours in animals. 80

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Figure 28. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour.

This section aims at investigating the effects of sustained distress on the quality of the decisional processes. For that, animals chronically treated with CORT will be evaluated in the translationally validated gambling task for mice. The effect of sustained distressed on interindividual variability will be also addressed. The behaviour of distressed mice will be systematically compared with that of a healthy, non-treated mice population. 100

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Behavioural [^]mGT performance in CORT-treated animals is characterized by a longer exploration phase in comparison with control individuals. In fact, reward responsiveness in CORT-treated animals was revealed after 40 choices, in the third gambling session (t-test: ###, $p < 0.001$; ####, $p = 0.0000$), whereas VEH-treated animals already chose preferentially the advantageous options after 20 choices, in the second session (t-test: ****, $p = 0.0000$). This difference in the emergence of reward responsiveness reveals a damaging effect of the pharmacological treatment on DM even if overall performances in both conditions are not significantly different (RM-ANOVA: ns, not significant). This negligible difference was suspected to be attributable to a veiled effect caused from behavioural interindividual variability. 101

Figure 30. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour.

This section aims at investigating the effects of sustained distress on choice strategy. The behavioural interindividual variability of a population of mice chronically treated with CORT will be compared with that of a healthy, non-treated mice population. 102

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Figure 34. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour. This section further investigates the effects of sustained distress on choice strategy. For that, the behavioural interindividual variability of a population of mice chronically treated with CORT will be studied through multiple behavioural domains that inform of outcome-based learning and outcome responsiveness. 105

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Figure 48. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour. This section aims at investigating the effects of sustained distress on spatial WM, which is a cognitive dimension required for associative learning processes. For that, mice chronically treated with CORT will be tested in a food-rewarding spatial WM task and their ability to succeed will be compared with that of a healthy, non-treated mice population. Understanding how chronic CORT exposure affects learning through this mnemonic dimension will help interpreting the observed delay in reward responsiveness in CORT-treated mice during the adapted gambling task. 118

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Figure 50. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour. This section aims at exploring the effects of sustained distress on effort allocation for food reward, which is considered a motivational component potentially influencing decisional processes. Understanding how chronic CORT exposure impacts effort valuation and processing will help comprehending the origin of suboptimal DM in CORT-treated mice during the adapted gambling task. 121

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contingency (extreme high flexible not included in the representation of data dispersion, n=3). The high-flexible cluster is conformed mostly by control animals, whereas low-flexible individuals are predominantly from the CORT condition, suggesting a deleterious effect of the pharmacological treatment on behavioural flexibility in the RL task. **(C)** Mice chronically treated with CORT obtained fewer rewards (total number of earned rewards \pm SEM, CORT, session 1: 34.20 \pm 4.40; session, 2: 26.80 \pm 3.66; session 3: 38.40 \pm 3.70; session 4: 26.20 \pm 3.87) than control individuals (VEH, session 1: 43.84 \pm 3.56; session 2: 42.93 \pm 3.113; session 3: 49.07 \pm 00.64; session 4: 41.87 \pm 2.59) irrespective of the session (Mann Whitney U: *, p<0.05; **, p<0.01; ***, p<0.001). 127

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Figure 56. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Physiology, neural systems and genetics. This section aims at investigating the effects of sustained distress on the expression of the brain GC receptors and the CRF neuropeptide in three cerebral areas relevant for DM and stress-related symptomatology. The expression of GR, MR and CRF will be calculated in the mPFC, DLS and VH of mice chronically treated with CORT. The objective is to understand how the brain physiologically adjusts to sustained distress. The levels of expression will be systematically reported to those of a healthy, non-treated mice population. The correspondence between protein expression and interindividual behavioural variability will be also explored. 133

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Figure 60. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Physiology, neural systems and genetics. This section aims at investigating the neurobiological underpinnings of effort valuation and processing in physiological conditions and under chronic distress. For that, the neural activation pattern of several discrete brain

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increasing the percentage of correct responses. This cluster predominantly comprises VEH-treated animals. Low-flexible animals, however, were less successful in adjusting their behaviour, thus improving significantly less their performance in the second RL session. This cluster predominantly comprises CORT-treated mice (see Third Chapter, Section 4). In the investigation of the neural activity patterns related to RL performance, **(A)** no differences were found in cFos expression between high- and low-flexible individuals (Mann Whitney U: ns, not significant) irrespective of the brain region of interest. Nevertheless, **(B)** sustained neural activation assessed by FosB expression was significantly different between subgroups in the BLA and VTA (Mann Whitney U: *, $p < 0.05$), but not in the other regions. Thus, these results support the hypothesis of the BLA and VTA-related computations implication on behavioural flexibility, and suggest that aberrant neural network activity patterns within and between these regions might result from sustained GC exposure..... 149

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ABBREVIATIONS

| | |
|--|---|
| ACC – anterior cingulate cortex | IC – insular cortex |
| ACh – acetylcholine | ICD – International Classification of Diseases |
| ACTH - adreno-corticotropic hormone | IGT – Iowa gambling task |
| ADHD – attention deficit/hyperactivity disorder | IL – infralimbic cortex |
| aIC – anterior insular cortex | LC – locus coeruleus |
| ANS – autonomic nervous system | LH – learned helplessness |
| AVP - vasopressin | MAPKs – mitogen activated protein kinases |
| BLA – basolateral amygdala | MD - mediodorsal thalamus |
| BNST – bed nucleus of the stria terminalis | MeA – medial amygdala |
| cAMP – cyclic adenosine monophosphat | MGB – medial geniculate body |
| CeA – central amygdala | mGT – mouse gambling task |
| CMS – chronic mild stress | MLT – motor learning task |
| CNS – central nervous system | mOFC – medial orbitofrontal cortex |
| CORT – corticosterone | mPFC – medial prefrontal cortex |
| CRF – corticotropin-releasing factor | MR – mineralocorticoid receptor |
| CRFR1 - CRF receptor type 1 | NA – noradrenaline (norepinephrine) |
| CRFR2 - CRF receptor type 2 | NAc – nucleus accumbens |
| CSDS – chronic social defeat stress | NAcC – nucleus accumbens core |
| D ₁ – dopamine receptor type 1 | NAcSh – nucleus accumbens shell |
| D ₂ – dopamine receptor type 2 | NIH – National Institute of Health |
| DA – dopamine | NSF – novelty suppressed feeding |
| DAT – dopamine transporter | NTS – nucleus tractus solitarii |
| dIPFC – dorsolateral prefrontal cortex | OFC – orbitofrontal cortex |
| DLS – dorsolateral striatum | PFC – prefrontal cortex |
| DM – decision-making | PL – prelimbic cortex |
| dmPFC – dorsomedial prefrontal cortex | PR – progressive ratio |
| DMs – decision-makers | PSTN – paraventricular nucleus |
| DMS – dorsomedial striatum | PVN – paraventricular nucleus of the hypothalamus |
| DREADDs – designer receptors exclusively activated by designer drugs | PVT – paraventricular thalamic nucleus |
| DSM - Diagnostic and Statistical Manual of Mental Disorders | RDoC – Research Domain Criteria |
| dWST – delayed win-shift task | RL – reversal learning |
| ELS – early life stress | RM-ANOVA – repeated measures analysis of variance |
| EPI – epinephrine (adrenaline) | RN – raphe nuclei |
| FCS – fur coat state | SPT – sucrose preference test |
| FFT – free feeding task | SSRIs – selective serotonin reuptake inhibitors |
| FR – fixed ratio | STN – subthalamic nucleus |
| FST – forced swim test | TRN – thalamic reticular nucleus |
| GABA - gamma-aminobutyric acid | UCN - urocortin |
| GC – glucocorticoid | VEH – vehicle |
| GLU - glutamate | VH – ventral hippocampus |
| GPCR – G protein-coupled receptor | vIPFC – ventrolateral prefrontal cortex |
| GR – glucocorticoid receptor | vmPFC – ventromedial prefrontal cortex |
| GRE – glucocorticoid response element | VTA – ventral tegmental area |
| HPA – | WM – working memory |
| Hypothalamo-pituitary-adrenal axis | βCD – β-cyclodextrine |
| | 5-HT – serotonin |

GLOSSARY

Abulia – reduced spontaneous motor, cognitive and emotional behaviours.

Allostasis – process by which a state of equilibrium is maintained through altering physiologic parameters to counteract challenges.

Ambiguity – a situation where the degree of uncertainty associated with a particular event is unknown to the subject; also called *unexpected uncertainty*.

Anergia – loss of energy.

Anhedonia – a state of general loss of interest and pleasure for rewarding items or activities.

Anticipation – a feeling of excitement about something that is going to happen in the near future.

Apathy – loss or reduction of motivation compared with an individual's previous state.

Appetitive behaviour – approaching or goal-seeking phase of behaviour.

Associative learning – the ability to perceive contingency relations between events in their environment.

Aversion to negative outcome – a tendency to avoid losses over the maximization of benefits.

Avolition – inability to perform self-directed, purposeful activities.

Behavioural flexibility – the adaptive change of behaviour in response to changing environmental contingencies.

Cognitive bias – a tendency to have selectivity in cognitive processes based on the biological state of the body, which results in distortion of the reality.

Cognitive flexibility – the mental ability to switch between thinking about two different concepts according to the context of a situation.

Cognitive interlock – a self-perpetuating processing configuration that occur through negative self-focused cognitive processing.

Consummatory behaviour – completion or consummation phase of behaviour.

Contingency – a future event or circumstance that is possible but cannot be predicted with certainty.

Decisional processing to option selection – the evaluation of the options through attribution of values.

Distress – state subsequent to events linked to uncomfortable feelings that predominantly lead to adverse outcomes.

Ecological validity – the idea that something measured with a laboratory test translates to performance in real-life settings.

Effort valuation – estimation of the worth of an effort to obtain a certain outcome.

Endophenotype – a genetic epidemiology term that is used to parse behavioural symptoms into more stable phenotypes with a clear genetic connection.

Eustress – positive form of stress having a beneficial effect on health, motivation and/or performance.

Exhaustion – a chronic state of physical and emotional depletion that results from continuous stress.

Fatigue – weariness or diminished ability following mental or physical activity.

Goal-directed behaviour – the behaviour resulting from the selection of actions on the basis of desired goals.

Habit – learnt dispositions to repeat past responses that not involve conscious thought.

Hedonic capacity – the ability to experience pleasure and reward from pleasant stimuli.

Hedonic impact – influence of pleasurable events on an individual's willingness to move towards or against a goal or threat.

Helplessness – the inability to defend oneself or to act effectively.

Homeostasis – self-regulating processes by which organisms tend to maintain stability.

Hopelessness – a feeling or state of despair.

Incentive value – the perceived value of a motivating stimulus or condition that varies among individuals.

Learning – acquisition of information that acts altering future behaviour.

Loss/penalty overappreciation – the tendency to overvalue a loss or penalty.

Option generation – the identification of present possibilities or options.

Option selection – the selection of an action based on the individual's needs.

Options exploitation – a directed behavioural strategy in which choices are explicitly biased toward information seeking.

Options exploration – a random behavioural strategy in which decisions lead to acquisition of new knowledge by chance.

Prepotent responses – automatic behaviours associated to immediate reinforcement.

Primary reinforcers – rewarding stimuli that facilitate the survival of an organism or its offspring.

Reinforcement learning - the process from which individuals learn from the outcomes of their actions to guide future behaviour.

Resilience – the ability to recover from or adjust easily to change.

Reward responsiveness – the development of a bias towards the option more frequently rewarded.

Reward underappreciation – the tendency to disvalue a reward.

Reward-prediction error – a computational quantity referring the difference between expected and actual outcomes.

Risk – a situation where there is a possibility of an incontestable negative outcome, and the probability of occurrence is known to the individual; also called *expected uncertainty*.

Rumination – the process of continuously thinking about the same thoughts.

Secondary reinforcers – rewarding stimuli that can provide pleasure and motivate behaviour once primary needs are fulfilled.

Sensitization – a non-associative learning process in which repeated exposure to a stimulus results in the progressive amplification of a response.

Set-shifting – the ability to switch back and forth between multiple tasks.

Uncertainty – a situation involving imperfect or limited knowledge where it is not possible to make exact predictions of future events.

PUBLICATIONS AND COMMUNICATIONS

Scientific journals

- L. Cabeza**, B. Ramadan, S. Cramoisy, C. Houdayer, E. Haffen, PY. Risold, D. Fellmann & Y. Peterschmitt, “Chronic distress in male mice impairs motivation compromising both effort and reward processing with altered anterior insular cortex and basolateral amygdala neuronal activation”, *Frontiers in Behavioural Neuroscience* (2021) 15. DOI: 10.3389/fnbeh.2021.717701.
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- L. Cabeza**, J. Giustiniani, T. Chabin, B. Ramadan, C. Joucla, M. Nicolier, L. Pazart, E. Haffen, D. Fellmann, D. Gabriel & Y. Peterschmitt, “Modelling decision-making under uncertainty: a direct comparison study between human and mouse gambling data”, *European Neuropsychopharmacology* (2020) 31, 58-68. DOI: 10.1016/j.euroneuro.2019.11.005 .
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- S. Cramoisy, **L. Cabeza**, B. Ramadan, C. Houdayer, E. Haffen, PY. Risold, D. Fellmann & Y. Peterschmitt, “Assessing impulsivity and risk-taking contributions to adaptive and maladaptive decision-making in distressed mice”, NeuroFrance (France), 11th-15th July 2021.
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Oral communications

PhD project diffusion - Interview in *Radio Campus Besançon* with Cécile Pollart, 17th October 2019.

L. Cabeza, “La prise de décision dans un modèle animal de dépression”, Annual competition of scientific communications, Besançon (France), 13th December 2018.

L. Cabeza, D. Gabriel, N. Galmes & Y. Peterschmitt, “Neurosciences et prise de décision: qu’est-ce qui détermine nos choix?”, Semaine du cerveau 2019 (organized by the French Neuroscience Society), Besançon (France), 13th March 2019.

Grants

Doctoral mobility grant, University of Bourgogne – Franche-Comté, 2020.

DAAD research fellowship, Max Planck Institute of Psychiatry, Germany, 2020.

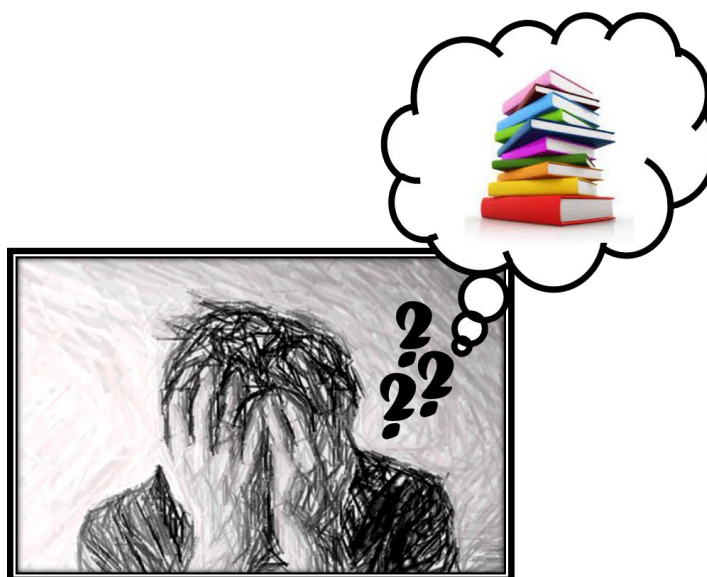
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PREAMBLE

Which book should I read now?

Here an easy decision to make, at least at first sight. Depending on the books available on a bookshelf, I will decide. I start by discarding the ones I have already read. Still many possibilities. But am I really in the mood for a historical novel? Or maybe better a bit of fantasy? Well, but this book looks a bit too thick, does it not?

Many alternatives can make us doubt and decisions may require a bit of time. Yet, in normal situations healthy people are able to choose what to do next... fortunately.



We are constantly making decisions. However, unexpected events can complicate the process of selecting between several alternatives. Being in a dynamic environment requires constant adaptation to new situations in order to continue making good decisions, or at least to behave in a way that does not disfavour ourselves. Nevertheless, major distressful events, especially if they happen in early-life periods or distributed over a prolonged time, threaten our mental stability and can hamper our ability to make decisions.

Extended bibliography highlights distress as a key factor for developing neuropsychiatric disorders. Combined, depression and anxiety together, for example, reach an alarming 25% of prevalence in the European population (*World Health Organization*). Patients frequently report comorbid cognitive alterations, including decision-making impairments, learning and memory deficits, and an important lack of motivation for daily normal activities. These symptoms clearly impinge on their quality of life, including the quality of social relationships and their ability to efficiently work. Moreover, in the particular case of decision-making, alterations are considered especially influential in prompting suicides.

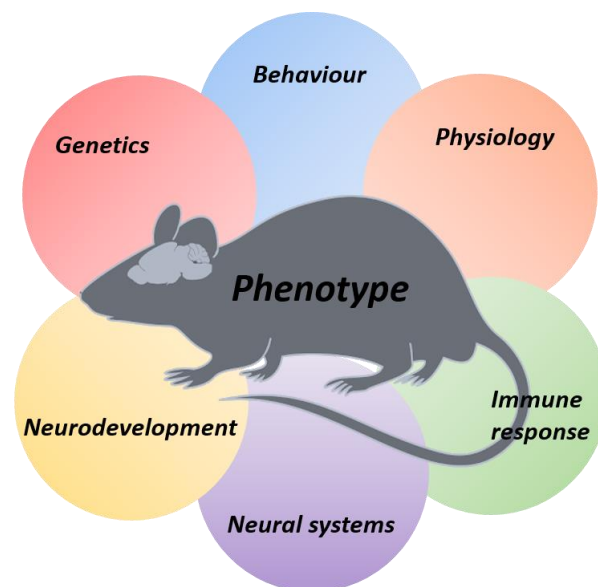
A major problem today concerns treatment for distress-related disorders such as depression. They generally target emotional symptoms rather than cognitive deficits, and their efficacy is limited. Besides, aetiology of many distress-related disorders is still not fully understood. Taken together, these factors place the pathologies caused by sustained exposure to stressful events into the spotlight of public health concerns.

Studying the biological foundations of human decisional dysfunction subsequent to sustained distress is not an easy task, since neuropsychiatric conditions such as depression and anxiety span a wide phenotypical spectrum. Not all patients suffering from these pathologies present the same emotional and cognitive symptoms, neither they suffer with the same severity. Their behaviour therefore differ significantly, and just as much does it differ for healthy individuals.

Understanding this interindividual behavioural variability in healthy and compromised populations is the main motivation of the present doctoral investigation. This study aims at shedding light onto the behavioural and neural mechanisms underlying decision-making in healthy subjects as well induced alterations in response to sustained distress and across different clinical conditions.

To address this challenging task, the working hypotheses of this investigation have been grounded based on extensive scientific evidence on the detrimental physiological and cognitive effects of chronically elevated circulating glucocorticoids (Wolkowitz et al., 2009). In the presence of stressors, organisms produce and release glucocorticoids into their general circulation where these operate as modulators of the physiological adjustments required to overcome the stressor-induced situation. Detrimental effects of sustained elevated levels of glucocorticoids therefore relate to the experience of chronic distressful events (Marin et al., 2011; McEwen, 2017) as well as chronic inflammatory diseases that are treated with exogenous glucocorticoids (Oray et al., 2016; Paragliola et al., 2017; Straub and Cutolo, 2016). In addition to that, the contribution of hypercortisolaemia to the general symptomatology reported in neuropsychiatric populations affected with severe cognitive dysfunction has also been confirmed (Gomez et al., 2009; Hinkelmann et al., 2009).

In this perspective, animal models are key tools to study the neurobiology underpinning the cognitive alterations caused by chronic exposure to distress. Human distress-related symptomatology can be modelled in rodents through a paradigm of chronic glucocorticoids administration (Darcet et al., 2016a). Animals chronically treated with glucocorticoids exhibit a behavioural spectrum reminiscent to emotional anxio-depressive symptoms (Darcet et al., 2014; David et al., 2009; Dieterich et al., 2020, 2019; Gourley et al., 2008). Current laboratory-techniques allow addressing multiple aspects behind this phenotype, ranging from physiological adjustments to stressors to the neurodevelopmental modulating factors. Preclinical tests investigating decisional processes have demonstrated that interindividual variability between healthy or ill animals is as high as in humans (Daniel et al., 2017; de Visser et al., 2011; Pittaras et al., 2016; Rivalan et al., 2013; Steingroever et al., 2013), and this is what the following investigations hinge upon.



By using a suited animal model for reproducing a human distress-related pathology, and by exploring behavioural paradigms specifically adapted to the animal nature, this doctoral investigation aims at providing novel insight into the mechanisms of maladaptive decision-making caused by sustained distress. This study seeks at identifying biological markers of vulnerability to decisional deficits caused by sustained distress, as well as at contributing to the understanding of the underlying pathophysiological mechanisms. Thus, this investigation's objective is the identification of alternative pharmacological targets towards precision medicine in biological psychiatry, and through that to aid in the elevation of the emotional and cognitive burden suffered by patients with clinical conditions.

The thesis introduction is structured in three main sections that present pivotal points of the present project. **Section A** introduces current knowledge and the state of the art regarding decisional

processes, including their neuronal bases and clinical and preclinical tools for their study. It also informs about specific decisional alterations in clinical populations. **Section B** of the introduction is dedicated to the current knowledge about distress, including the underlying mechanisms of the physiological stress-response. It also informs about how cognitive functioning is altered upon sustained distress and the current possibilities to model chronic distress-related disorders in the laboratory. Finally, **section C** of the introduction illustrates how decisional processes change upon sustained distress, and how transdiagnostic approaches could help disentangling the complexity of their relationship.

Following the introduction, the investigation is presented in four chapters. The **first chapter** presents the results concerning the modelling of a decision-making task for a murine population, with especial emphasis on its suitability to address human behaviour. The **second chapter** presents the results concerning the modelling of chronic distress-related symptomatology in a murine population and its impact on decisional processes. The **third chapter** provides the results of the study on five potential behavioural modulators of decision-making: hedonic appreciation, working-memory, motivation, behavioural flexibility and psychomotricity. Finally, the **fourth chapter** presents the results of the investigation on the neurobiological correlates of behavioural alterations identified in the second and third chapters. It aims a neurobiological explanation for the emergence, maintenance and aggravation of distress-related human symptomatology.

An overall discussion of the findings of this investigation is provided afterwards.

GENERAL INTRODUCTION

A. Making decisions

Decision-making (DM) is a cognitive process resulting in the selection of a course of action among several alternatives. Thus, a decision is a deliberate process resulting in the commitment to a precise possibility. In everyday life, encompassing choices vary in difficulty, especially as a function of the relative subjective value of their outcomes that change through context sensitivity. Decision-making is therefore a motivated behaviour, where specific goals will guide individuals to achieve desired outcomes while avoiding the undesired. Since making decisions is an intended process, the goals' context is crucial and actions might need according adjustment. Thus, optimal DM concerning particular goals would lead to behaviours resulting in a maximization of the accuracy to achieve the desired outcomes.

In all its complexity, the adaptive process of DM is mostly decomposed in four sequential stages (Doya, 2008; Gold and Shadlen, 2007; Husain and Roiser, 2018): (i) the identification of the present possibilities or options (*option generation*), (ii) the evaluation of the options through attribution of values, thus making predictions about actions' outcomes (*decisional processing to option selection*), (iii) the selection of an action based on the individual's needs (*option selection*), and (iv) the evaluation of consequences that resulted from the selected action (*hedonic impact and learning*) (see **Figure 1**).

- *Option generation*. The process of making decisions involves the enunciation, consciously or unconsciously, of (at least two) competing hypotheses that represent a state of the individual's reality. Options can be self-generated, e.g., guided by physiological or emotional drives, or cued by environmental stimuli, e.g., the presentation of associative perceptive stimuli.
- *Decisional processing for option selection*. Each generated hypothesis will be processed before the initiation of an action, and will probably be affected by many factors, such as the subjective value of the expected outcome. As a consequence, they would have an associated probability estimated to be true before receiving any related evidence, i.e., any information strengthening the commitment to a hypothesis.
- *Option selection*. The former decisional elements (hypotheses, evidences and attributed values) are collectively interpreted to produce a discrete choice and initiate the course of action (*appetitive behaviour*), which is normally necessary for reattribution of values in response to the evaluation of potential outcomes. Once the choice is made and prior to outcome encounter, individuals usually experience an emotional reaction related to the awaiting of the expected outcome (*anticipation*).
- *Hedonic impact and learning*. The relationship between hypotheses and associated evidences is useful if interpretations about the likelihood are possible, i.e., if individuals are able to monitor evidence and its impact on the hypotheses' probabilities. The subjective valuation of outcomes, i.e., the attribution of subjective costs and benefits, is then a dynamic process since values continuously are updated through feedback, and either increase or decrease in accordance with their *hedonic capacities*. Feedback, moreover, can be explicit, i.e., if it involves physical presentation of the output to the individual (e.g., a monetary reward or a distasteful food), or implicit, i.e., if costs and benefits reflect tacit factors (e.g., invested time or effort). Ultimately, decisions are then categorized as more or less adapted, as it may be reflected by the intention to pursue or avoid the course of action previously selected (*learning*). And it is at

this point when the connection between DM and other high-order capacities, such as planning, are revealed themselves as of relevance.

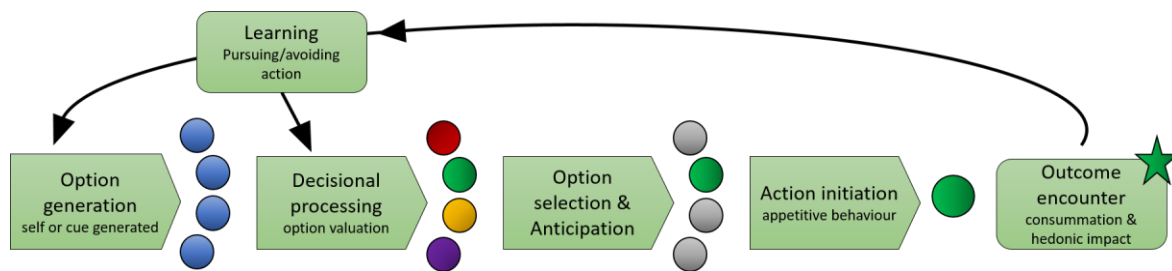


Figure 1. Sequential stages of DM. Before the initiation of a course of action, potential options for behaviour (blue disks) might be elaborated either by internal states (self-generated) or cued by the possibilities offered in the environment. Once the potential options have been generated, they need to be evaluated in terms of their benefits or costs before an option for behaviour is selected. The act of choosing a potentially rewarding option (green disk) often induces a motivational arousal, the so-called anticipation effect. Then, the initiation of the action is promoted by approaching or appetitive behaviour that leads to outcome encounter (green star). In the consummation of the choice, outcomes exert a hedonic impact on the individual that will influence the process of learning in order to adaptively adjust the behaviour. Subsequently, options are reevaluated before the selection of a new option. The process of learning can also motivate the generation of new options. Adapted from Husain and Roiser, 2018.

According to the *Somatic marker hypothesis* (Damasio and Everitt, 1996; Damasio et al., 1994), DM depends on many neural operation levels, of among which cognitive operations depend themselves on support processes such as working-memory (WM), attention and emotion. In addition, DM also more or less strongly depends on the accessibility to knowledge concerning an individuals' spatio-temporal context, choice possibilities and potential outcomes. This intrinsic complexity of DM has motivated a growing body of studies in cognitive neurosciences, in an effort for disentangling its underlying neural mechanisms.

a.1. Decision-making and reinforcement learning

Making decisions is not a homogeneous process. Organisms are usually compared with different kind of decisions, ranging from the selection of an alternative based on cost-benefit predictions, to making inferences or even moral decisions that are not necessarily the most beneficial for the single individual. In addition, decisions can also differ in complexity in function of their relative degree of *uncertainty*, *risk* and/or *ambiguity* (Gardenfors and Sahlin, 1982). An uncertain situation is one involving imperfect or limited knowledge, where it is not possible to make exact predictions of future events as well as future outcomes. Risky situations refer to those where there exists the possibility of an incontestable negative outcome, for example getting hurt, the loss of something valuable or being defeated. Ambiguity applies to situations where an identical fact can have more than one possible meaning and therefore possibly causing confusion (Winstanley and Floresco, 2016). In such cases, the knowledge or inference of the outcome probability rule allows the development of decisional strategies. Decision-making is consequently a future-oriented process that seems to rely on high-order functions including planning, cognitive flexibility and memory.

The connection between feedback processing and DM creates a prediction-choice-outcome loop that might be fundamental in associative learning and *reinforcement learning*, i.e., the process from which individuals learn from the outcomes of their actions to guide future behaviour (Husain and

Roiser, 2018). The prediction-choice-output loop in associative learning concurs with the *Somatic marker hypothesis* (Bechara et al., 1994; Damasio and Everitt, 1996), wherein experiencing rewarding or unpleasant outcomes after a decision under uncertainty will stimulate emotional responses that will guide following decisions to be taken after such an event. Orienting future behaviour towards optimal output therefore necessitates learning and memory processes to enable behavioural adjustment relative to a changing environment. Simultaneously, output hedonic capacity plays a central role in guiding future choices by influencing the valence attribution to the choice alternatives.

In the study of DM, reward-related actions are primarily investigated in relation to the interaction of two different learning processes: on one side, a learning process controlling the acquisition of goal-directed actions, and on the other side, one controlling the acquisition of habits. In the first case, the selection of an action is governed by the association between a particular action (or response) and its consequence (or output). In the case of habits, the selection of an action is motivated by the association between a particular stimulus and a response that it is not linked to the outcome of that action. At this point, *Pavlovian* and *Skinnerian* conditioning experiments should be discerned: *Pavlovian* learning or classical conditioning alludes to the association of initially meaningless somatosensory cues that acquire predictive value as they signal behavioural relevant events (Pavlov, 2010). *Skinnerian* or operant/instrumental conditioning is a learning process based on associations between deliberate behavioural actions and their consequences, and where behaviours can be strengthened by reinforcers or punishment (Skinner, 1988). Most relevant, Pavlovian and Skinnerian conditioning experiments have demonstrated the different sensitivities of goal-directed and habit-based learning processes to the value dynamics of the outputs, and to the action-outcome contingency. In reinforcement learning, therefore, all actions associated to consequences for an individual ultimately rely on the hedonic capacity of the rewarding or unpleasant stimuli.

a.1.1. Action value and contingency rule

The value of an action depends on one hand on the outcome's desirability, and on the other hand on the contingency related to a particular outcome (i.e., a provision for a future event). This is easy to illustrate with food-rewarding: at least to the certain extent, the value of a specific food reward is a function of the individual's satiation state. In this regard, the output's nature is considered a major modulator of DM, since it leads to differential behavioural reinforcement. Food-rewarding, for example, acts as a primary reinforcer through maintaining and invigorating the pursue of an action and satiating a basic biological drive: hunger. Money, on the contrary, is a secondary reinforcer since its value is relative to primary reinforcers. However, the value of a reward is also sensitive to its causal relationship with the action required to obtain it.

Outcome devaluation paradigms demonstrated that animals, just as humans, are able to encode action-outcome associations (contrary to the first assumptions limiting their capabilities to sensorimotor learning), but also that reward-value loss causes a reduction in performance (see for instance, Balleine and Dickinson, 1998). Thus, after training individuals are able to integrate new (sensory, motivational, hedonic) information about the reward value experienced in order to adapt their subsequent behaviour. The devaluation effect has also been demonstrated to depend on the amount of training, consistent with the view of habitual behaviour acquisition (stimulus-action association) (Dickinson et al., 1995; Killcross and Coutureau, 2003).

Contingency degradation paradigms have also demonstrated the relevance of the causal relationship between a predictive stimulus/action and the obtention of a specific reward. When this contingency relationship is reduced or *degraded*, behavioural performance worsens, proving that new experience information related to ineffective instrumental action is updated (Ostlund and Balleine,

2007). Similarly as for outcome devaluation, the sensitivity to contingency changes depends on training, with habitual behaviour being mostly insensitive (Balleine and Dickinson, 1998).

Furthermore, as a learning process concerning action-outcome associations, behavioural conditioning can influence DM processing through output (mostly reward) anticipation. Indeed, the emergence of anticipation processes following a cue association of positive outcomes can bias conditioned behaviour towards inflexible choices. Similarly, *aversion to negative outcome* (i.e., a tendency to avoid losses over the maximization of benefits) can also bias decisional strategies leading to maladaptive choice behaviour. Thus, the *reward-prediction error* (i.e., a computational quantity referring the difference between expected and actual outcomes) reflects the degree of emotional agreement with the encountered outcome following an option selection, and influences conditioned responding.

a.1.2. Behavioural flexibility and habits

Flexibly updating cue-reward associations is a necessary cognitive process for capturing the central tendency of outcomes in a given environment and in order to improve predictions prior to new decisions. Thereupon, a failure to integrate information resulting from learning and new selection of actions would probably lead to aberrant DM and subsequent increment of losses or unpleasant outcomes. Under stable conditions and non-changing environments however, the revaluation of outcomes does not require continuous updating, resulting in habitual actions consistent with invariable action-outcome associations. In terms of efficacy, this habitual behaviour demands less cognitive investment and is usually displayed more rapidly. These two associative elements, goal-directed and habit-based processing, which are pivotal in instrumental behaviour, conduct the behavioural control during adaptive DM. Dysregulated habit-based behavioural control, for example, often results in greater cognitive inflexibility. *Prepotent responses*, i.e., automatic behaviours associated to immediate reinforcement, do therefore necessitate of executive functions to be overridden (Uddin, 2021).

In uncertain and/or risky contexts, *reward responsiveness* or the development of a bias towards the option more frequently rewarded, does not only depend on the formation of adaptive action-outcome associations, but also on the individual's ability to generate options. In fact, cognitive flexibility allows searching for potentially better alternatives (*options exploration*) for decisional strategy, even if initially this is in conflict with taking advantage of familiar alternatives (*options exploitation*) and hence exposes individuals to risky outcomes.

a.1.3. Memory

Decision-making is a future-oriented dynamic process relying on reinforcement learning. Past experiences therefore influence the subjective value of each predicted outcome prior to a new course of action. The integration of memories is therefore necessary in adaptive DM, especially interventions of short-term memory systems when dealing with uncertainty. When the environment dynamically changes, then learning rates for instrumental associations mostly depend on the time spent by the individual on learning, as well as on the availability of memories concerning past experiences. In the management of relevant information, WM, i.e., a cognitive function that allows holding a limited amount of information in the focus of attention (Smith and Jonides, 1999), influences the process of making decisions by contributing to the development and attribution of incentive values, i.e., the individual perceived value of a motivating stimulus (Hinson et al., 2002; Jameson et al., 2004). Indeed,

the more dimensions of benefit and loss are involved in the decisional learning process, the more necessary are the informative resources hold by WM systems.

Reinforcement learning is therefore a complex process relying on perceptive and cognitive functions that are differentially routed in brain substrates and networks. These brain networks in their turn operate in a coordinated manner to orient behaviour and to shape new decisions.

a.2. Anatomical basis of decision-making

The neurobiology underlying DM has been extensively studied during the past decades (see for instance Khani and Rainer, 2016; O'Doherty et al., 2017). Behavioural examinations, in combination with physiological measurements and functional brain imaging techniques, have been used for assessing not only human DM, but also in preclinical studies based on rodents and non-human primates, substantiating translational findings.

In general terms, in the process of making adaptive decisions, multiple sources of somatosensory information are integrated to efficiently select the most advantageous options. Behavioural performance in human decisional tasks is thought to depend on learning processes that control goal-directed and habitual behaviour, and therefore on the encoding of the relationship between actions and their consequences, and the formation of stimulus-response associations respectively. These processes are in turn apparently governed by corticostriatal networks. Specifically,

human goal-directed behaviour has been described to primarily rely on activity of the prefrontal cortex (PFC) and the dorsal striatum (anterior caudate), while the emergence and maintenance of habits depend on striatal functioning (posterior lateral putamen) (Balleine and O'Doherty, 2010; Walton et al., 2004). These learning processes responsible of shaping decisions apparently compete for performance control and are modulated by other operators, as it is the case for the cortico-basal ganglia network.

Other anatomically complex network systems have also been described as players in decisional processes. The particular case of the human PFC, for instance, includes four main subregions: the orbitofrontal cortex (OFC), the anterior cingulate cortex (ACC), the dorsolateral PFC (dlPFC) and the ventromedial PFC (vmPFC). The PFC is intimately connected with thalamic

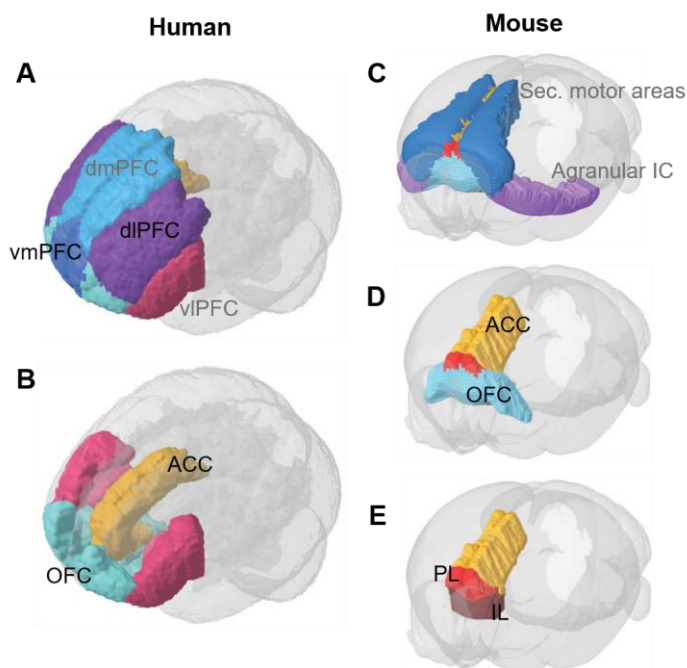


Figure 2. Functional division of the human and mouse PFC. (A,B) The common functional divisions of the PFC are illustrated in a tilted frontal-side view of the human brain. Besides the dlPFC, vmPFC, ACC and OFC, the dorsomedial (dmPFC) and the ventrolateral PFC (vlPFC) are also illustrated (in grey). **(C-E)** Illustration of the PFC in a tilted frontal-side view of the mouse brain. Besides the ACC, OFC, PL and IL regions of the mPFC, secondary motor areas and the agranular insular cortex (IC) are also illustrated. Adapted from Carlén 2017.

nuclei, acting as gate of sensory information from the external environment (Wolff et al., 2021). An OFC–vmPFC–limbic system network has been linked to reward and emotional processing during DM, while the dlPFC would predominantly integrate context information, and the ACC would manage conflictive information and process feedback information during reinforcement learning (Khani and Rainer, 2016).

Fortunately, preclinical studies have found rodent homologies that have strongly contributed, and still do, to disentangle the intricate details of the neurobiology underpinning DM. Today, the functional correspondences between the human dlPFC, vmPFC, anterior caudate and posterior lateral putamen, and the rodent prelimbic (PL) cortex, dorsomedial (DMS) and dorsolateral striatum (DLS) are well established (Balleine and O’Doherty, 2010; Carlén, 2017; Seamans et al., 2008; Uylings et al., 2003) (see **Figure 2**). Anatomical descriptions in the present manuscript will hereafter refer to rodent brain regions.

a.2.1. Medial prefrontal cortex

The rodent medial PFC (mPFC) is a structurally complex brain region comprising four structurally and functionally heterogeneous subregions: the PL cortex, infralimbic (IL) cortex, ACC and medial OFC (mOFC) (Dalley et al., 2004). Extensive brain-tracing studies have pointed to the mPFC’ pivotal role on goal-directed behaviour, which is due to its role in integrating an important amount of information from a wide range of afferent projections (Hoover and Vertes, 2007). Each subregion of the mPFC is strongly connected to adjacent regions, as well as intimately interconnected. There is scientific evidence supporting a dorsal-ventral functional gradient within the mPFC, where dorsal regions (dorsal PL cortex and ACC) are specialised in the control of actions, while ventral regions (ventral PL and IL cortices) predominantly manage autonomic and emotional information (Euston et al., 2012). According to their relevance for the present doctoral project, the subregions mentioned above are thenceforth detailed.

Prelimbic cortex

The role of the PL cortex in DM is fundamental. It detects instrumental contingencies, i.e., the formation of action-outcome associations. The importance of the PL cortex in initiating goal-directed actions was firstly revealed thanks to drug self-administration and fear conditioning studies, but it seems that it can also act oppositely, i.e., inhibiting instrumental action. Indeed, it seems to distinctly operate in accordance with the context’s valence: appetitive contexts will primarily motivate, whereas aversive contexts would restrain the initiation of actions (Capuzzo and Floresco, 2020). The PL cortex activity is apparently also critical in situations in which flexible behaviour is necessary to adapt in face of new environmental elements (Floresco et al., 2009; Young and Shapiro, 2009). In fact, PL-function is required to actively avoid previously punished behaviour in favour of safety, especially in situations involving motivational conflicts, e.g., when reward-seeking and physical security are in confrontation (Capuzzo and Floresco, 2020). Recent investigations have demonstrated the contribution of PL-thalamic nuclei pathways in instrumental behaviour and DM. The thalamic reticular nucleus (TRN), in coordination with the striatum, modulates PL activity. This enables the inhibition of irrelevant information and therefore allows selective attention to conditioned stimuli, as well as a contribution to flexible performance (Wolff et al., 2021). The PL cortex acts also in coordination with the paraventricular thalamic nucleus (PVT), a brain region playing a pivotal role in associative learning through representation of salient cues. Indeed, there exists a top-down control of the PL-PVT pathway involved in incentive value attribution, with the anterior PVT participating in appetitive information processing, while the posterior PVT does so for aversive information (Wolff et al., 2021). The functional connectivity between the PL cortex and the mediodorsal thalamus (MD) has also been proposed to be

essential for adaptive DM, particularly during updating reward value and for the revaluation of action-outcome associations (Alcaraz et al., 2018).

The PL cortex does not only detect instrumental contingencies; it is also necessary for the extinction of old associations in favour of the formation of new ones. Thus, the process of extinction in instrumental behaviour constitutes an integral part of behavioural inhibition (Cardinal et al., 2002). Accordingly, the PL cortex has also been proposed to be necessary in the emergence and development of new decisional strategies.

In terms of connectivity, the PL cortex is intimately connected to the OFC and strongly innervated by the hippocampus. It also shares projections with key structures of the limbic system (the nucleus accumbens -NAc, amygdala and ventral tegmental area -VTA) and receives information from the insular cortex (IC), locus coeruleus (LC), and dorsal and median raphe nuclei (RN) among others. Hence, the PL cortex is strategically well placed to integrate information of different nature (Hoover and Vertes, 2007).

Infralimbic cortex

The IL cortex participates in decisional processes in a manner complementary to the PL cortex. Owing its key role in behavioural adaptation by suppressing instrumental behaviour irrespective of the valence of the context, this telencephalic region was firstly associated to the suppression of goal-directed actions. Hence, the IL cortex inhibits action-initiation both in appetitive and aversive contexts, but can facilitate goal-directed behaviours in the particular context of instrumental avoidance. Specifically, reward devaluation experiments have demonstrated that temporary pharmacological inactivation of the rat IL cortex after extensive training, provokes selective sensitivity to devaluation during instrumental responding. Goal-directed behaviour was reinstated in animals that would otherwise behave habitually and should be resistant to the devaluation protocol (Coutureau and Killcross, 2003). Comparable to the PL cortex, the activity of the IL cortex seems essential in flexible behaviours. Indeed, its pattern of neural activity changes profoundly after strategy switches, suggesting its implication in later learning processing compared to PL activity (Rich and Shapiro, 2009).

The IL cortex is considerably less innervated than the PL cortex, with the limbic system being the origin of almost the totality of its inputs. Within the mPFC, the IL cortex receives information essentially from the PL cortex and beyond, from the hippocampus, IC, basolateral amygdala (BLA) and the midline thalamus. Noteworthy, the limbic-innervation allows the IL cortex to exert a profound influence on visceral and autonomic activity (Hoover and Vertes, 2007).

Combined, the PL and IL cortices have been demonstrated to be essential in a wide range of cognitive processes, subserving instrumental behaviour including WM processes, reward anticipation, behavioural flexibility and attention (Delatour and Gisquet-Verrier, 2000; Orsini et al., 2018). Most directly, the inactivation of, both, the PL and IL cortices have been demonstrated to impair DM under uncertainty, with individuals tending to more frequently select long-term disadvantageous options (Paine et al., 2013; Zeeb et al., 2015).

Anterior cingulate cortex

It is generally accepted that the ACC is involved in multiple motivationally oriented behaviours as well as in emotional processing. The ACC is considered an important brain region for cost-benefit DM, required for the learning of contingencies associated to choice alternatives. This region seems to act through the integration of explicit cues in order to guide behaviour in dynamic contexts, i.e., it guides decisional processes under risk or uncertainty when discriminative and informative cues are

available for the individual. This point is of importance since scientific findings are controversial and ACC impairments do not always result in suboptimal DM (Rivalan et al., 2011; van Holstein and Floresco, 2020). It has been suggested that the ACC mediates error and conflict processing through modulating the aversive effect of unpleasant outcomes on new decisions. In fact, ACC lesions impair the acquisition of stimulus-reinforcer associations in operant tasks (Parkinson et al., 2000). Additionally, the ACC, by encoding the worthiness of an effort to obtain an outcome of a precise value, has also been suggested to be a key player in effort-based choice (*effort valuation*).

Concerning neural connectivity, the ACC receives vast projections from cortical and thalamic areas. Besides from being highly interconnected, it is strongly innervated from within the mPFC (mostly the PL cortex) and OFC (Wang and Shyu, 2004). It also receives subcortical projections from the hippocampal formation, bed nucleus of stria terminalis (BNST), BLA and VTA among others (Hoover and Vertes, 2007). In turn, the ACC principally projects to the basal ganglia, motor cortex and spinal cord.

a.2.2. Orbitofrontal cortex

Being involved in signalling the value of the expected outcomes, the rodent OFC plays a significant role in decisional processes (Stalnaker et al., 2009). An adequate valuation of a decision's potential outcomes is an essential step prior to the evaluation of its consequences. In fact, the OFC activity is necessary for the perception of dynamics concerning contingencies associated to the subjective outcome valuation. The OFC has therefore been suggested to be essential in DM under uncertainty, and specifically in gambling performance. Lesions in the lateral OFC, but not in the mOFC impair an individuals' ability to discriminate between choice contingencies by accelerating switching between consecutive selections, which prevents the adequate integration of their reinforcing load (Noonan et al., 2012).

Interestingly, OFC lesions increase the effort individuals expend for rewards, suggesting that its role in DM could be modulating an individuals' motivational state (Gourley et al., 2010). The OFC has also been associated to behavioural flexibility in studies where local lesions or drug infusions impair performance in reversal learning (RL) tasks (Bohn, 2003).

The OFC's connectivity supports the hypothesis about its essential role in integration of contextual salient information. The OFC is strongly innervated from sensory-associative cortical regions, and from the hypothalamus and the amygdala. Specifically, it is intimately connected with the BLA, both via efferent and afferent projections (Cardinal et al., 2002). The relationship between the OFC and the BLA has stimulated scientific attention and some studies have highlighted its relevance in decisional processing. Specifically the disconnection between these two regions impairs instrumental acquisition in probabilistic valued-based DM tasks, demonstrating the relevance of its functional connectivity (Zeeb and Winstanley, 2013). The OFC projects itself to the ACC, hypothalamus, VTA, and dorsal and ventral (NAc) striatum.

a.2.3. Insular cortex

The IC is a key telencephalic area involved in interoception and cognition, ranging from emotion to motivation (Gehrlach, 2019; Gogolla, 2017; Nieuwenhuys, 2012) (**Figure 3**). The anatomical analogy between the human insula and the rodent IC is well established (Allen et al., 1991; Singer et al., 2009). More specifically, the human agranular insula is analogous to the rodent anterior IC (aIC), while the human granular insula is that of the rodent posterior IC. In regards to the process of making decisions, rodent studies have demonstrated the engagement of the IC in a variety of functions including memory, associative learning and valence representation (Balleine and Dickinson, 1998;

Parkes and Balleine, 2013). Specifically the PFC-ACC-IC network contributes to reward and effort valuation and consequently, the IC has been proposed to participate in the integration of sensory information resulting from rewarding or unpleasant experiences (Husain and Roiser, 2018; Pushparaj et al., 2015; zu Eulenburg et al., 2013). Bilateral lesions of a rat's aIC produce bidirectional effects in probabilistic cost-benefit DM (Daniel et al., 2017), such that individuals with previously strong option preferences switched responding toward opposite alternatives. This switch on preference causes former long-term optimal performers to impair their DM strategy, whereas former long-term suboptimal performers obtain an improved DM for the same reason. Therefore, the aIC appears to facilitate reward responsiveness when facing uncertainty or risk depending on the individual's current state (Parkes et al., 2016).

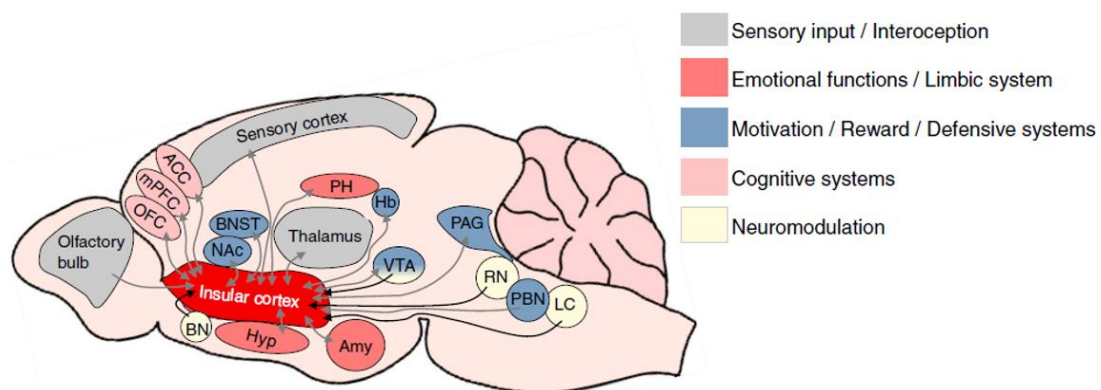


Figure 3. Schematic representation of the rodent insular basic connectivity. The IC is reciprocally connected (grey arrows) with sensory, emotional, motivational and cognitive brain systems. It receives neuromodulatory input (black arrows) from the basal nucleus (cholinergic, BN), from the VTA (dopaminergic), from the raphe nuclei (serotonergic, RN), and from the LC (adrenergic). Other relevant projections are also illustrated: ACC, amygdala (Amy), BNST, habenula (Hb), hypothalamus (Hyp), mPFC, NAc, OFC, periaqueductal grey (PAG), parabrachial nucleus (PBN) and parahippocampus (PH). Image from Gogolla, 2017.

The network connecting the IC, somatosensory and motor cortical areas, including prefrontal and ACC areas, is involved in higher cognitive processes (Nieuwenhuys, 2012), in intimate cooperation with the striatum and subthalamamic nuclei (STN) (Albin et al., 1989). The IC is also highly interconnected with the amygdala (particularly to the central amygdala -CeA), and their connectivity has been proposed as essential for valence processing (Gehrlach et al., 2020) and hence for shaping emotional outputs and subsequent behaviour (Nieuwenhuys, 2012). Importantly, IC and CeA projections target the STN-adjacent parabrachial nucleus of the hypothalamus (PSTN), which is involved in the regulation of food intake and satiation (Barbier et al., 2020). Thus, the IC-CeA-PSTN network has a prominent role in the control of feeding behaviours. Besides, the IC strongly innervates the NAc, especially its core (NAcC), and their circuitry participates in addictive behaviours and might operate as a functional interface for emotional and instrumental processing (Mogenson et al., 1980). In parallel, the IC sends and receives thalamic projections.

a.2.4. Striatum

The striatum, as a major input region of the basal ganglia, plays an essential role in action control and learning. It has been linked to reward responsiveness by refining the selection of course of actions, while participating in outcome information processing and habit formation (Graybiel and Grafton, 2015; Lovinger, 2010). The striatum is commonly divided into the so-called dorsal and ventral striatum regions. Generally, the dorsal striatum is associated to instrumental learning and the

development of instrumental habits, whereas the ventral striatum participates in motivational processes. These striatal subregions modulate decisional and learning processes within a network of brain structures, forming three main distinct circuits: (i) in interaction with cortical regions including the mPFC, the DMS constitutes an associative circuit; (ii) in connection with sensory and motor cortical areas, the DLS forms a sensorimotor circuit; and (iii) together with the BLA, hippocampus and limbic cortical connections, the ventral striatum or NAc forms a limbic circuit (Lovinger, 2010).

The dorsal striatum participates in the initiation of actions and in learning from their consequences (Belin et al., 2009) via the integration of multimodal information (Balleine et al., 2007; Balleine and O'Doherty, 2010). Together with the PL cortex, the DMS is involved in goal-directed learning, as shown by lesion experiments in rodents. Instrumental acquisition is prevented after lesion in the DMS, which renders individuals' performance promptly habitual (Balleine and Dickinson, 1998; Corbit and Janak, 2010). It also participates in processing output information and is especially sensitive to magnitude variations of the rewards and losses (Starcke and Brand, 2012). Indeed, pharmacological inactivation of the DMS in rats has been recently demonstrated to impact performance in decisional tasks relying on cognitive effort (Silveira et al., 2018) (Figure 4). The DLS is essential in the development of habitual behaviours, i.e., the emergence of a routine or automatism from a goal-directed

behaviour, and it consequently has been implicated in the emergence of compulsions (Everitt and Robbins, 2005). Lesions in the rodent DLS render animals goal-directed even after extensive training, as demonstrated in outcome devaluation and contingency degradation experiments (for review, see Balleine and O'Doherty, 2010).

The ventral striatum, more commonly named NAc, is composed of two structurally distinct subregions that function differently but in a complementary manner during reward-seeking (Singh et al., 2010). On one hand, the NAcC promotes appetitive behaviours and may facilitate flexible actions (Floresco, 2015; McGinty et al., 2013). On the other hand, the NAc shell (NAcS) acts through suppression of certain behavioural patterns that may interfere in goal pursuit, and therefore acts as a

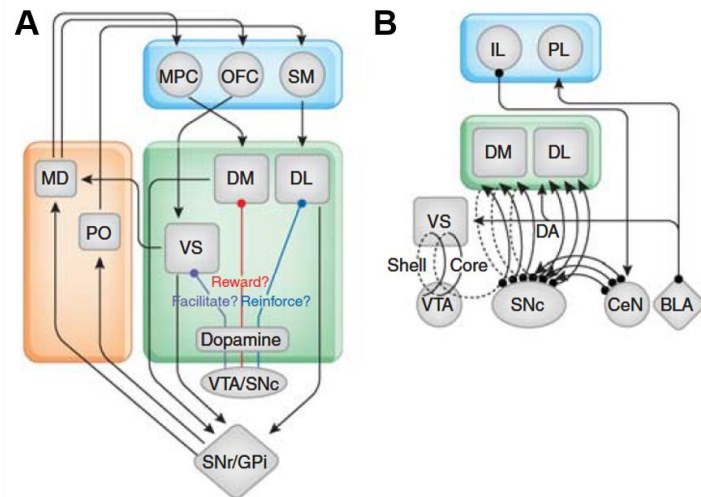


Figure 4. Corticostriatal determinants of goal-directed and habitual behaviours. (A) Schematic representation of distinct neural networks mediating goal-directed actions and habits, in interaction with neural substrates underlying motivational aspects of behaviour. Habitual behaviour is encoded in a network involving sensory-motor (SM) cortical inputs to the DLS (here DL), with feedback to cortex from the substantia nigra reticulata/internal segment of the globus pallidus (SNr/GPi). Goal-directed actions during rewarding tasks might rely on a parallel circuit linking the mPFC (here MPC), DMS (here DM), SNr and mediodorsal thalamus (MD). The hedonic impact of the outcomes may either facilitate or obstruct new choices, and operates through the connectivity between the amygdala and the ventral striatum (VS), mPFC and DM, and between the OFC and the VS. (posterior thalamus –PO; substantia nigra pars compacta –SNc). (B) Cortical, midbrain and limbic structures interact with the striatum to control goal-directed behaviour and habits. The schematic representation also illustrates dopaminergic reciprocal projections within a VTA-NAc and SNc-striatal networks, as well as the contribution of the BLA in reward processing. (central nucleus of the amygdala –CeN). Image from Balleine & O'Doherty 2010.

controller of inappropriate behaviours towards motivational cues (Piantadosi et al., 2017). Furthermore, the NAcS is involved in exploratory seeking in complex environments.

Combined, vast scientific data have demonstrated the NAc's contribution in goal-directed behaviour and DM through modulation of action selection. Indeed, in order to successfully obtain the most beneficial output, adaptively approaching or avoiding behaviours is of relevance in DM, especially in versatile environments. Decisions made under uncertainty mostly rely on brain networks involved in output processing, which therefore are sensitive to rewards and penalties, such it is the case of the striatum (Starcke and Brand, 2012).

a.2.5. Amygdala

Concerning DM, the amygdala plays a pivotal role as a hub for emotional processing for, both, pleasant and unpleasant experiences. Extensive scientific literature identifies the amygdala as a key contributor to reinforcement learning, and particularly in reward-guided DM (see for instance Baxter and Murray, 2002). Neuroanatomically, the amygdala is mainly divided into the BLA and the central medial nuclei group that includes the CeA and the medial amygdala (MeA). Forming part of a functional network including other brain structures such as the mPFC, the BLA and CeA have been involved in reward-value encoding and processing through complementary but different mechanisms (Balleine and Killcross, 2006; Seo et al., 2016). The BLA's role is of particular interest in DM since it is directly implicated in adaptive goal-directed behaviour. It is commonly accepted that the BLA acts through encoding motivationally-relevant representations of specific outputs from instrumental associations, ultimately guiding behaviour (Pignatelli and Beyeler, 2020). Indeed, the role of the BLA as a sensor of incentive value changes has been demonstrated through studies including devaluation experiences (S.H. Wang, 2005) and reward discriminatory tasks (Schoenbaum et al., 1998).

In dynamic environments, the BLA is required to face contingency changes in order to adapt responses after an outcome-conflict, i.e., when an outcome does not correspond to a specific instrumental association. The BLA therefore reacts to prediction errors and is likely involved in flexibility processes required for reorienting actions towards adaptive behaviour. Along this line, lesion or pharmacological inactivation of the BLA usually inclines DM towards safer strategies in cost-benefit paradigms, with eventual shifts towards risky choices after explicit penalties (Wassum and Izquierdo, 2015). However, the BLA's implication in effort-based DM has not been yet clarified, with studies showing divergent results (Floresco and Ghods-Sharifi, 2006; Hosking et al., 2014; McGregor et al., 1994).

The amygdala receives environmental information from sensory-cortical and thalamic areas. Reciprocally-connected BLA networks including the OFC, IC and hippocampus, are thought to strongly contribute in decisional processes. The functioning of a BLA–OFC–auditory thalamus (medial geniculate body -MGB) network in particular is necessary for successful conditioning (Wolff et al., 2021). The BLA also unidirectionally innervates the striatum (especially the NAc), BNST and CeA, structures that are thought to act as major translators of BLA-signalling to behavioural outputs (Janak and Tye, 2015). Apparently the BLA–NAc connectivity is directly involved in the response regulation resulting from rewarding and unpleasant outcomes, and BLA–BNST interactions contribute in modulating motivation-based behaviours (Beyeler and Dabrowska, 2020; Wassum and Izquierdo, 2015). The VTA and RN also send projections to the BLA. In parallel, the CeA projects to the VTA, substantia nigra pars compacta, LC and RN.

a.2.6. Hippocampus

Planning is a relevant process within DM. It is explicitly required for predicting the outcome of a course of action, since a mental representation of a potential future situation is necessary. At the same time, representing a potential future situation also requires of a mental representation of the individual current state. The hippocampus provides the neural substrate for both mental activities through self-projection processes and memory formation (Goodman et al., 2010), the so-called cognitive mapping (Johnson et al., 2007; Yu and Frank, 2015).

In the processes of cognitive mapping, memories are recalled to provide information for evaluation and comparison of possible options, and during this process, the hippocampus operates in intimate coordination with other brain regions. In particular, lesion studies compromising the hippocampal connectivity with the OFC and ventral striatum have demonstrated its relevance in outcoming value encoding. Therefore, the hippocampus likely supports valued-based DM by contributing to the revaluation of outcomes and subsequent reconstruction of state representations through memory recalls. This way of functioning has been proposed to engage flexible learning, since memory information needs to be leveraged in order to adjust behaviour to adaptive future choices (Palombo et al., 2015).

a.3. Neuromodulatory systems in decision-making

The underlying neurochemistry of decisional processes is of utmost importance to fully understand their neural mechanisms. Brain neurotransmission is predominantly glutamate (GLU) and gamma-aminobutyric acid (GABA)-mediated, leading to excitatory and inhibitory effects respectively in a balanced manner (Petroff, 2002; Zhang and Sun, 2011). The transmission of learnt and recalled information is therefore driven by GLU and GABA-related neural activity, but DM-related brain networks also utilize other neurotransmitters to effectively communicate and generate adequate behavioural responses. Besides, the release of other substances during the synaptic communication, so-called neuromodulators, tends to activate or inhibit entire neural circuits involved in specific cerebral functions through widespread projections over the entire cortex and subcortical targets. Neuropeptides also participate in neurotransmission, either by modulation of the synaptic communication or directly as neurotransmitters, as is the case of the corticotropin-releasing factor (CRF) (Deussing and Chen, 2018). Pharmacological evidence has revealed the essential role of two neuromodulatory systems in DM-related synaptic activity, the dopamine (DA) and serotonin (5-HT) systems (for review see for instance Rogers, 2011), which are further detailed below.

a.3.1. Dopamine

Dopamine is a brain neurotransmitter of the catecholamine family, synthesized via its direct precursor L-DOPA from the non-essential amino-acid tyrosine via tyrosine hydroxylase and dopamine decarboxylase action. Importantly, DA is synthesized in the substantia nigra and the VTA, and the blood-brain barrier is impermeable to it. Dopamine projections from the substantia nigra innervate the dorsal striatum, acting predominantly in motor control. The VTA sends dopaminergic projections to the NAc, amygdala and hippocampus, constituting the so-called dopaminergic mesolimbic circuit. Finally, the VTA also innervates the PFC in a mesocortical circuit (Haber, 2014).

The DA system plays distinct significant roles in DM. Most importantly, the DA system is essential for motivated behaviours by directly participating in option generation, prediction of rewards and losses in instrumental learning, outcome reevaluation and output-related learning necessary for behavioural adjustment (Ang et al., 2018; Le Heron et al., 2020; Salamone and Correa, 2012; Schultz, 2016). Dopamine expressing neurons participate in reward-prediction error encoding (for literature review see Khani and Rainer, 2016; Rogers, 2011), affecting plasticity and action value learning in cortico-basal networks (Cinotti et al., 2019). Noteworthy, the studies of Schultz and colleagues were seminal in understanding the mesolimbic DA contribution in reward prediction (Schultz et al., 1997). Dopamine release in the NAcC is involved in the formation of instrumental associations during value-based DM, while in the NAcS it encodes motivational information. Recent studies have also highlighted that higher DA tones are associated to higher exploration rates in rodents. The DA system is therefore directly involved in the modulation of incentive choice, and engaged in reward responsiveness in cost-benefit and effort-based DM tasks (Salamone et al., 1994). It does so by participating in risk-taking and effort valuation associated behaviours (Cinotti et al., 2019). Besides, it has been proposed that DA receptors type 1 and 2 (presynaptic-D₁ and postsynaptic-D₂) play dissociable roles in reward-based DM. Striatal neural activity resulting from reward encountering would facilitate or inhibit responses depending on the relative distribution of D₁ and D₂ receptors, respectively.

a.3.2. Serotonin

Serotonin is an indolamine neurotransmitter synthesized from the amino-acid tryptophan via tryptophan hydroxylase and amino-acid decarboxylase action. In the brain, it is synthesized in the RN of the brainstem.

Serotonin has been suggested to be involved in DM through modulation of neuronal signalling during cost-benefit assessment and penalty aversion primarily, but it has also been linked to impulsive choice behaviour (Asher et al., 2013; Khani and Rainer, 2016). Its contribution to DM is likely to be complex and be directly connected to DA activity. Serotonin participates in outcome-value processing, and reduced 5-HT brain levels likely impair DM through exacerbation of the hedonic impact of negative outcomes. In fact, after depletion of the 5-HT precursor, rodents show poor performances in decisional tasks in face of uncertainty (Koot et al., 2012). In general, scientific evidence suggests that 5-HT influences learning from negative output, as well as might alter choice strategies through modulation of midbrain DA neurons activity involved in reward processing (Rogers, 2011).

The decisional process is remarkably complex. It comprises a sequence of interconnected and interdependent operations that allow individuals, before making a discrete choice, to recognize the available options and to evaluate them. After experiencing the consequences of their choice, individuals can learn from the outcomes and adjust their behaviour to maximize their benefits. The neural networks and neuromodulators underlying these operations are also remarkably complex. Frontostriatal networks including the PFC and the dorsal striatum act in synchrony during associative learning, and their activity is modulated by several other cortical and subcortical brain regions. The learnt information is transmitted through various neurotransmitters and the activity of the implicated brain regions is modulated by different neuromodulators. In combination with the adaptive relevance of DM as a biological function, all this structural and functional complexity has contributed to make the study of decisional processes a constant challenge. Accordingly, several behavioural approaches and tasks have been developed in order to assess specific DM modalities (under risk or uncertainty, effort-based, with devaluation of outcomes...), both for human and preclinical models. Through these

behavioural approaches, the presented doctoral project aims at contributing to the understanding of DM, both in physiological and pathological conditions, at the behavioural and also at the neural level.

a.4. Human assessment of decision-making under uncertainty

Examining human DM capabilities has become relevant in the fields of psychiatry and neuropsychology. Deficits in judgement and choice-related behaviours have been reported in patients of a wide range of conditions, ranging from brain injuries to neuropsychiatric disorders. For that, several tasks addressing individuals' decisional abilities under specific conditions have been developed, with special interest in featuring DM in dynamic ambiguous environments. In *The Wisconsin Card Sorting Test* and its computerized versions (Brand et al., 2007; Grant and Berg, 1948), for instance, participants are required to sort a deck of cards of different colours, shapes and numbers according to a predetermined rule. Participants, who receive feedback regarding their accuracy after sorting each card are usually required to repeat the process for each rule at least twice. This way, different scores (e.g., number of items correctly sorted) can be calculated in order to measure distinct DM-related cognitive components such as flexibility and abstract thinking. Gambling tasks are also very useful to address DM in dynamic contexts. The *Game of Dice Task* (Brand et al., 2006, 2005) is, for example, an explicit gambling task to assess the participants' attraction or aversion to risky decisions. In this task, participants are asked to predict the outcome of dice throws, and to face choices between several alternatives that are associated to explicit monetary outcomes and winning probabilities. In order to maximize their gains and since outcome contingencies are explicitly provided, participants calculate the risk associated with each possible option and define profitable strategies.

Another commonly used gambling task addressing risk is the *Cambridge Gamble Task* (Rogers et al., 1999), a computerized task where choice options are also associated to explicit probabilities of monetary gains and losses. Participants are presented with a row of blue and red boxes across the top of a screen and they have to decide the colour of the box they think it hides a yellow token. After each decision, participants are required to place a bet according to the confidence in their decision. In this particular case and contrary to the *Game of Dice Task*, participants do not deal with long-term beneficial strategies, but outcome measures allow evaluating risk-taking proclivity, impulsivity and DM adaptiveness. The *Ballon Analogue Risk Task* has also been developed to address risky DM (Lejuez et al., 2002). It is a computerized task that simulates real-world situations involving risky behaviours that, up to a certain extent, can yield monetary rewards. Participants are presented with multiple balloons of different colours and have to decide whether or not inflating them will result in a reward. As they are not informed regarding the amount of virtual money accumulated until they finish, they need to decide whether continue to inflate balloons, with the risk of causing the balloons to burst and to lose everything, or to stop inflating.

Decisional performance can also be evaluated using *Multi-Armed Bandit Tasks* (Daw et al., 2006). Participants are presented with pictures of different-coloured slot machines and, trial by trial, they have to select one in order to obtain money-points. Each option or *bandit* is associated with a different amount of reward that gradually and independently changes from trial to trial. Thus, these tasks evaluate participants' DM in terms of their pursuit of known resources and in relation to their exploratory tendency. Among DM tasks, another behavioural paradigm involving uncertainty and the possibility of risk-taking is the *Iowa Gambling Task* (IGT), which nowadays is one of the neuropsychological tests used most frequently.

The Iowa Gambling Task

This task was developed by Damasio and Bechara (Bechara et al., 1994) with the objective to better understand the behavioural dysfunction they had observed in patients with injuries in their vmPFC. In fact, they observed that such patients experienced difficulties in emotional DM and risk evaluation, but performed normally in tests such as the Wisconsin Card Sort Test. To study these behavioural variations, they designed a task where participants make a series of choices from 4 card decks that lead to consecutive money wins or losses. In the original version of the IGT, 2 of the 4 decks of cards offered to the participants are disadvantageous or risky and generate large immediate rewards but more frequently larger losses. The other 2 decks are advantageous or safe, and in the long-run they result in larger accumulation of profit. In this probabilistic DM paradigm, patients with vmPFC damage could not decipher the choice contingencies and ultimately were unable to develop an advantageous strategy, hence they were deficient in reward responsiveness. In practical terms, such patients persisted in choosing from the risky decks throughout the task based on their immediate attractiveness. These results conceived the basis of the *Somatic marker hypothesis* and gave the first impulse for turning the IGT into a highly influential DM test within psychiatry. At present, computerized versions of the task are predominantly used, with virtual money gain and losses as reinforcers. Interestingly, no differences between the original and new versions of the IGT have been evidenced in terms of overall performance (Buelow and Suhr, 2009). Both, the original and the virtual IGT adaptations have been useful in demonstrating decisional impairments and risky behaviours in many neuropsychiatric conditions such as psychopathy (van Honk et al., 2002), substance abuse disorder (Bechara and Damasio, 2002) and depression (Must et al., 2013).

In the IGT, participants are told to repeatedly select from the decks in order to optimize their benefit, but they only succeed when they learn to refrain from choosing high immediate rewards and preferentially choose the safe options. In fact, IGT performance in clinical groups (e.g., patients of substance abuse disorders) is usually referred to a control group whose choice-pattern is characterized by selecting advantageous long-term over immediate outcomes (Figure 5). According to the *Somatic marker hypothesis*, healthy participants rely on emotional cues to anticipate bodily states associated

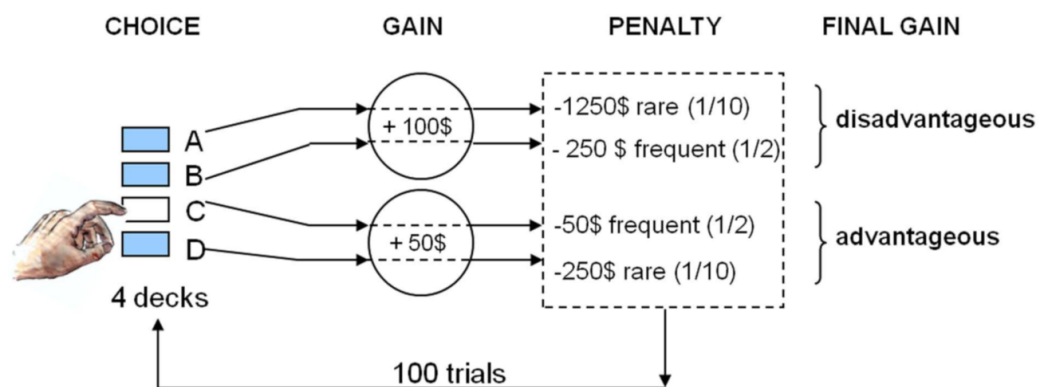


Figure 5. Schematic representation of the IGT set-up. Participants choose 100 consecutive times between four decks of cards yielding monetary gains and losses. Two of the decks are disadvantageous on the long-run, yet offer bigger immediate rewards. The other two decks are advantageous on the long-run, but offer smaller immediate rewards. For a successful IGT performance, participants need to learn refraining from choosing the bigger immediate rewards in order to favour gains on the long-run. Image from de Visser et al., 2011.

to the potential IGT outcomes, and use these expectations to redirect their choices towards the options associated to positive affective feedback. Yet, scientific evidence suggests that IGT

performance is highly sensitive to other factors such as instructions availability. Since its introduction, the original protocol from Bechara and Damasio has been repeatedly adapted through incorporation of more or less slight variations including differential instructiveness among them. Indeed, participants' IGT performance improves when more details regarding the task's goal or the existence of advantageous and disadvantageous decks are provided. Therefore, comparisons between IGT studies in clinical populations require caution and should explicitly refer to the amount and quality of the information given during the presentation of the task, so as to allow further interpretation (Balodis et al., 2006).

Scientific production has also been prolific in what concerns the neural basis of IGT-related DM. The dual-processing theory of self-regulation or dual-processing theory of cognition (Djulgovic et al., 2012; Everitt and Robbins, 2005), asserts that adaptive DM in face of short-term and long-term outcomes hinges on the optimal activation of two main neural networks. On the one hand, an amygdala-striatum dependent network would promote habitual behaviours, as it is essential for the subjective valuation of outcomes. On the other hand, a prefrontal dependent network would predict and evaluate potential future consequences of precise course of actions, allowing for inhibitory control of habitual responses. The latter in turn relies on two neural circuits implicated in executive functioning: a "cool" circuit involving frontostriatal and frontoparietal networks, and a "hot circuit" including the vmPFC and the OFC. The determination of risks and benefits associated to the IGT options, as well as their recall from memory, seem to depend on the "cool" circuit. The regulation of emotional responses and the inhibition of impulsive reactions during the task, however, rely on the "hot" circuit. Thus, an adequate coordinated functioning between both networks is necessary for reward responsiveness through the IGT, which is supported by clinical evidence in patients of for example, pathological gambling disorder. The behaviour of these patients is driven by powerful impulsive motivational-habits directed to task-cues, biasing their DM toward the most immediate and attractive short-term rewards (Brevers et al., 2013). Moreover, IGT disadvantageous performance in pathological gamblers is associated to ventral striatal activity.

The ecological validity of the IGT, i.e., its approximation to real-life DM, is generally accepted. Indeed, as in the everyday-life, performing optimally in this task requires dealing with uncertainty in a dynamic environment that presents choices associated with rewards or penalties in a temporally differential manner. Nevertheless, the adequacy of the IGT as a clinical diagnostic tool is a current topic of scientific discussion. The outcome measures, for example, usually report to the overall proportion of advantageous choices (selection of safe decks), or to the difference score between the overall proportion of advantageous versus disadvantageous choices (selection of safe vs risky decks), even if they do not reflect the dynamics in the selection pattern (or preference) of decks across the task. To overcome this limitation, performance is also commonly split into blocks of a limited number of choices (10 or 20 preferentially). In doing so the power of the associated statistical analyses is influenced and the results' interpretation is biased given the single-session nature of the IGT (Steingroever et al., 2013). The ecological value of the IGT has also been questioned in view of the important interindividual variability evidenced in healthy populations. Indeed, besides a majority of individuals performing advantageously, a non-negligible number of healthy individuals perform poorly in the IGT, but they do not show DM deficits in real-life (Dunn et al., 2006). Another point of criticism concerns genders behavioural dimorphism. In the IGT, men and women perform differently, with the former usually making more advantageous options than the latter. These differences have been associated to DM-related brain lateralisation: men have been described as more right-lateralised than women and at the same time, advantageous IGT performance involves preferentially the right brain hemisphere. Thus, if true, it is an arguable statement that advantageous IGT performance mainly reflects cognitive control,

which is actually substantially right-lateralized (Singh, 2016). In any case, the IGT has significantly contributed, and still does, to the understanding of the neurobiology underpinning the substrates and mechanisms of DM under uncertainty.

a.5. Preclinical models

Animal models are crucial to understand in detail the neurobiology of DM processes as an essential part of human and non-human adaptive behaviour. Mammals, and more specifically rodents offer some advantages in behavioural preclinical approaches: (i) they allow the dissection of specific neural mechanisms to decipher the brain substrates and networks involved in DM; (ii) in respect to humans, the use of rodents efficiently reduces time constraints in longitudinal approaches; (iii) rodents allow the study of interindividual variability; and (iv) their environmental conditions and (v) their genetic background can be controlled. Since the IGT captures essential features of real-life DM, such as the presentation of options associated to short- and long-term outcomes of different value, developing animal models of this task was a judicious and well-grounded step in the investigation of this cognitive function. Thus, for ethical and methodological reasons, rodent IGT adaptations have been privileged.

After the first rodent adaptation of the IGT (van den Bos et al., 2006), multiple other versions of the task that had significantly contributed to understand the basis of DM in several fields have been designed. For example, rodent IGT versions have been used to approach decisional gender dimorphism (Orsini and Setlow, 2017; van den Bos et al., 2013), physiological aspects of DM (de Visser et al., 2011; Koot et al., 2013), decisional interindividual variability (Pittaras et al., 2016; Rivalan et al., 2013, 2009), environmental modulators (Zeeb and Winstanley, 2013), and of course, neurobiological correlates (Daniel et al., 2017; de Visser et al., 2011; Fitoussi et al., 2015; Pushparaj et al., 2015; Rivalan et al., 2013, 2011; van Enkhuizen et al., 2014; Zeeb et al., 2009). Until today, all IGT rodent adaptations can be grouped into different task-types based on the following key specificities: equipment, task schedule, nature of the rewards and penalties, and outcome contingency.

a.5.1. Equipment: mazes vs operant chambers

The first rodent IGT protocol was a maze-based task, which was afterwards followed by other protocols (Pittaras et al., 2016; van den Bos et al., 2006; Visser et al., 2011). In this type of tasks, animals find themselves inside a device divided in two or three zones: a start-point zone, a choice zone and the arms zone. Sometimes the start-point and the choice zone are identical and primarily serve to avoid early orientation and spatial bias. The arms of the maze can be parallel or radial, transparent or opaque. In scientific literature, the maze's arms are usually labelled as A, B, C and D, and can be provided with visual cues to help animals in their differentiation. Like for human IGT decks, two arms are advantageous in the long-run, and two are disadvantageous. Thus, rodents face the choice of visiting one out of four arms, with food rewards or penalties located at the extremes of each arm and usually hidden from sight. When a choice is made, i.e., the animal introduces a considerable part of its body in one of the arms, a door usually closes the arm to prevent the animal from changing its choice without investigating the extreme where the reward or penalty is located.

More recent IGT rodent adaptations use operant automatized chambers (Rivalan et al., 2009). Animals are trained to associate a specific action (a nose-poke or a lever-press) with the delivery of a food reward. After this first phase of instrumental acquisition, animals can be tested in the rodent IGT

version. For that, animals usually have to choose to nose-poke (or lever-press) between four holes (or lever-pressers) located at one side of the chamber, in order to provoke the delivery of the reward in a food-tray located at the opposite side of the chamber. The course of actions and reward-penalty deliveries are commonly accompanied with the illumination of the components (holes and tray) to help guiding the animals, especially indicating the time when new choices are possible (all holes illuminated or lever pressers accessible). Throughout the task, animals learn the contingencies associated to each option by trial and error. Behavioural approaches in operant chambers offer an important advantage in comparison with maze-versions, since they allow constant free exploration of the space with simultaneous recording of multiple secondary parameters that can prove useful in ulterior interpretation.

a.5.2. Task schedule: single vs multi-sessions tasks

Rodent IGT adaptations are especially variable with respect to the task schedule (Visser et al., 2011). Maze and operant IGT protocols have been modified in order to suit performance in single and multi-session tasks of different duration. Operant single-session tasks usually last a maximum time period (e.g., an hour) or until a total number of choices has been made (Daniel et al., 2017; van Enkhuizen et al., 2014). Maze-adapted protocols usually rely on a specific number of choices that animals have to complete, and even if single-session protocols exist (Pais-Vieira et al., 2007), they more commonly last 5-10 daily sessions of a variable number of trials (Pittaras et al., 2016; van den Bos et al., 2006). The most important reason to favor multi-session protocols in rodent IGT versions is to maintain animals' motivation during the entire task, since the nature of the reward (food pellets or palatable liquid solutions) entails satiation effects that could influence DM performance.

a.5.3. Outcome nature

To adapt the monetary gains of the IGT, rodent versions of the task use palatable rewards in solid or liquid forms. Most commonly, small grain-based pellets or sugar/chocolate pellets are used, but milkshake solutions of different flavours are also frequent. In the case of solid reinforcers, the value of the immediate reward is perceived based on the amount of delivered pieces, whereas the value of liquid solutions usually depends on its delivery frequency. Interestingly, liquid solutions in comparison with solid rewards have a lesser satiety effect, allowing protocols with higher number of trials (Kim et al., 2017). In the case of penalties, monetary losses have been adapted to non-palatable food rewards, such as quinine pellets (Pittaras et al., 2016; van den Bos et al., 2006), to periods of inactivity when no reward is delivered (time-outs) (Rivalan et al., 2009; Zeeb et al., 2009), or simply to the absence of reward (Pais-Vieira et al., 2007). Thus, the value of these penalties is associated to the amount of pieces delivered, or the time spent without a reward delivery.

The nature of rewards, and to a lesser extent that of penalties, is considered a general limitation of rodents IGT adaptations. As primary reinforcer, food outcome is subjected to its incentive value (Cardinal et al., 2002). However, no better adapted reinforcers have been identified until now to model the monetary outcomes of the IGT. Based on that, behavioural approaches utilizing food reinforcers usually integrate alimentary deprivation protocols in order to maintain the animals' motivation to complete the tasks. Furthermore, modelling monetary losses in rodent protocols is not an easy objective, and neither time-outs nor quinine pellets completely convince the scientific community about their adequacy to reproduce monetary deficits.

a.5.4. Outcome contingency

In early phases of the rodent tasks, animals learn about outcome contingencies by exploring the different options (*exploration*). Optimal performers will associate smaller immediate rewards to larger long-term benefits, and therefore refrain selecting from the options offering larger immediate rewards (*exploitation*). Similar to the rest of specificities, task contingencies also vary across rodent IGT versions, with probability schedules for gain

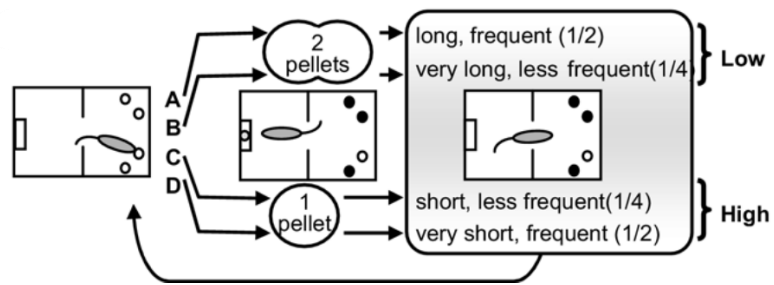


Figure 6. Experimental design of a rodent operant single-session version of the IGT. Animals learn to nose-poke among four different holes (A-D) to obtain food reward during one hour. The selection of options yields delivery of 1 or 2 food pellets, or is followed by a time-out of variable duration according to two different probabilities (1/2 or 1/4 times). Holes A and B are disadvantageous, while holes C and D are advantageous on the long-run. Image from Rivalan et al., 2009.

and losses adapted to the amount of outcome delivered. Hence, the long-term compensation conflict that animals face will induce more or less drastic behavioural shifts as a function of the different frequencies of gain and losses attributed to each option, and in combination with the amount of outcomes. However, the human IGT differentiates the safe from the risky options only through the amount of the outcome, while the frequency of penalty delivery is the same between both advantageous or disadvantageous decks. As an exception, the rodent version developed by Rivalan and colleagues is more reliable to the original task contingencies, since it combines solid food rewards and time-outs in an operant single-session protocol, where only one or two food pieces are delivered at a time (Rivalan et al., 2009) (see **Figure 6**).

Despite the current limitations of the rodent IGT versions, continuous methodological protocol refinement has permitted to produce significant scientific work in DM investigation. In general terms, these adaptations have translational value since similar behavioural patterns between humans and rodents are evidenced. Animals as much as humans are able to learn the contingencies of the tasks and to develop a long-term advantageous decisional strategy. Moreover, rodent IGT adaptations have evidenced gender behavioural dimorphism, with females performing poorer than males, as it is the case for the human IGT (de Visser et al., 2010; van den Bos et al., 2006). Further evidence supporting the translational value of these rodent versions appeal to their utility in assessing interindividual variability, a useful tool to investigate individual vulnerability for example, to pathological gambling. Most importantly, however, rodent IGT adaptations have allowed to deepen investigations of the neural basis of DM. Animal studies ranging from lesion and pharmacological inactivation approaches to imaging or optogenetic strategies are used in order to dissect the key brain substrates and networks underlying IGT performance. The involvement of neural networks and mechanisms on the rodent IGT has been investigated, questioned and confirmed by multiple studies in the last decades. Rodents lesioned in their OFC or BLA, for example, show retarded learning and delayed onset of reward responsiveness (Zeeb and Winstanley, 2013, 2011). Lesions in the rodent mPFC are also able to impoverish animals' decisions (Pushparaj et al., 2015; Rivalan et al., 2011), and the implication of the IC in modulating IGT performance has also been confirmed (Daniel et al., 2017). Additionally, the rodent ventral striatum seems to be differentially recruited in good and poor performers of the task, and specifically higher levels of NAcC activation have been related to higher reward responsiveness

(de Visser et al., 2011). Collectively, neural correlates obtained through rodent models are overall consistent with human data, comprising the mPFC, amygdala and striatum as major modulators of DM in the IGT-based adapted tasks.

During the last decades, considerable advances in the understanding of the neural networks and mechanisms underlying DM have been achieved, yet much remains to be explored. More particularly, decisional capabilities have been demonstrated to be compromised in the presence of stressors, especially after long-lasting exposure.

B. Chronic distress

In a general sense, the term *stress* refers to an event or a series of events that are perceived as a threat for the integrity of an individual. These events, ranging from disease to predation, to unpredicted context changes (uncertain, risky and ambiguous environments), elicit behavioural, structural and physiological adjustments in the organisms necessary to maintain internal physiological stability. Stress prompts allostasis to preserve biological homeostasis (Houweling et al., 2005; McEwen, 2017). Physiological adjustment and continuous adaptation in face of stressors is of utmost importance for ensuring survival. Living beings are constantly challenged by environmental changes of a different nature, yet not all sorts of stressors are necessarily prejudicial. Commonly referred as *eustress*, some events lead initially to challenging experiences, which through adaptive and resilience evolvment ultimately become rewarding. *Distress*, in a contrary manner, refers to events linked to uncomfortable feelings that predominantly lead to adverse outcomes. When individuals lack of the necessary physiological, behavioural, psychological and/or cognitive support to control distress, events become prejudicial by overloading their allostatic pressure. This situation is indeed typical of stressors operating with long-lasting effects, commonly named chronic stressors.

Chronic distress responses contribute to the cumulative allostatic load and can involve biological pathways different from those activated during acute stress. When allostatic load increases, it leads to physiological consequences resulting from heightened neural and neuroendocrine responses. Chronic distress can operate with *sensitization*, a phenomenon resulting from overexposure to a particular stimulus that triggers a state of hyperresponsiveness to the identical (homotypic) or other stimuli (heterotypic), which can last in a long-term modification (Belda et al., 2015). Thus, an intimate interaction between the physiological and behavioural adjustments underlying the stress responses is crucial when facing persistent environmental threats, and when disrupted, can propitiate several neuropsychiatric conditions.

The biological stress response system, in the presence of stressors, operates through its two main effectors: (i) the autonomic nervous system (ANS), which provides an immediate response to a stressful event (*alarm phase*) via its sympathetic and parasympathetic branches resulting in fast adjustments of the organism's physiological state; and (ii) the hypothalamic-pituitary-adrenal (HPA) axis, whose activation promotes the increase of circulating glucocorticoids (GC), which operate as direct or indirect activity modulators of specific brain regions involved in stress-dependent behaviour (*maintenance phase*). When the adjustments of these two effectors succeed in reducing the allostatic load, organisms encounter homeostasis or homeostatic relief (*resilience*). However, when these effectors are beyond the limits of homeostatic mechanisms and become unbalanced, organisms are able to sustain the current state only for a limited time without suffering from negative effects. Thus, a prolonged imbalance of the biological stress responses results in allostatic overload (*exhaustion*) (McEwen, 2017; Selye, 1950).

b.1. The stress response system: the Autonomic Nervous System

In the presence of stressors, the sympatho-neuronal and sympatho-adrenomedullary branches of the ANS promote different physiological adjustments and the immediate release of catecholamines, which contribute to the organism general stress response. Particularly, the activation of the sympatho-adrenomedullary branch of the ANS results in increased circulating levels of adrenaline (epinephrine - EPI) from the adrenal medulla (Joëls et al., 2007), and of noradrenaline (norepinephrine -NA)

predominantly from sympathetic nerve terminals. Stressful events usually enhance activity in the LC (the brain structure with the largest concentration of noradrenergic neurons) inducing NA release (Smith and Vale, 2006). Increased levels of NA then elicit activation of specific adrenoceptors in other brain regions such as the amygdala, PFC and hippocampus. Noradrenaline has been described to inhibit or enhance excitatory transmission in these brain regions depending on the type of binding interaction. In parallel, the sympatho-adrenomedullary branch of the ANS, via neuronal innervation, can provoke fast adjustments in the heart rate or the blood pressure, as well as to promote hepatic glycogenolysis (Ulrich-Lai and Herman, 2009).

b.2. The stress response system: the Hypothalamic-Pituitary-Adrenal axis

The HPA axis response to stress starts with the activation of parvocellular neurons located in the PVN. These neurons synthesize and secrete CRF, a neuropeptide considered the major activator of the HPA axis. In response to stress, CRF is released into the portal circulation in the median eminence, together with vasopressin (AVP) and other secretagogues, to reach the anterior pituitary gland. There, CRF acts on corticotrope cells by binding to the CRF type 1 receptor (CRFR1), and stimulates the synthesis and secretion of adreno-corticotrophic hormone (ACTH), which is potentiated at the same time by the presence of AVP. After release in the systemic circulation, ACTH targets the zona fasciculata of the adrenal cortex, where it binds to the melanocortin type 2 receptor and in turn promotes the synthesis and secretion of GC, mainly cortisol in humans and corticosterone (CORT) in rodents (Belda et al., 2015; McEwen, 2017; Smith and Vale, 2006). Glucocorticoids regulate the physiological changes necessary to cope with the stressor by acting on specific intracellular receptors that are ubiquitously distributed. They are therefore considered the downstream effectors of the HPA axis: they contribute to the mobilisation of resources and the preparation of the organism to further stress and they are key players in the consolidation of stress-related memories. In parallel, GC also regulate HPA axis activation avoiding excessive responses that may contribute to the emergence of pathological states. For that, GC operate on negative feedback mechanisms involving glucocorticoid- and mineralocorticoid receptors (GR and MR, respectively) localized in specific brain regions (Herman et al., 2003; Smith and Vale, 2006; Ulrich-Lai and Herman, 2009) (see **Figure 7**).

The HPA axis-mediated response to stress is tightly controlled by neuronal and endocrine mechanisms. In the first case, the firing activity of parvocellular neurons from the PVN is modulated by afferent projections from different brain regions (mainly from the lamina terminalis, extra-PVN hypothalamic nuclei, forebrain limbic regions and brainstem neurons). By regulating the activity of these hypophysiotropic neurons, the activation of the HPA axis is ultimately inhibited since the synthesis and release of CRF is limited (see for instance Smith and Vale, 2006). The endocrine regulation of the HPA axis is mostly driven by GC, but circulating CRF-binding proteins also contribute to the duration and magnitude of the HPA axis' response (Deussing and Chen, 2018). Glucocorticoids are involved in two main mechanisms of negative feedback: the first is a delayed feedback system responsive to GC circulating levels leading to genomic alterations, the second is a fast non-genomic feedback system responsive to the rate of GC secretion. In the genomic feedback system, GC bind to GRs localized in several stress-responsive brain regions that act via transcriptional alteration of HPA axis components by binding glucocorticoid response elements (GRE) or by interacting with transcriptional factors (Dallman et al., 2004; Herman, 2018; Herman et al., 2003; Ulrich-Lai and Herman, 2009).

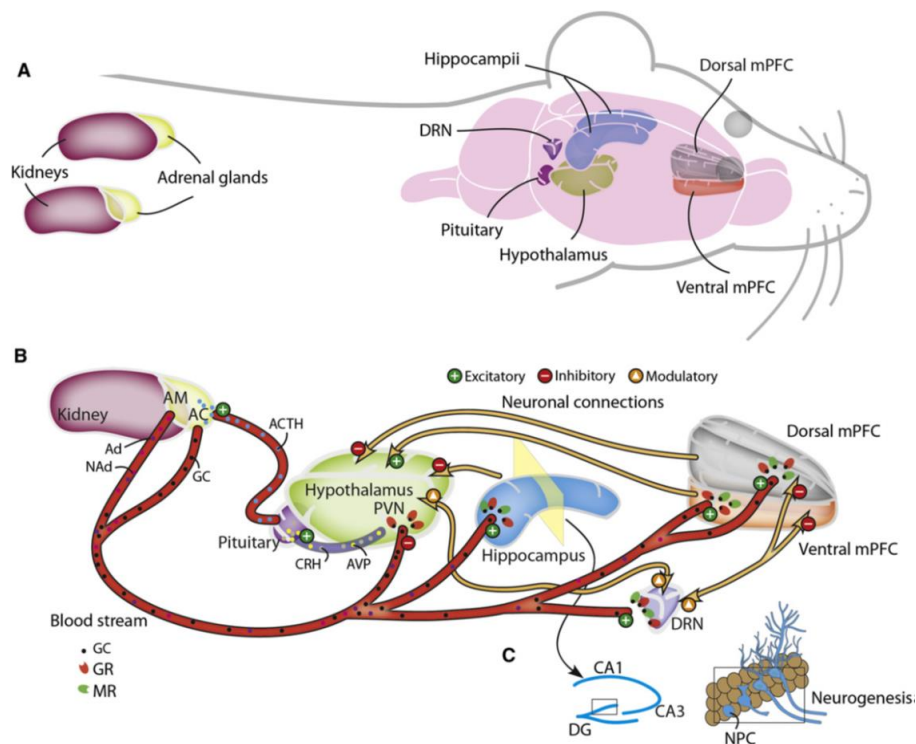


Figure 7. Anatomical and functional connectivity of the HPA axis and stress-sensitive brain substrates. (A) Major components of the HPA axis and connected stress-sensitive brain regions (dorsal raphe nucleus, DRN). **(B)** The HPA axis activation is initiated by stimulation of parvocellular neurons of the PVN and subsequent secretion of CRF and AVP in the portal circulation. Importantly, AVP amplifies the CRF effects. The pituitary gland secretes ACTH, which in turn stimulates the release of GC from the adrenal cortex (AC), and EPI and NA from the adrenal medulla (AM) into blood circulation. When the stressful event reaches its end, the HPA axis returns to its baseline state by the action of regulatory negative feedback systems. Glucocorticoids are directly involved in the suppression of the hypothalamic and pituitary response to stress, and therefore contribute to stopping the release of CRF and ACTH. Indirectly, they regulate the stress-response by activating GRs in the hippocampus and cortical areas that project back to the hypothalamus. Mineralocorticoid receptors are also activated by GC and participate with GRs in a coordinated manner in the stress response. + and - signs indicate positive and negative regulation, while triangles indicate excitatory/inhibitory modulation. Image from Franklin et al. 2012.

b.2.1. Corticotropin-releasing factor

Besides coordinating the neuroendocrine response to stress, the CRF is the principal hypothalamic activator of the HPA axis. This 41-amino acid peptide is widely expressed in the brain and in several peripheral tissues. Within the central nervous system (CNS), CRF is strongly expressed in the PVN, but among others also in the olfactory bulb, NAcS, cortical structures, piriform cortex, CeA, BNST and lateral hypothalamus (Deussing and Chen, 2018). Regarding the periphery, CRF is expressed in the adrenal glands, testis, skin, thymus, placenta and gastrointestinal tract (Smith and Vale, 2006). The CRF is also the most-expressed member of the CRF peptide family, which includes Urocortin 1 (UCN1), UCN2 and UCN3. Urocortins expression is more restricted than CRF expression since urocortins-expressing neurons are more discretely distributed than CRF-expressing neurons, but all signal through the activation of two G-protein-coupled receptors (GPCR): CRFR1 and CRFR2. These receptors are abundant in the mammalian brain, yet their expression pattern does not entirely overlap between humans and rodents. They are strongly expressed in the pituitary and also present in the amygdala, thalamus, hippocampus, cerebellum and cortex of humans. Importantly, CRF has a much higher affinity for CRFR1 than for CRFR2. Indeed, principal CRF neuroendocrine properties are

mediated through binding to anterior pituitary CRFR1, which activates cyclic adenosine monophosphate (cAMP) pathway events through adenylate cyclase stimulation, necessary for ACTH release. The initiation of the acute stress-response is then assumed to be mostly mediated via CRF-CRFR1 signalling. The role of CRFR2 is more ambiguous, with UCN-CRFR2 activity controlling later

adaptive phases of the physiological response (Dedic et al., 2018) (Figure 8). Under sustained distress, however, extra-hypothalamic CRF-CRFR1 activity is disrupted and increases in arousal, altered locomotor activity and diminished food consumption have been linked, among others, to a hyperactivity of the system (Dedic et al., 2018).

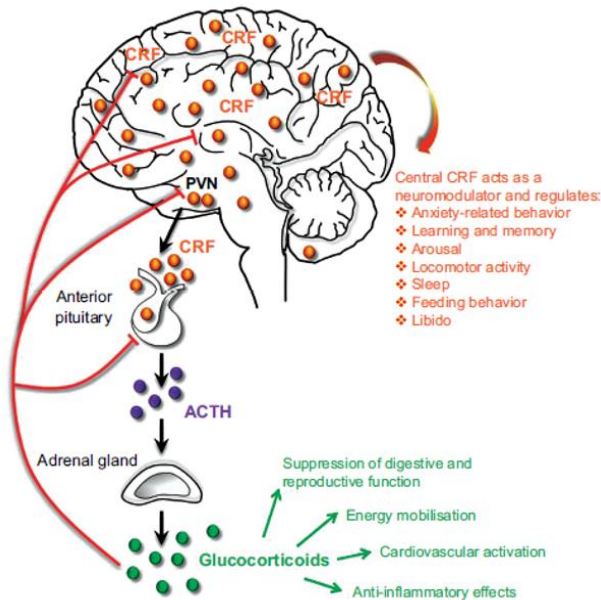


Figure 8. Schematic representation of the CRF role in the regulation of the neuroendocrine and behavioural responses to stress. Corticotropin-releasing factor regulates peripheral HPA axis function and modulates synaptic transmission in the CNS. Through these regulatory mechanisms, CRF integrates neuroendocrine and executive behavioural responses. Image from Dedic et al., 2018.

b.2.2. Glucocorticoid receptor

Increased release GC tone occurs in face of every stressful event, originated either internally or externally, and leads to variation of GR expression. Glucocorticoid receptors are ubiquitously distributed in the CNS, and within the brain they are expressed in different cellular bodies (neurons and glia) and regions, including the PVN and the PFC-hippocampus-amygdala network (Joëls et al., 2007). Importantly, the affinity of GRs to GC is low compared to MRs: in the case of CORT, for example, GR affinity is 10-fold lower (Reul and Kloet, 1985). Independent of ligand sensitivity, GRs signal through two main pathways named genomic and non-genomic (Oakley and Cidlowski, 2013). The principal effects of GC occur in temporal limits on the scale of minutes or hours, and are mediated by GR-related genomic mechanisms. In this genomic pathway, where GRs are found mostly in the cellular cytoplasm and are associated to binding proteins, they undergo conformational changes following GC binding. As a result, binding proteins dissociate and GRs are rapidly translocated into the cellular nucleus, where they bind to GREs and regulate the expression of targeted genes. Alternatively, GRs can also regulate gene expression by directly interacting with other transcription factors, e.g., GRs modulate inflammatory responses by directly interacting with proinflammatory factors. In the non-genomic pathway, however, cellular responses are faster, on the order of seconds or minutes. These non-genomic mechanisms seem to rely on the activity of different kinases, such as mitogen-activated protein kinases (MAPKs). Thereupon, activation of GRs is considered a crucial step in physiological and subsequent behavioural responses to stress.

b.2.3. Mineralocorticoid receptor

Contrary to GRs, MRs are involved in the HPA axis basal activity and in the onset of the stress response, but do not substantially contribute to its termination. The distribution of MRs in the brain is more restricted than for GRs, and they are especially abundant in the limbic regions, notably the hippocampus and amygdala (de Kloet et al., 2018). Hippocampal MRs exert an important control on HPA axis activity via a GC-mediated feedback mechanism (Berardelli et al., 2013). These receptors have a very high affinity for the endogenous GC, which explains that in absence of stressful events, i.e., at low presence of circulating GC, they are already considerably occupied (Joëls et al., 2007). Thus, MR activity is very sensitive to the circadian release rhythm of endogenous GC. As for GRs, the genomic signalling pathway of MRs starts with ligand binding inducing conformational changes in the cytosolic MRs structure, which ultimately results in their translocation to the cellular nucleus and the subsequent modulation of gene transcription. Mineralocorticoid-receptors have also been described to participate in a non-genomic signalling pathway (Berardelli et al., 2013).

The effects of GC following a stressful event or series of events partially depend on their functional interactions, i.e., their capacity to form homo- and heterodimers. This is because they differently modulate gene transcription, which is mostly determined through the asymmetric distribution of MRs and GRs in the brain. Additionally, MR activity is usually related to excitatory neurotransmission (via GLU release potentiation in presynaptic neurons or via excitability enhancement in postsynaptic neurons), while GR activity is primarily associated to neural inhibition via GABAergic or endocannabinoid pathways (Tasker, 2006). Hence, the degree of occupation of these receptors is particularly relevant when they co-localize. Subsequently, their cooperation has been proposed to be primordial to maintain brain health integrity in face of stressors, resulting in the formulation of the *MR:GR balance hypothesis* (de Kloet et al., 2018; de Kloet et al., 1998) (Figure 9). This hypothesis states that imbalances of MR- and GR-mediated actions compromise normal stress responses, which could lead to neuroendocrine dysregulation and consequently to maladaptive behaviour with detrimental allostatic stress-related effects. Thus, brain areas activated following the presentation of stressors should be especially sensitive to receptor imbalances since prolonged exposure to GC could ultimately lead to HPA axis hyperactivity and, through that to inadequate HPA response.

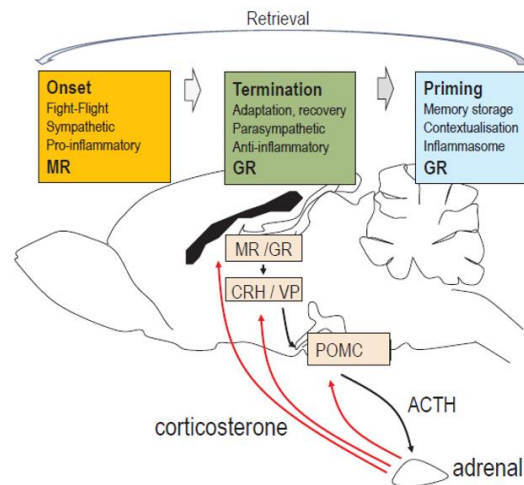


Figure 9. Schematic representation of the complementary roles of GRs and MRs during the stress response. Stressful events activate CRF and AVP release from the median eminence terminals of the parvocellular neurons of the PVN, which in turn stimulate the synthesis of pro-opiomelanocortin (POMC) and its product ACTH. The latter promotes adrenocortical secretion of GC that naturally bind to GRs and MRs. The affinity of GC for MRs is significantly higher than for GRs, and therefore the onset of the stress response is predominantly mediated by MRs. Yet, the response termination and its priming effects are mediated by GRs. Image from de Kloet et al. 2018.

b.3. Stress-sensitive brain regions and networks

The brain regions and networks mainly activated by stressful events are integrated in the limbic circuits and include the PFC, amygdala and hippocampus (Godoy et al., 2018). Importantly, these stress-sensitive networks receive associative information from higher-order sensory areas (e.g., the insula), and from regions participating in memory processing (e.g., the cingulate cortex) or/and in arousal and attentional processing (e.g., LC and RN). In their turn, the neural networks activated during the stress target subcortical sites necessary for limbic processing. These networks of interacting brain regions therefore cooperate in emotion and cognition, and support neuroendocrine responses.

b.3.1. Medial prefrontal cortex

The mPFC exerts strong regulatory action on an organism's stress-response through the complementary roles of its dorsal and ventral areas. The PL cortex regulates HPA axis activity by inhibiting its responses to psychogenic stressors without affecting the magnitude of the GC release. At the same time, the involvement of the PL cortex in the inhibition of the autonomic stress response has been also demonstrated, for example via the effects of NA local injection. It contributes to increases of the heart rate following stressful events (Akana et al., 2001). Contrary to that, the IL cortex is involved in the activation of the HPA axis and autonomic responses in face to psychogenic stressors. Indeed, pharmacological activation of the IL cortex increases basal levels of circulating CORT in rodents, while inhibition reduces CORT release following an adverse event (Ronzoni et al., 2016). Complementary, stimulation but not inhibition of the IL cortex leads to increased blood pressure and heart rate in rodents (Resstel et al., 2006). Besides, given its vast interconnectivity with the hippocampus and the amygdala, the mPFC is considered a key player in the coordination between the limbic and the autonomous reactivity to stress.

b.3.2. Amygdala

The amygdala modulates the autonomic and neuroendocrine stress responses, and, in correspondence to its structural complexity, its principal components respond differently according to the nature and extent of the stressor. The BLA is primarily activated by psychological stressors and leads to HPA axis adjustments. In addition, there is extensive evidence for the importance of NA release in the BLA for memory consolidation after stressful events (Roozendaal et al., 2009). There is also evidence for a stress-induced reduction in inhibitory regulation in the BLA that might facilitate memory consolidation through increased plasticity in its excitatory synapses. Furthermore, CRF release in the BLA after stress exposure seems to modulate memory consolidation through interaction with NA receptors. Finally, it has also been proposed that the BLA is necessary for habituation of the HPA axis to chronic intermittent stress (McCarty, 2016). In its turn, the CeA is mainly activated by allostatic pressure and systemic stressors. It is considered a key node for integration of stress-related information, importantly contributing to the autonomic response to stress, and is crucial in fear responses (Ulrich-Lai and Herman, 2009).

b.3.3. Bed nucleus of the stria terminalis

The role of the BNST is essential to what concerns the integration of stress-related information. Indeed, a majority of limbic-PVN connections project through GABA-neurons in the BNST, providing final inhibitory action. In the particular case of the amygdala, however, upstream projections are mostly GABAergic, which act by inhibiting the activity of BNST projections and, therefore, promoting

PVN disinhibition (Herman et al., 2003). The integrative influence of BNST is also region specific: anteroventral subregions mostly enhance HPA axis activity, whereas the posteromedial subregion preferentially inhibits it. Posteromedial lesions have been related to increased PVN neural activity and GC release, as well as enhanced CRF expression (Choi et al., 2007; Ulrich-Lai and Herman, 2009). Tracing approaches in rodents have also demonstrated that anterolateral BNST projections to the PVN include CRF-expressing neurons, while posteromedial projections mainly comprise GABAergic neurons (Cullinan et al., 1993).

b.3.4. Hippocampus

The hippocampus participates in the HPA axis regulatory negative feedback, and influences HPA axis activity in a region specific manner adjusted to the nature of the stressor (Ulrich-Lai and Herman, 2009). Notably, a population of neurons from the ventral subiculum is responsible of the regulatory inhibition on PVN neural activity. Therefore, hippocampal lesions, especially in the ventral subiculum, result in augmented GC release and increased PVN CRF expression after stress exposure (Herman and Mueller, 2006). Moreover, the majority of hippocampal projections to hypothalamic regions originate in the ventral subiculum. The hippocampus also modulates to a certain extent the autonomic stress response, e.g., it limits the heart rate and blood pressure when stimulated, yet it is apparently more reactive to psychogenic rather than to systemic stressors. Importantly, the hippocampus likely influences the autonomic stress response via its connections with the nucleus tractus solitarii (NTS) in the brainstem, which is considered the principal visceral sensory nucleus in humans and is connected to prefrontal brain regions such as the IL cortex (Godoy et al., 2018; Ulrich-Lai and Herman, 2009).

Under chronic distress, the structure and functioning of these stress-sensitive brain regions are known to change. The morphology of their dendrites is altered, for example, following chronic restraint in rodents. Nevertheless, the sense and magnitude of the change depends on the brain region. In the hippocampus and mPFC, pyramidal dendrites usually retract after chronic restraint in parallel to a reduction of neuronal spine density. In the BLA, however, chronic distress increases dendritic ramification (Radley et al., 2008; Vyas et al., 2002). Importantly, the expression pattern of HPA axis components (CRF, GR), as well as of catecholamines, is also altered after chronic distress. An ineffective action of GC resulting from chronic distress exposure (*GC brain resistance*), could then reflect HPA axis hyperactivity (Pariante and Lightman, 2008). Subsequently, reactivity to novel acute stressors following chronic distress usually differs, leading to either its facilitation or to a blunted stress response. Interestingly, chronic distress seems to undermine hippocampal HPA axis regulation, since lesions do not result in altered HPA axis responses (Herman and Mueller, 2006). Besides, the role of the BNST in the HPA axis response also changes upon chronic distress, by facilitating its reactivity rather than inhibiting it (Choi et al., 2007).

There is scientific evidence that chronic distress is significantly regulated by the CeA, which is highly sensitive to GC. Actually, chronic distress-induced elevated GC levels result in increased CRF expression in the CeA, which enhances PVN reactivity via indirect projections. Other brain regions might also contribute to the chronic distress-induced blunted HPA axis activity, as is likely the case of the mPFC and, to a lesser extent, of the hippocampus. High persistent circulating levels of GC can lead to down-regulation of GC receptors expression, consequently impacting non-genomic GC negative feedback modulation (Mizoguchi et al., 2003). Concerning the ANS response, chronic distress usually promotes long-term physiological adjustments such as increased heart rate, but the neuronal mechanisms underlying these adaptations have not yet been fully described.

b.4. Chronic distress and pathological conditions: anxiety and depression

Disorders subsequent to sustained distress do not only include mental health disorders. Fibromyalgia, irritable bowel syndrome and chronic fatigue syndrome are examples of clinical conditions deriving from chronic exposure to stressors and physiological maladaptation. Distress-related neuropsychiatric conditions may yet attract much popular attention. Obsessive-compulsive disorder and post-traumatic stress disorder have been catalogued as stress-related disorders (American Psychiatric Association, 2013), but the potentially most explored and stated relationship between chronic distress and mental health is illustrated by mood disorders.

The involvement of stress in the development of mood disorders, in particular of anxiety and depression, has been extensively documented (for review see Pêgo et al., 2009). In a significant proportion of cases, depressive episodes are preceded by major stressful events, and chronic exposure to stressors has been related to symptomatology deterioration and treatment resistance (Pizzagalli, 2014). Although anxious responses cannot be related to the discrete presence of specific stressors, their correlation with chronic distress has also been consistently demonstrated (Pêgo et al., 2009).

Anxiety-related disorders represent a heterogeneous group of neuropsychiatric conditions, generally characterized by psychophysical sensations of discomfort and apprehension in response to unconditioned diffuse cues. Depression is a psychiatric condition characterized by a complex range of behavioural, emotional and cognitive symptoms, including decreased interest for pleasure, or *anhedonia*, diminished motivation for physical, cognitive and/or emotional activity, or *apathy*, and executive dysfunction. The aetiology of, both, anxiety and depression often overlaps and relates to altered patterns of the stress-response system, yet they are not identical. In particular, anxiety shows a wider spectrum of HPA axis activity patterns than depression, probably due to the highly heterogeneous nature of the pathology. Anxiety and depression can also be understood as consecutive and connected phenotypes in a physiological to pathological continuum, where the former would precede the latter (Cuthbert, 2014). However, for the present doctoral study the neurobiology underlying depressive phenotypes is of greater interest, hence the following information is focused in scientific literature on depression.

Multiple studies have been carried out focusing on the potential role of a dysregulated stress-response system in the emergence and development of depression, and within that, the role of the HPA axis has attracted much of the attention. In the presence of chronic stressors, organisms adapt their stress response to maintain an increased secretion of GC despite the regulation by negative feedback. For that, CRF and AVP expression are increased in the parvocellular PVN cells and/or in their terminals in the median eminence. Preclinical studies have also reported a down-regulation of GC receptors, GR and MR, as a common consequence of chronic distress, especially in stress-sensitive brain regions such as the hippocampus (Herman et al., 1995). Finally, another adaptation of the HPA axis to chronic distress is the hypertrophy of the adrenals glands (Checkley, 1996). In agreement to that, elevated plasma levels of ACTH and GC are often reported in depressive patients (Gillespie and Nemeroff, 2005). Growing evidence points towards deep changes in the HPA axis functionality as the origin of depressive episodes. Particularly an anomalous GR signalling appears to trigger chronic CRF hypersecretion within the HPA axis, thus resulting in persistent HPA axis activity. Increased levels of CRF, for example, have been reported in the cerebrospinal fluid of many patients suffering from depression (Banki et al., 1987). It is important to note that elevated GC levels have been associated to greater cognitive impairments within depressive-related symptomatology, including memory and executive dysfunction (Gomez et al., 2009; Wolkowitz et al., 2009). Yet, the relationship between long-

lasting circulating GC and depression apparently hinge on diverse factors, such as the severity of the illness and the recurrent nature of the depressive episodes. Remitted patients, for example, generate high GC levels in response to acute stressors, but this exacerbated HPA responsiveness is not observed in patients of long-lasting depression (Nandam et al., 2020). The *Diagnostic and Statistical Manual of Mental Disorders, fifth edition* (DSM-5) defines two main types of depression based on the severity of the illness: the atypical forms of depression, with a less severe symptomatology, and the melancholic forms, with a more hampering symptomatology (American Psychiatric Association, 2013). Importantly, in melancholic patients, which usually experience higher levels of basal GC and enhanced HPA reactivity, more severe and varied cognitive dysfunction is reported. Thus, high GC levels relate to the severity of the depressive form (Dudek et al., 2021; Hinkelmann et al., 2009).

Hopelessness is apparently the final result of the most depressogenic stressors. In combination with *cognitive vulnerability*, chronic distressful events modulate the physiological stress response in order to predict self-reported goal-directed behaviour. Yet, since they are perceived as uncontrollable, they act through reflective inhibition. Thus, chronic distress and hopelessness interact to induce *avolition*, i.e., the inability to perform self-directed purposeful activity, and trigger first episodes of depression (Pizzagalli, 2014). Noteworthy, exposure to major stressful events during early stages in life (childhood, adolescence) is considered a major factor of risk to depression in adulthood (Heim and Binder, 2012). Another important risk factor for developing depression after chronic distress is gender. Women are more vulnerable to the illness and scientific evidence suggests that its origin is in the intragender variability of the GC-mediated response to stressors. However, a simple neuroendocrine causal relationship has not been found (Young and Korszun, 2010). Epidemiologic data indicate that genetics can reach 50% of the total risk for depression (Nestler et al., 2002), and epigenetic mechanisms are likely contributing to the vulnerability of individuals (Hodes et al., 2017).

The neurobiology underlying depression

Many brain networks seem to contribute to the emotional and cognitive dysfunction that characterizes depression. Human evidence relates mostly to brain imaging and post-mortem studies, which have evidenced changes in blood circulation and anatomical alterations in several brain areas, including frontal cortices, hippocampus, striatum, insula, amygdala and thalamic areas (Belzung et al., 2015; Griffiths et al., 2014; Krishnan and Nestler, 2008; Nestler et al., 2002) (**Figure 10**). These anatomo-functional alterations affect simultaneously distinct capacities and contribute to the emergence of different emotional-cognitive symptoms. Therefore, they are usually framed based on core symptoms of the disorder.

Anhedonia, apathy and reward underappreciation. The general loss of interest and pleasure for rewarding items or activities –*anhedonia*– is considered one of the core symptoms of depression. Patients commonly neglect pleasurable stimuli at the same time that they respond poorly to rewarding experiences. Patients of depression lack motivation for pursuing new goals, so their ability to form new stimulus-response associations is diminished. Such loss of general motivation, also referred as *apathy*, likely stands at the origin of their frequent states of *avolition* and *abulia* (i.e., a state of reduced spontaneous behaviour). It stands to reason, that the neurobiology underlying anhedonia, apathy and hedonic underappreciation corresponds to the neurobiology of pleasure integration, reward valuation and motivation. An altered OFC neural activity including a reduced connectivity with the BLA is likely the major orchestrator of the blunted responses to pleasant stimuli in depressed patients. They also present reduced activity patterns in the ventral striatum in response to positive stimuli. Noteworthy, bilateral deep brain stimulation in the NAc of depressed patients has been successful in reducing anhedonia-related symptomatology (Schlaepfer et al., 2008). A reduced VTA activity in face of pleasant

stimuli is reported in depressed patients, so that VTA DA projections to the NAc might be involved in these alterations. The vmPFC and dlPFC, players of the network mediating reward valuation, are also involved in anhedonia- and apathy-related states. The level of hypofunctionality in these areas has been associated to the severity of the symptomatology (Alloy et al., 2016; Der-Avakian and Markou, 2012).

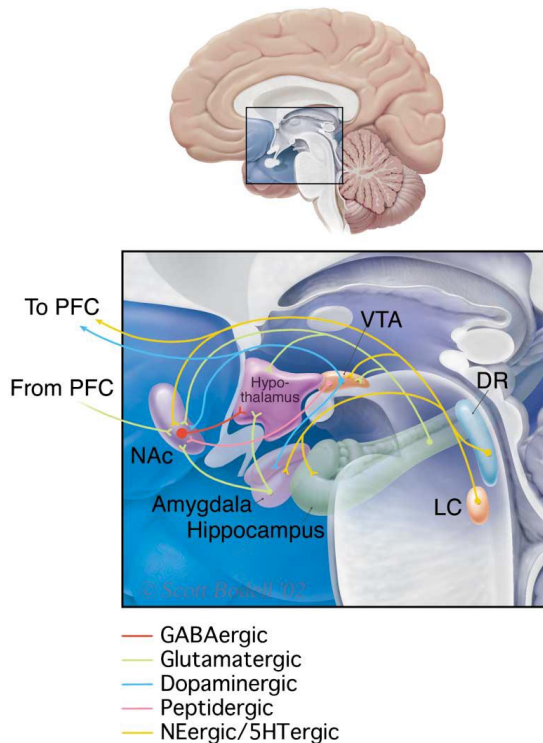


Figure 10. Neural substrates underlying depression. Highly simplified schematic summary of a series of neural circuits in the human brain presumably contributing to mood regulation. The figure shows only a subset of the many known interconnections among various brain regions, as well as the innervation of several of these brain regions by monoaminergic neurons. In interaction with the hippocampus and the PFC, subcortical structures including the NAc, amygdala, VTA and hypothalamus are essential in the regulation of mood. Image from Nestler et al., 2002.

information. Concerning specifically the memory biases, the amygdala-hippocampus connectivity appears reinforced in depressed patients, suggesting its involvement in the enhancement of memories of affective value.

Rumination. Focusing the attention on the distress symptoms is typical from depressed patients and is also considered a core feature of the illness. In relation with the cognitive biases, rumination in depression originates from the inability to switch attention away from negative information. This alteration in executive functioning ultimately leads patients towards memory impairments and misjudgements that directly affect their contingency awareness. The neurobiology of rumination overlaps with brain networks operating in cognitive biases, with potentially special contributions of the ACC, vmPFC and hippocampus (Belzung et al., 2015).

Loss/penalty overappreciation.

Contributing to the hedonic general misappreciation, individuals suffering from depression excessively respond to negative valence stimuli, indicating aberrant processing and evaluation of general stimuli. These alterations promote attentional orientation towards negative emotional states, a mechanism that becomes a habit through reinforcement and that is called *cognitive interlock*. Within the network underlying this biased information processing through negative valence stimuli, the OFC and ACC are major players, yet other participating interoceptive brain regions, such as the insula, are also dysfunctional in depression (Belzung et al., 2015).

Cognitive biases.

Altered hedonic appreciation, also commonly referred in scientific literature as hyposensitivity to reward and hypersensitivity to loss/penalty, ultimately engenders cognitive biases in depressed patients, and memory and attentional biases in particular. The amygdala, in intimate coordination with the lateral habenula, has been suggested to be the principal brain substrate of these cognitive biases. Indeed, depressed individuals show increased patterns of neural activation in the amygdala in response to negative stimuli (Victor et al., 2010). Altered patterns of activity in the ACC might also contribute to these cognitive biases, since this region acts by modulating the activity of subcortical regions implicated in processing of negative

Being a major factor concerning neuromodulation, serotonergic neural activity is thought to play a prominent role in depression. The *monoamine hypothesis of depression* postulates that this disorder is associated to a deficient transmission within monoamine systems, and in particular to low brain levels of 5-HT (Cosci and Chouinard, 2019). Indeed, selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, are the pharmacological treatment of reference for depression, notably in severe cases. Monoamine-based pharmacological agents have potent antidepressant effects (e.g., they enhance mood states), but they require long-time periods to start being effective and their remission rates are considerably low (Krishnan and Nestler, 2008). Thus, monoamine-agents have permitted to uncover the contribution of monoamines in the aetiology of depression, but are a centre of discussion concerning its treatment, revealing the existence of more intricate and complex mechanisms underpinning its symptomatology.

b.5. Animal models of chronic distress-related disorders

Preclinical investigations on the physiological and behavioural effects of chronic distress started soon, and Katz, if not the first, was one of the pioneers in modelling a stress-induced animal model of depression-like symptomatology (Katz and Baldighi, 1982). In his investigations, he exposed rats to various severe stressors for weeks until animals developed a collection of signs and symptoms reminiscent to those of human depression. Among them, rats showed for instance signs of reward underappreciation or anhedonia from decreased intake of palatable solutions. From that moment on, a multitude of rodent models have been developed aiming to reproduce human symptomatology of chronic distress-related disorders, ultimately searching to optimally fit validity criteria.

What is an animal model of a human disorder? In accord with the scientific literature, an animal model is “an experimental preparation developed in one species (mouse, rat, non-human primate, etc.) to study phenomena occurring in another species (human)”. It should also possess the same structure as the human correlate, i.e., a correspondence between the human pathological elements and those expressed in the animal model should exist (Belzung and Griebel, 2001; McKinney, 1984). The validity of animal models is generally evaluated in relation to the following terms: (i) isomorphism (*face validity*), (ii) homology of underlying neurobiological mechanisms (*construct validity*) and (iii) pharmacological correlation (*predictive validity*). Thus, in the case of chronic distress-related disorders, animal models should reproduce typical physiological and behavioural responses of the human pathology (e.g., self-negligence-related appearance, altered heart rate), which should be grounded in similar neurobiological disturbances (e.g., altered HPA activity) and should be sensitive to pharmacological agents adequate for clinical treatment (e.g., fluoxetine administration should ameliorate depressed-like symptomatology).

The scientific path towards the development of animal models of chronic distress-related disorders has not been easy due to a multitude of factors, including the lack of reliable biomarkers and the subjective nature of multiple symptoms (e.g., rumination). In spite of these difficulties, some of the developed rodent models show outstanding face and predictive validities that qualify them to experimentally address the neural basis of chronic distress-related disorders.

b.5.1. Chronic mild stress (or unpredictable chronic mild stress)

The chronic mild stress (CMS) model was developed by Willner, who inspired himself from the first model developed by Katz (Willner, 2005). With the purpose of particularly reproducing the anhedonic state of depression, animals are subjected to multiple mild stressors (e.g., tail suspension,

water deprivation, predators scent cues) over a prolonged period of time. This sustained exposure to mild stressors effectively brings animals to develop a depressive-like phenotype, evidenced by consistent behavioural tests. The CMS model is broadly considered the animal model of depression with the strongest validity and translational value. In terms of its predictive validity, for example, the CMS model is sensitive to chronic administration of multiple antidepressant drugs, as well as to other alternative treatments for depression, such as deep brain and transcranial magnetic stimulations (Papp et al., 2019, 2017; Willner, 2017). Regarding its face validity, the CMS model reproduces anhedonia-like behaviours when tested in reward-motivated paradigms, and shows a decreased preference for palatable solutions and a reduced place preference conditioning (Pizzagalli, 2014). Symptoms of a disrupted circadian rhythm reminiscent to the observed in melancholic patients of depression are also observed in CMS animals. Most interestingly, even if the model has often been criticized for the unreliability of the procedure, the neurobiological homology with the human pathology is considerable. Animals, for example, show decreased NAc dopaminergic activity after encountering palatable food in rewarding behavioural tasks, decreased hippocampal GR expression and increased PVN CRF expression.

b.5.2. Learned helplessness

The learned helplessness (LH) model has proven to be a useful tool to address depression-like coping deficits in aversive but avoidable situations (Seligman and Beagley, 1975). Animals are firstly exposed to uncontrollable and inescapable stressful events, such as mild electroshocks, and later on, reintroduced into the same environment but with the possibility to escape from the stressors. Stressed animals become unable to learn how to escape from the stressful events, called *helplessness*, which is considered a typical depressive-like behaviour. In terms of validity, the LH model successfully reproduce phenotypical features of the human pathology, like increased plasma GC levels and altered reward appreciation and valuation. Electroconvulsive seizures, a therapeutic approach used in clinic for treatment-resistant depression, and a wide range of antidepressant drugs reverse LH-typical behaviour when administered chronically (Vollmayr and Gass, 2013). However, the validity of this model is limited on female rats, since contrary to males, they are able to learn how to escape the stressful events and therefore do not always express helplessness-related behaviours.

b.5.3. Chronic social defeat stress

Chronic social defeat stress (CSDS), proposed by Miczek and collaborators (Miczek et al., 2004) and addressing the biology of social conflict, is one of the most frequently used rodent models of chronic distress-related disorders. In this paradigm, animals (*intruders*) are introduced individually into the territory of a higher body-weight and aggressive conspecific (*aggressor*), where they are rapidly explored, attacked and defeated. After some minutes of interaction, intruders and aggressors are usually separated by a holed barrier allowing visual, olfactory and auditory contact. Over the course of several days, the intruders are repeatedly exposed to different aggressors, and the intruders' behaviour during confrontation is studied. A majority of animals exposed to CSDS show significant social avoidance when tested in social interaction tasks, and they are typically called *stress-susceptible* individuals. The rest of animals, called *stress-resilient*, do not develop neither social avoidance nor anhedonia-like behaviours. Interestingly, although only stress-susceptible individuals show depressive-like behaviours (e.g., decreased preference for palatable solutions or social avoidance), both susceptible and stress-resilient individuals show increased anxiety-related behaviours when tested in behavioural tasks such as the elevated plus maze or the stress-induced polydipsia test (Golden et al., 2011). Thus, this discriminative ability of the model to distinguish animals from the theoretical

depression or anxiety parts of the physio-pathological continuum, makes it highly relevant for studies addressing interindividual variability and pathological vulnerability to chronic distress. This model has also good predictive validity, with social avoidance behaviours being suppressed after chronic antidepressant drugs administration. However, this paradigm cannot be implemented in female rodents without modifications. Indeed, in order to motivate aggression, which naturally it would not happen, female intruders need to be previously exposed to male outsiders' urine (Yohn et al., 2019b).

b.5.4. Early-life stress

Based on the scientific knowledge that early-life exposure to stressful events is a risk factor for mood disorders in adult humans (see for instance Heim et al., 2008), animal models of early-life adversity (ELS) have been developed. Among the possibilities, the most common manipulation in rodents is the interruption of maternal interaction, either quantitatively (maternal separation during hours) or qualitatively (limited nesting and bedding material during early postnatal days). These early stressed animals developed depression- or anxiety-like behaviours in adulthood, substantiating the translational validity of the model (**Figure 11**). However, many studies have failed in reproducing chronic distress-related symptomatology in this model, a fact that might be attributable to protocol variations. Indeed, the duration and moment of the stressful events during the postnatal period in rodents are likely critical for the efficiency of the protocol (Murthy and Gould, 2018).

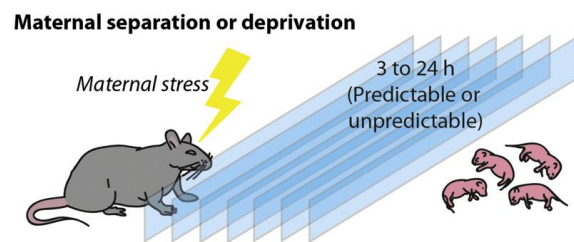


Figure 11. Experimental paradigm based on maternal deprivation to investigate distress-related pathology in rodents. Maternal separation or deprivation periods can be predictable (fixed schedule) or unpredictable and are usually combined with maternal stress. As a consequence, the continuity and quality of the maternal care are perturbed and pups experience stress. Image from Franklin et al. 2012.

However, many studies have failed in reproducing chronic distress-related symptomatology in this model, a fact that might be attributable to protocol variations. Indeed, the duration and moment of the stressful events during the postnatal period in rodents are likely critical for the efficiency of the protocol (Murthy and Gould, 2018).

b.5.5. Chronic corticosterone administration

Chronic CORT administration in rodents represents a tractable mean to address chronic distress-related human pathology (Darcet et al., 2016a). As an important advantage, this model allows examining the direct influence of GC on the emergence and development of the pathological symptomatology. Contrary to other animal models of chronic distress-related disorders based on the external application of stressors by the researcher, the CORT model offers the possibility to produce reliable and robust behavioural symptomatology, and therefore limits variability. A variability in the effect of stressful stimuli on individuals is inherent in paradigms such as the CMS and LH, since individual responsivity is not a parameter that can be controlled by the experimenter. Therefore, in face of a same stressor, the HPA response and subsequent CORT secretion rates will differ between individuals, leading to experimental variability. Moreover, habituation mechanisms after repeated exposure to stressors have been described in animals, which naturally contribute to the interindividual variability of the response (Grissom et al., 2007). This variability can certainly be advantageous in studies seeking, for example, for behavioural markers of resilience or vulnerability to stress. But it also can be counterproductive for addressing other scientific questions relying on a more-framed symptomatology.

This experimental approach allows mimicking human chronic distress-related hypercortisolaemia by sustained exogenous CORT administration, which can last from some weeks to

months. Corticosterone is usually administered through the drinking water, but can also be subcutaneously injected or gradually released from a pellet implant. Indifferently, all these methods permit a rigorous control over CORT levels. Different concentrations of the hormone can be used to induce emotional stress-related symptomatology (e.g., 25 µg/mL and 50 µg/mL) but most frequently, 35 µg/mL suffice to reach steady-state moderate levels of the GC (David et al., 2009; Gasparini et al., 2016; Gourley et al., 2008; Shahanoor et al., 2017). In fact, chronic CORT-treated animals exhibit a behavioural spectrum reminiscent to emotional anxio-depressive symptoms that substantiate its translational value (Darcet et al., 2014; David et al., 2009; Dieterich et al., 2020, 2019; Gourley et al., 2008; Gourley and Taylor, 2009). Among the behavioural disturbances reported, chronically CORT-treated animals show increased passive behaviour when tested in the force swim test (FST), a first-line paradigm for testing antidepressant action of drugs. The effect of exogenous CORT on depressive-like behaviour seems to be dose-dependent, with more passive swim styles following greater doses, as many studies have reported (Johnson et al., 2006; Zhao et al., 2008). Thus, the magnitude of the hormonal exposure in terms of duration and concentration might be crucial for the development of the pathological phenotype.

Corticosterone-treated animals also show different signs of disrupted reward-processing or anhedonia-like behaviours, such as reduced preference for palatable solutions or decreased self-directed behaviours like grooming (Darcet et al., 2014; David et al., 2009; Gorzalka et al., 2003; Gourley et al., 2008). Anxiety-like behaviours are also reported in the CORT model, supporting the idea of a physiological-to-pathological continuum in mood disorders and strengthening the translational value of the model, in particular its face validity. As an illustration, animals have been reported to behave in a typical anxious way in the open field and elevated plus maze tests, the light/dark box task and the novelty suppressed feeding (NSF) task (Darcet et al., 2016b; David et al., 2009; Dieterich et al., 2019; Murray et al., 2008). Together, the symptomatology observed in animals chronically exposed to exogenous CORT points to the development of a negative affective state concurrent with a hedonic imbalance. Chronic CORT exposure also results in physiological allostatic alterations homologous to those in humans, with especial relevance of a deregulated HPA function and hypotrophy of the adrenal glands (Bush et al., 2003; Johnson et al., 2006). These changes subsequent to sustained exogenous CORT exposure further support the construct validity of the model. In the case of its predictive validity for chronic distress-related disorders, the model has been reported sensitive to different antidepressant treatments (Ago et al., 2008; Darcet et al., 2016b; David et al., 2009). However, a major limitation is a general insensitivity of female rodents to the effects of chronic CORT administration, in terms of its efficacy to induce anxio-depressive-like behaviours. Fluctuating gonadal hormones characterizing the phases of the oestrous cycle in females might contribute to this difference, but a direct causal relationship has not been yet proposed. Moreover, since increasing doses of CORT have been demonstrated ineffective in females to induce a pathological phenotype, more complex and intricate mechanisms are thought to operate behind these phenomena (Mekiri et al., 2017).

The study of cognitive deficits in animal models has attracted much attention the last decades, especially since they are considered core features in the symptomatology of human chronic distress-related disorders. Consistent with the clinical evidence, chronically stressed rodents exhibit impaired or suboptimal cognitive function in various behavioural paradigms. A non-systematic overview of the cognitive deficits reported in animal models of chronic distress can be found in **Table 1**. In the case of chronic CORT administration, and for its relevance in the present project, several spatial memory and executive deficits merit particular attention. Impaired instrumental acquisition and deficits in reward processing have been evidenced in animals tested in operant paradigms, including reduced motivation for food rewards in effort-based tasks and insensitivity to outcome devaluation (Dieterich et al., 2020, 2019; Gourley et al., 2008; Olausson et al., 2013). Furthermore, animals chronically exposed to CORT

also show deficits in memory-based learning and in behavioural flexibility (Cerqueira, 2005; Coburn-Litvak et al., 2003; Dachir et al., 1993; Darcet et al., 2014; Sousa et al., 2000). Overall, empirical data support the suitability of the CORT model for addressing chronic distress-based emotional and cognitive dysfunction.

In addition to the models mentioned above as well as other models based on the external application of stressors, genetic models for anxiety- and depression-like symptomatology have also been generated via selective breeding. Animals naturally expressing desired features are bred over several generations in order to obtain inbred strains that robustly exhibit the physiological and/or behavioural alterations of interest. *Flinders sensitive* line rats and *Wistar-Kyoto* rats, for example, are characterized by their depressive-like behavioural repertoire, as well as for augmented basal levels of circulating CORT (Malkesman et al., 2006; Nam et al., 2014). Other genetic models used in the study of depression-related dysfunction are, for instance, congenitally LH animals and swim high- and low-active animals (Elyacoubi and Vaugeois, 2007). Besides, in the wake of scientific evidence of depressive-like behaviours and physiological symptoms on conditional knockout mice and viral approaches, other genetic mouse models targeting CRF activity have been defined. Despite the fact that they are currently widely used, findings in CRF-deficient/excessive mice remain controversial (Deussing and Chen, 2018).

| Cognitive deficits | Reference | Animal model | Animal manifestations | Protocol |
|-------------------------------|------------------------------|---------------------------------------|---------------------------------|------------------------------------|
| <i>Spatial working memory</i> | (Meunier et al., 2004) | ELS | Reduced learning | Morris Water Task |
| | (Bardgett et al., 1994) | Subcutaneous CORT | Reduced spontaneous alternation | T-maze |
| | (Coburn-Litvak et al., 2003) | Subcutaneous CORT | Increased Nb of errors | Barnes maze |
| | (Patki et al., 2013) | CSDS | Increased Nb of errors | Radial Arm Water Maze |
| | (Dachir et al., 1997) | Subcutaneous CORT pellet implantation | Impaired acquisition | Radial Arm Maze |
| | (Tagliari et al., 2011) | CMS | Reduced learning | Morris Water Task |
| <i>Inhibitory control</i> | (Wilson et al., 2012) | ELS | Decreased accuracy | 5-choice serial reaction time task |
| | (Comeau et al., 2014) | CMS | | |
| <i>Attention</i> | (Bondi et al., 2008) | CMS | Reduced performance | Attentional set-shifting task |
| | (Baudin et al., 2012) | ELS | | |
| <i>Flexibility</i> | (Cerqueira, 2005) | Subcutaneous CORT | Impaired learning | Reversal learning |
| | (Dieterich et al., 2019) | Oral CORT | | |
| | (Bisaz et al., 2011) | CMS | | |
| | (Laredo et al., 2015) | CSDS | | |
| <i>Motivation</i> | (Gourley et al., 2008) | Oral CORT | Reduced breakpoint | Progressive Ratio |
| | (Dieterich et al., 2020) | | | |

Table 1. Overview of cognitive deficits reported in different animal models of distress-related disorders.

Collectively, rodent models of chronic distress-related disorders share behavioural, physiological and neurobiological similarities to those observed in humans, but how they contribute in the emergence, development and maintenance of the resulting phenotype remains elusive. Thus, animal models are mostly used in combination with genetic manipulations to better isolate and decorticate the underlying mechanisms of the related pathologies.

C. Decision-making under chronic distress

Major cognitive impairments are reported in patients suffering from chronic distress-related disorders. Among a wide-ranged cognitive dysfunctionality, decrements in memory (WM, visuospatial, verbal), learning, selective attention, psychomotor learning and executive functioning have been described. In the case of executive functioning, patients of depression for instance encounter problems during planning and problem solving, while inhibitory control and flexibility are also compromised (Darcet et al., 2016a; Hinkelmann et al., 2009). Combined, these executive dysfunctions seem to explain why during depressive episodes patients also have problems in making decisions (Baeza-Velasco et al., 2020; Griffiths et al., 2014).

Making decisions under stress is a common situation. Circumstances in real life can be stressful and influence the process of DM and the quality of the choices. Furthermore, some decisions are, by themselves, stressful. The neural networks involved in DM comprise brain regions highly sensitive to stress-associated changes. It is then justified to suggest that the effects on DM will aggravate when stressors are present for longer periods and/or in greater magnitudes. Therefore, to investigate the effects of chronic distress on DM it is necessary to characterize the nature and extent of the stressors, notably in the case of experimental stress-induced protocols. Clinical studies usually address stress through personal questionnaires (e.g., State Trait Anxiety Inventory) or physiological measures (e.g., heart rate, GC circulating levels), whereas a battery of ethological and physiological measurements are regularly used in animals (e.g., evaluation of the fur coat state, presence of mechanical stereotypes, CORT circulating levels). Based on the neurobiology underlying DM and the allostatic adaptation to chronic distress, some assumptions can be formulated:

- (i) Distress-induced abnormal VTA activity should affect decisions involving rewards. The motivational dimensions of DM, identified as appetitive and consummatory behaviours, are considered to reflect reward appreciation. Scientific evidence points to the central role of the VTA, within the mesolimbic DA circuit-OFC network, in reward anticipation and recognition. Abnormal VTA activity might result from sustained distress since this structure is intimately connected to other stress sensitive regions such as the BNST.
- (ii) Distress-induced abnormal PFC and limbic activity should affect decisions made under uncertain situations. Uncertainty triggers expectation and to certain extent acts as a stressor. Therefore, it favours the arousal of emotional reactions involving limbic structures that modulate prefrontal activity. Under sustained distress, this emotional state might be enhanced. The PFC essentially favours goal-directed behaviour, driving a constant reevaluation of options and expected consequences. Abnormal PFC activity would therefore affect the balance between immediate emotional responses and reflective behaviour, and impact their computational products and, subsequently, final decisions.
- (iii) Distress-induced abnormal PL cortex (or human dlPCF) activity should affect decisions made under risk or high uncertainty. Under this kind of situations, individuals' adaptability rely on their inhibitory control and in their ability to acquire flexible behavioural strategies. Disrupted PL cortex activity might affect the adequacy of decisions since it is essential for both behavioural components and, therefore, for adapting behaviour in dynamic environments.

The effects of chronic distress on human decisional capabilities have been mostly studied through patients of stress-related disorders, particularly of anxiety and depression. These individuals

usually perform poorly in DM tasks and show lower correspondent neural activation than healthy individuals (Hartley and Phelps, 2012; Lenow et al., 2017; Treadway et al., 2012). For instance, they spent less time deliberating before making a decision, but feel more conflicted during the process, which translates into more frequent experiences of post-decisional regret. At their origin, typical reward underappreciation and penalty overappreciation have been proposed (Cella et al., 2010; Giustiniani et al., 2020). Different studies using the IGT paradigm have evidenced DM impairments in depressed patients (Baeza-Velasco et al., 2020; Must et al., 2013), but normal performance is also reported in a non-negligible proportion of cases. The severity and the state of the illness are apparently key determinants in the emergence of decisional alterations, with higher probabilities of cognitive dysfunction in correlation with the accumulation of depressive episodes.

Preclinical studies have also offered valuable insight into the neural mechanisms orchestrating decisional changes under stress. The detrimental effect of repeated stressful events on mPFC-dependent cognition has been demonstrated, for example, in mice exposed to persistent noise and in intracerebroventricularly CRF-injected mice. These animals show degraded information processing in tasks evaluating goal-directed behavioural strategies, concomitant to disrupted outcome evaluation (Devilbiss et al., 2016). Since mPFC-dependent goal-directed behaviour is proved necessary for optimal DM in dynamic environments, these results point to prefrontal CRF signalling as a potential determinant of DM quality, in agreement with additional evidence in acute stress (Bryce and Floresco, 2016). Poor decisional performance in a rodent IGT version has been also associated to individuals with higher anxiety levels in physiological conditions (de Visser et al., 2011), and repetitive CORT injections in the IL cortex of rats reduce reward responsiveness with respect to vehicle-treated animals (Koot et al., 2014). Other studies of translational value have addressed the role of monoamines in DM impairments reminiscent to depressive symptomatology. Manipulations of the DA transporter (DAT) leading to neural hyperdopaminergia result in impaired DM in a rat version of the IGT, consistent with poor IGT performance in depressed patients (van Enkhuizen et al., 2014). All these findings further support the notion of distress, and in particular of chronic distress, as key determinant of the adaptive value of the decisional processes, but the underlying neural mechanisms remain still to be elucidated. The experimental hypotheses formulated in this doctoral project aim to contribute to the general understanding of these pathophysiological mechanisms, with the ultimate objective of identifying biological targets for the development of new therapeutic approaches in biological psychiatry. For that, the effects of sustained distress on behavioural adaptability, and specifically on DM under uncertainty, will be evaluated in a murin model, allowing individual variability characterisation. Behavioural phenotypes are expected to reflect specific physiological and neuronal adjustments to sustained distress.

About transdiagnostic approaches

The *Diagnostic and Statistical Manual of Mental Disorders* (DSM) and the *International Classification of Diseases* (ICD) (American Psychiatric Association, 2013; World Health Organization, 2018) are international standards for reporting diseases and health conditions. They usefully provide an organizing framework for psychiatric and clinical psychology literature, guiding professional mental health training worldwide. In spite of their diagnostic advantages, the taxonomic approach of the DSM and ICD has been increasingly questioned over the last decades. There is a growing consensus about the unsuitability of their psychiatric nosology approach for research and clinical purposes (Insel, 2014; Kotov et al., 2017). Alternatively, this sceptical movement supports transdiagnostic approaches for the study and treatment of mental disorders. A transdiagnostic treatment, for example, is a treatment that

specifically targets psychological processes or core vulnerabilities that contribute to the development and maintenance of classes of disorders. Substantial scientific evidence has been presented demonstrating that different psychiatric conditions share core underlying vulnerabilities. This transdiagnostic approach in particular has received special support regarding the study of chronic distress-related disorders such as anxiety and depression, which have predominantly relied on transdiagnostic categories defined by the National Institute of Health (NIH) in the Research Domain Criteria (RDoC) initiative.

The feasibility and reliability of these relatively recent conceptions need further substantiation in order to more generally assure the scientific community. Still, they are interesting approaches, which if successful, are likely offering opportunities towards new therapeutic interventions that could potentially contribute to alleviate the burden of patients of neuropsychiatric conditions. Thus, the present study draws on the evidence of shared core vulnerabilities across distinct clinical conditions, without denying the contribution by nosology codes used in classical approaches of human neuropathology. Furthermore, the utility of a transdiagnostic methodology is genuine in the present project, since sustained elevated circulating GC levels do not only relate to chronic distress-related disorders, but also to other clinical conditions attracting special attention in the medico-scientific community. Administration of exogenous GC, for example, is used for treatment of inflammatory diseases. Chronic activation of the immune system can lead to neuroinflammation of the CNS, which has been described to be mostly mediated by cytokines. Most interestingly, the physiopathology of depression and the inefficiency of antidepressant drugs has been linked to dysregulating effects of cytokines on cerebral activities (see for instance Carlessi et al., 2021; Pariante, 2017). Besides, core symptoms of chronic distress-related disorders like anhedonia and apathy, are often present in other psychiatric conditions such as Alzheimer's disease, dementia and schizophrenia (Horan et al., 2006; Staekenborg et al., 2010; Zhao et al., 2016). Therefore, such an approach provides an opportunity to learn more about how DM operates under chronic distress as well as under many other circumstances.

WORKING HYPOTHESES

The main goal of this study is to obtain a better insight into the neurobiological underpinnings of DM under uncertainty. In humans as well as in mice, paradigms of DM for monetary or food rewards contribute to better understand adaptive goal-directed behaviour acquired through reinforcement learning. The behaviour of individuals is expressed in an adaptive-to-maladaptive behavioural continuum for, both, physiological and pathological conditions. Therefore, behavioural evaluations for individual variability characterization are essential to decipher the stoichiometry of the different behavioural dimensions that contribute to adaptive DM under uncertainty.

This study therefore rests on the working hypotheses detailed below. A general overview of the paradigms used to evaluate specific processes within DM can be found in **Table 2**:

First working hypothesis – Decision-making under uncertainty and characterizing a healthy human population can be modelled in mice based on a gambling paradigm specifically adapted to facilitate motivational-based behaviours.

Decision-making under uncertainty was thereafter evaluated with respect to enduring distress. An in depth characterization focusing on the effects of chronic exposure to high levels of GC in mice (CORT model) was conducted, with the aim to understand the contribution and interaction of multiple biological domains to adaptive DM processes. That leads to the following working hypothesis:

Second working hypothesis – Human distress-induced DM impairments can be modelled in mice through a paradigm of chronic CORT administration.

To better understand the physiological and neural adjustments to sustained distress, further characterisations were then undertaken based on the third working hypothesis.

Third working hypothesis – Distress-induced suboptimal DM in mice grounds on plastic anatomofunctional adaptations in telencephalic areas sensitive to stress and relevant for DM processing: study on GR, MR and CRF signalling in the mPFC, DLS and ventral hippocampus (VH) areas.

To better appraise the contribution of the motivational dimensions to DM, the consequences of enduring distress on relevant effort and reward processing dimensions and their neurobiological correlates were investigated. Experiments were designed based on the following working hypotheses:

Fourth working hypothesis – Distress-induced suboptimal DM in mice relies on appetitive-related motivational deficits.

Fifth working hypothesis – Distress-induced suboptimal DM in mice relies on consummatory-related motivational deficits.

Sixth working hypothesis – Distress-induced motivational deficits in mice ascribe to brain networks within the reward system, including the NAc, amygdala, IC, PSTN and VTA.

Following the evaluation of the motivational domains and their neurobiological correlates, sensorimotor and cognitive dimensions involved in learning and relevant for DM under uncertainty, as well as their neurobiological correlates, were investigated. That leads to the last working hypotheses of this investigation:

Seventh working hypothesis – Distress-induced suboptimal DM in mice relies on learning deficits.

Eighth working hypothesis – Distress-induced learning deficits in mice ascribe to (fronto)corticostriatal networks including the mPFC, OFC, IC and DLS.

| <i>Decisional process</i> | <i>Behavioural paradigm</i> | <i>Parameters</i> | <i>Interrogated neurobiology</i> |
|---|--------------------------------|--|---|
| <i>Overall DM</i> | ^A mGT | Reward responsiveness & final performance | GR/MR & CRF expression in the mPFC, DLS & VH / plasma CORT levels |
| <i>Appetitive behaviour/behavioural initiation</i> | FCS | FCS degradation | Plasma CORT levels |
| | NSF test | Latency to feed | - |
| | FFT | Latency to feed | GR/MR & CRF expression in the mPFC, DLS & VH / plasma CORT levels |
| | PR schedule of reinforcement | Breakpoint (effort allocation) | cFos* and FosB expression in telencephalic and diencephalic areas / IL CRF activity modulation |
| | RL task* | Obtained rewards | |
| <i>Consummatory behaviour/hedonic appreciation/reward valuation</i> | ^A mGT | Total intake | GR/MR & CRF expression in the mPFC, DLS & VH / plasma CORT levels |
| | | Lose-shift | |
| | | Win-stay | |
| | FST | Immobility latency & immobility duration | |
| | SPT | Sucrose solution consumption | |
| | FFT | Choice & total intake | |
| <i>Reinforcement learning/psychomotor learning</i> | ^A mGT | Rigidity score | GR/MR & CRF expression in the mPFC, DLS & VH / plasma CORT levels |
| | | Flexibility score | |
| | dWST | Total errors & test latency | |
| | FR-1 schedule of reinforcement | Instrumental acquisition | cFos* and FosB expression in telencephalic and diencephalic areas / IL CRF activity modulation* |
| | RL task* | Correct responses & learning rates – behavioural flexibility | |
| | MLT | Latency to fall | GR/MR & CRF expression in the mPFC, DLS & VH / plasma CORT levels |

Table 2. General overview of the behavioural paradigms and parameters used, and the neurobiology interrogated during the evaluation of the specific processes directly or indirectly subserving DM under uncertainty.

COMMON METHODOLOGY BETWEEN CHAPTERS

Ethical statements

The present work has been conducted under the guiding principles for animal scientific research, so-called principles of the 3Rs (**R**eplacement, **R**eduction and **R**efinement), following the standards of the Ethical Committee in Animal Experimentation from Besançon (CEBEA-58; A-25-056-02) and according to the Directive from the European Council at 22nd of September 2010 (2010/63/EU).

In order to limit the number of animals used in the presented work without compromising the statistical power of the comparisons, sample sizes were identified *a priori* by statistical power analysis (see *Statistical analyses*). This point is of utmost importance since studying interindividual differences requires a sufficient number of animals to obtain representative subgroups. Experiments never had to be stopped because of reaching an endpoint, i.e., an upper limit for the pain, suffering or harm of the animals to be tolerated.

Animals

Three mouse strains were used during the presented doctoral work: *C57BL/6JRj* (all *Chapters*), *crf* floxed mice sensitive to Cre recombinase-mediated modulation (Crh-IRES-Cre (ZJH), referred here as *CRF-cre*) (*Fourth Chapter*) and *CRF-cre x Ai9* reporter mice expressing tdTomato fluorescence in CRF positive neurons and also sensitive to Cre recombinase-mediated modulation (*Second and Third Chapters*) (*EtsJanvier Labs, Saint-Berthevin, & Jackson Laboratories, Domaine des Oncins, France; Max Planck Institute of Psychiatry, Munich, Germany*). *C57BL/6JRj* is a widely used mouse strain in neurobiological studies that allows for the expression of a wide variety of mutations and, therefore, frequently used as genetic background in transgenic models for neuropsychiatric and neurological conditions. *C57BL/6JRj* mice are easy-to-handle animals even though they are relatively aggressive, and have a life expectancy of about two years. The *CRF-cre* transgenic mouse line has Cre recombinase expression directed to CRF positive neurons by the endogenous promoter/enhancer elements of the *Crf* locus. These mice are fertile, normal in size, and physical or behavioural abnormalities have not been reported. Finally, *CRF-cre x Ai9* mice were generated by crossing *CRF-cre* mice with *Ai9* mice. The *Ai9* strain, which is congenic on the *C57BL/6J* genetic background, is a Cre reporter tool strain expressing robust tdTomato fluorescence following Cre-mediated recombination.

The three inbred mouse strains selected to carry out this research are highly uniform in terms of inherited characteristics including appearance and behaviour, making them adequate to study the neurobiological bases of DM and behavioural interindividual variability (Festing, 2014); but see (Tuttle et al., 2018). Particular reasons have motivated the use of different mouse strains in the present project. Behavioural experiments that did not include causal manipulations of neurobiological molecular players were carried out in *C57BL/6JRj* mice. On the contrary, behavioural approaches including causal manipulations of the CRF system needed of genetically modified animals. The results presented in this manuscript include preliminary data of these causal investigations, which have importantly contributed to “pave the way” for ongoing projects addressing the role of the CRF system on distress-induced suboptimal DM, in collaboration with PhD Jan Deussing from the *Max Planck Institute of Psychiatry* in Munich, Germany.

All mice were male aged 6-14 weeks at the beginning of the experiments. In this doctoral project, male individuals were chosen since they have been described to more advantageously perform than females in rodent gambling tasks (van den Bos et al., 2006). Besides this point, published data on females indicate that chronic CORT administration is not equally effective in altering emotional negative valence behaviours (Mekiri et al., 2017; Yohn et al., 2019a). Future studies will examine the effect of chronic CORT administration on gambling performance in females.

- Housing conditions

All animals were group-housed (3-6 animals per cage; 552 cm² surface) and maintained under a normal 12-hour light/dark cycle (light switched on at 7:00 a.m.; 270-300 lux) with constant temperature (22±2 °C) and controlled humidity (55±10 %). Animals had access to standard chow (Kliga Nafag 3430PMS10, Serlab, CH-4303 Kaiserau, Germany) *ad libitum* until one week before the beginning of the behavioural experiments, when the food restriction protocol started. Drinking bottles containing vehicle or treatment were available at all times.

- Food deprivation

As a standard procedure for behavioural approaches relying on food-rewarding (Jensen et al., 2013), animals were subjected to an alimentary deprivation protocol to 80-90% of their free-feeding weight from the last week before the start of, and throughout the whole behavioural testing. For this, mice were weighted daily at the beginning of the light cycle (8:00 a.m.), and the amount of food presented in the home cage was progressively decreased (starting from 3 g of food per animal) until all mice met the weight limit criteria. From a pilot study previously carried on (data not shown), one week-long was judged the necessary duration of the protocol to reach the weight limit criteria through progressively decreasing the amount of food no more than 1 g per day. From that point, the amount of food in the home cage was maintained but, whenever necessary, recalculated to better adjust the weight of the animals. This *in continuum* food restriction protocol allowed to maintain the animals' motivation for exploring and completing the behavioural tasks offering food reinforcers. It furthermore reduced animals' stress levels linked to manipulation, since a daily handling was performed at the moment of weighing. See Annex I: Supplementary results, Animals weight monitoring during food deprivation.

- Pharmacological treatment

The animal model used for the present investigation is based on chronic oral administration of corticosterone (CORT, 4-Pregnene-11 β -diol-3,20-dione-21-dione, *Sigma-Aldrich*, France). For this, CORT was suspended in vehicle (VEH), a 0.45% solution of hydroxypropyl- β -cyclodextrine, using two consecutive 10-minute cycles of sonication at 40.8 Watts (Fisherbrand™ Q500 Sonicator with Probe, *Thermo Fisher Scientific*, Illkirch-Graffenstaden, France) at 4°C. The solution was gently vortexed between and after sonication cycles. Once in suspension, it was diluted in drinking water (35 μ g CORT/ml solution). Liquid intake during the procedures was monitored (data not shown), mice drinking 3-4 ml/day on average, thus an equivalent to 5 mg/kg/day per 25g animal.

- Experimental conditions

All behavioural evaluations were conducted during the light phase of the cycle, starting at 8 a.m., and after at least four weeks of CORT/VEH treatment when steady-state CORT blood titers were

reached (data not shown). Prior to each experimental session, animals were acclimatized to the experimental rooms for 30 min. Low luminous intensity (30-50 lux) was used during the experiments (Peirson et al., 2018). Different kinds of food reinforcers were used in food reward-based tasks. In the adapted mouse Gambling Task (^AmGT), the delayed Win-Shift Task (dWST) and the operant tasks (Fixed Ratio –FR, Progressive Ratio schedule of reinforcement –PR, and Reversal Learning –RL), 20 mg grain-based pellets were used (Dustless Precision Pellets® Grain-Based Diet, *PHYMEP s.a.r.l.*, Paris, France). In the Free Feeding Task (FFT), besides the 20 mg grain-based pellets, ~20 mg chocolate pellets (Choco Pops, *Kellogg's*®) were also presented. Finally, for the Novelty Suppressed Feeding (NSF) test, standard chow was used as reinforcer. In order to reduce the putative influence of neophobia (except for when it was specifically evaluated; see results from *Third Chapter, Section 1*), all animals were presented to food reinforcers in their home cages for several days (habituation). At the end of each behavioural session, animals were transferred one by one in new home cages in order to avoid any influence of social transmission from the already tested mice.

Operant training and testing: *Equipment and software.*

Operant behaviour was evaluated using five identical two-hole-operant chambers (*Med Associates*, Hertfordshire, UK; Retailer – *BIO-CONCEPT Scientific*, CLS, France). All operant chambers were individually housed inside sound-attenuating cubicles with ventilating fans and were connected to a PC computer with MedPC-5 software via a *Smart Control* interface cabinet. The MedPC programs used in this study were written in Medstate notation code.

Sacrifice and tissue preparation for histological analysis

Animals were deeply anesthetized with an intraperitoneal injection of Dolethal (1 ml/kg, *Vetoquinol S.A.*), then transcardially perfused with 0.9% NaCl followed by ice-cold 4% paraformaldehyde (PFA, *Roth*®, *Carl Roth GmbH*, Karlsruhe, Germany) fixative in 0.1 M phosphate buffer pH 7.4. Once extracted, brains were post-fixed in a 4% PFA solution overnight at 4 °C and cryoprotected by immersion in a 15% sucrose solution (D(+)-Saccharose, *Roth*®, Karlsruhe, Germany) in 0.1 M phosphate buffer 24 hours at 4 °C. Brains were then frozen by immersion in isopentane (2-methylbutane, *Roth*®, Karlsruhe, Germany) at -74 °C using a Snap-Frost™ system (*Excilone*, France) and stored at -80 °C until processed. Brains were cut in serial 30 µm-thick coronal sections, collected in a cryoprotector solution (1:1:2 glycerol/ethylene glycol/phosphate buffered saline –PBS 0.2M at pH 7.2; *Roth*®, Karlsruhe, Germany) and stored at -40° C.

Image acquisition and processing

Western-blot images were acquired with either a Bio-Rad ChemiDoc XRS+ System (Life-Science, *Bio-Rad*, France) or with autoradiographic films (Hyperfilm ECL, *GE Healthcare*, Velizy-Villacoublay, France). All quantifications were made blind to the experimental conditions using *ImageJ* software (*National Institutes of Health*, Bethesda, MD, USA) (Schneider et al., 2012). All details about the protocol for Western-blot images quantification can be found in [Annex II: Image analysis, Western Blot](#).

Microscopic brain images from enzymatic immunohistochemistry were acquired using 4x objectives of an Olympus microscope Bx51 equipped with a camera Olympus DP50. *ImageJ* software was used to count cFos- and FosB- labelled nuclei over the regions of interest. All details about the

immunohistochemical images analysis protocol can be found in [Annex II: Image analysis, Immunohistochemistry](#).

Data and statistical analyses

Data are presented as means \pm SEM. Statistical analyses were conducted using STATISTICA 10 (Statsoft, Palo Alto, USA) and figures were designed using GraphPad Prism 9 software (GraphPad Inc., San Diego, USA). Statistical power analyses (G*Power software, Heinrich Heine Universität, Dusseldorf, Germany) preceded behavioural experiments in order to *a priori* identify sample sizes yielding reproducible outcomes with $1-\beta > 0.8$ and $\alpha < 0.05$.

Assumptions for parametric analysis were verified prior to each analysis: (1) Shapiro-Wilk tests to study the normality of data distribution, (2) Levene's tests to address the homogeneity of variance, and (3) Mauchly's tests to study sphericity data effects. When datasets did not meet assumptions for parametric analyses, non-parametric analyses were used: Kruskal-Wallis, Friedman X^2 , Wilcoxon or Mann Whitney U tests. Upon significant main effects, further comparisons were performed with Duncan or Bonferroni corrections.

Behavioural time-dependent measures assessed during the Δ mGT, dWST, FR schedule of reinforcement, RL task and Motor Learning Task (MLT) were analysed by Repeated Measures ANOVA (RM-ANOVA) with sessions and blocks as within-subject factors, and species (human vs mice), treatment (VEH vs CORT) and DM clusters (good, intermediate and poor decision-makers) as between-subject factors. Student t-tests were used to compare groups' performance to chance level (50% of advantageous choices) in the Δ mGT, as well as to compare other non-time-dependent measures, e.g., FST scores. Survival curves for NSF and FFT scores were compared using the Gehan-Breslow-Wilcoxon (GBW- X^2) method. The degradation of the coat state due to the treatment was analysed by ANOVA with factors being weeks (1 to 13) and treatment (VEH vs CORT).

In the particular case of the comparative study between human and mice gambling data aiming to validate the animal model, a Bayesian approach was also performed. Learning rates (the ratio of the 'risk' of success – humans/mice) were calculated and compared by Beta laws, whose parameters were interpreted in terms of numbers of successes and failures, establishing the probability law of success rates' ratio. A credibility interval was then calculated.

The assumption of independent and normally distribution of treatment populations within DM clusters, FFT choices and flexibility clusters was tested with Chi-squared tests (X^2). Dimensional relationships between cognitive behavioural markers and final behavioural performance variable in the Δ mGT and operant tasks, and protein levels and immunohistological markers in the various brain structures under investigation were analysed using Pearson correlations.

For all analyses, the significance level was $p < 0.05$ and analysis with $p \leq 0.1$ were described as trends. Effect sizes are reported as partial η^2 ($p\eta^2$).

RESULTS

FIRST CHAPTER – Modelling decision-making under uncertainty

1.1. Adaptation of a mouse Gambling Task protocol to overcome satiation bias

Objective: The first objective of the present work was to adapt already published gambling tasks for mice (Pittaras et al., 2016; van den Bos et al., 2006), aiming at limiting the intrasession effect of satiation on animals' behaviour (**Figure 12**).

Continuous observation of CORT-treated animals' behaviour (data not shown) has permitted the confirmation and extension of already published data concerning their feeding habits. Particularly, chronic exposure to CORT exacerbates hyponeophagia in mice (see *Second Chapter, Section 1*; David et al., 2009; Dieterich et al., 2019), by increasing the periodicity and reducing the consumption during each intake episode. Even though previous studies showed that acute satiation in non-treated/drug-free animals does not apparently alter DM preferences on cognitive tasks (Cardinal and Howes, 2005; Floresco et al., 2008; Simon et al., 2009), the feeding behaviour of CORT-treated animals indicates that satiation could be a limiting factor during a multi-choice gambling task. This sets the rationale followed in the present project justifying the modifications applied to the original protocol, with the aim to limit acute satiation within testing sessions and to maintain animals' motivation for food reinforcers.

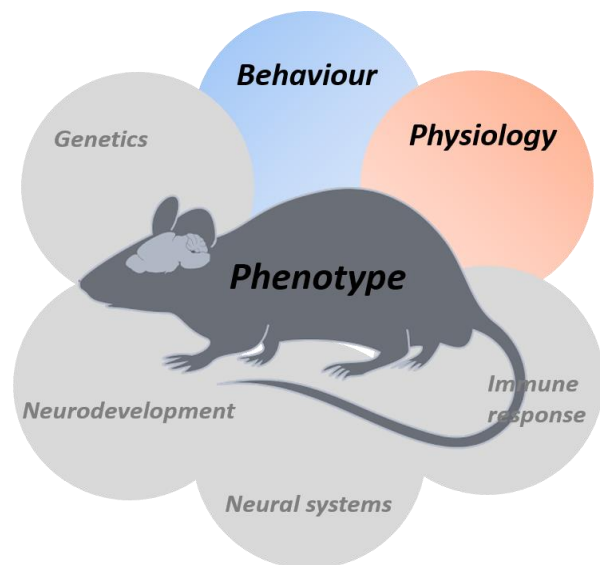


Figure 12. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour and physiology. This section aims at adapting a behavioural paradigm to evaluate DM in a murine model of human chronic distress-related disorders. Gambling tasks for mice rely on food rewards to motivate goal-directed behaviours, however, scientific literature reports aberrant feeding behaviour in the CORT model. The modifications of the gambling task aim at facilitating appetitive and consummatory behaviours in animals.

A. Materials and Methods

Animals: Eighty adult male C57BL/6J mice were used for this study (mean weight (g) \pm SEM = 26.20 \pm 0.26). Half the individuals received CORT, the remaining 40 animals received vehicle. The behavioural evaluation started after seven weeks of treatment. Six mice displaying immediate spatial preference among options (choice proportion different from expected in absence of spatial preference, thus 25% of choices for each of the 4 available options; χ^2 , $p < 0.05$) were discarded from the analyses (VEH, $n=39$; CORT, $n=35$).

Experimental protocol: adapted mouse Gambling Task (AmGT)

Decision-making was evaluated using a fully opaque 4-arm radial maze (*Penlab*, LE760/2) with identical and equidistant arms (length: 37 cm, width: 5.7 cm) and a common central zone used as a start-point. Mice were rewarded with 20 mg grain-based pellets or punished with grain-based pellets previously treated with quinine (180mM quinine hydrochloride, *Sigma-Aldrich*, Schnelldorf, Germany). Quinine pellets are not palatable but edible.

Mice were tested twice daily for five consecutive days, with each daily session comprising 20 choice trials (10 choices in the morning and 10 in the afternoon). At the end of the fifth session mice had chosen 100 times. During each trial, positive and negative reinforcers were allocated to the arms following a probabilistic rule such that visiting arms *A* and *B* was overall *disadvantageous* while visiting arms *C* and *D* was overall *advantageous* (as described by Pittaras et al., 2016). Before the first trial of the first session, mice had 3 min to explore and eat inside the maze (*first habituation period*). From the second session onwards, mice had 2 min to explore the maze before the first trial but no food was provided (*general habituation period*). These habituation periods helped reducing the stressful effect on animals that might be induced through the exposure to the device.

In order to limit the intrasession satiation, specific intended changes were made in relation to the original protocol:

- After habituation, mice were individually placed at the start-point inside an opaque cylindrical structure that prevented visual assessment of the maze by the animals. The cylinder was removed after 5 sec and animals had 1 min to choose an arm, explore it and eat the reinforcer. The choice was considered once the body of the animal except for the tail had completely crossed the gate. An extra-minute was given if the choice was not made in time.
- Two arms gave access to a small reward (1 pellet) in the first choice trial of each half session (trials 1, 11, 21, 31, 41, 51, 61, 71, 81 and 91) and bigger rewards (3–4 pellets) in the other 18 choices of each session with a small probability of presenting a penalty (3–4 quinine pellets, twice in 18 possible choices). *Advantageous arms C* and *D* provided a maximal amount of 50 and 66 rewards per session, respectively. The other two arms, *disadvantageous arms A* and *B* gave access to a bigger reward (2 pellets) during the first trial of each half session, but bigger penalties (4–5 quinine pellets) and a smaller reward-possibility (4–5 pellets, once in 18 possible choices) in the remaining 18 choices of each session. Arm *A* gave access to a maximal amount of 8 rewards per session, arm *B* up to 9 rewards.

Mice were placed in their home cages for 90 s between consecutive trials. The location of advantageous and disadvantageous arms was randomized with different reward and penalty sequences for each animal (see **Figure 13 A,B**). In order to evaluate the adequacy of the proposed modifications on animals' satiation, the proportions of total intake for each condition (CORT and VEH treated animals) were calculated ($\frac{\text{number of eaten rewards}}{\text{number of obtained rewards}} \times 100$) and compared.

B. Results

Minor acute satiation in animals during the adapted mouse Gambling Task

In the ^AmGT, as in the other food rewards- and penalties-based rodent versions of the IGT, animals learn from the outcome of their choices to guide future selections. Such reinforcement learning requires reevaluation of choice alternatives, which depends to a considerable extent on the hedonic impact of the outputs. For the generation of options and DM processing animals therefore

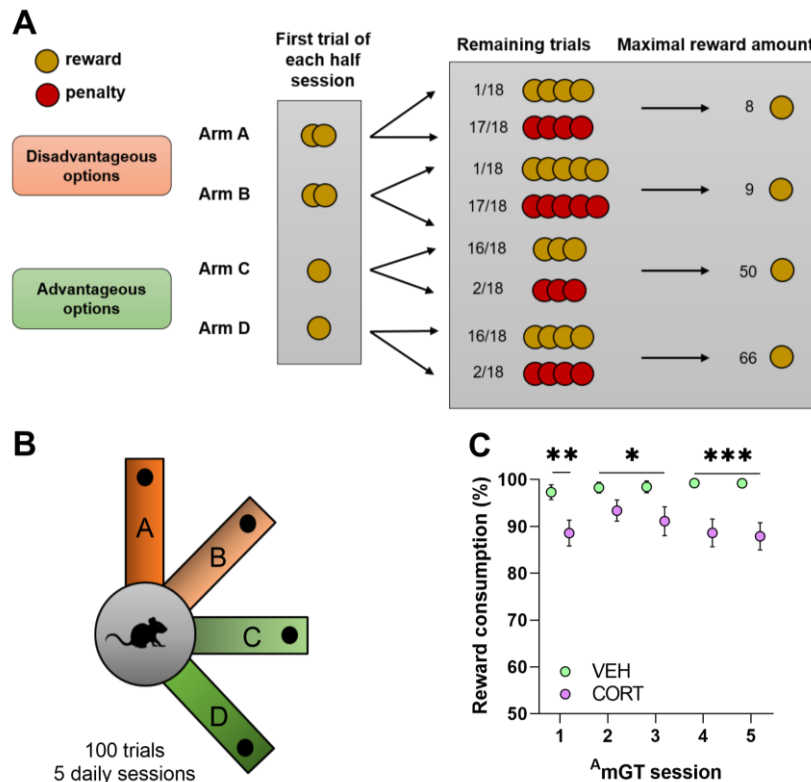


Figure 13. The adapted mouse Gambling Task minimizes intrasession satiation in mice. (A) Schematic representation of the ^AmGT experimental design. Mice were allowed to choose visiting one of the four arms in a radial maze, and to explore and encounter the associated reward or penalty during a minute. They were exposed to consecutively choose 20 times for 5 consecutive days, 10 times in the morning and 10 in the afternoon. Advantageous arms C and D gave access to one food pellet in the first trial of each half session, and then to 3 or 4 pellets (reward) in the other 18 choices. These arms were associated to a small probability of presenting the same amount of quinine pellets (penalty, twice in 18 choices). Disadvantageous arms A and B gave access to 2 food pellets in the first trial of each half session, but 4 or 5 quinine pellets in the other 18 choices. These disadvantageous arms however, were associated with a small probability of presenting the same amount of reward (once in 18 choices). **(B) Schematic representation of the 4-arm radial maze.** The image represent a possible location of the advantageous (green, C and D) and disadvantageous (orange, A and B) arms. The start-point is represented in grey. **(C) Minor satiation in mice during the ^AmGT.** The gambling protocol was adapted from the original of Pittaras et al. 2016, in order to minimize intrasession satiation. The adaptation of the protocol to this effect was essential since significant alterations of appetitive and consummatory behaviours are reported in the animal model selected to study DM-related alterations subsequent to chronic exposure to stress (CORT model). The results on consummatory behaviour during the ^AmGT showed that all animals, independent of the pharmacological condition, consumed the majority of earned rewards in each task session. However, CORT-treated animals ate significantly less than control individuals irrespective of the session (mean percentage of reward consumption (%) ± SEM; sessions 1-5, VEH: 98.51±0.96; CORT: 89.80±2.57; session 1, VEH: 97.32±1.55; CORT: 88.57±2.75; session 2, VEH: 98.29±1.13; CORT: 93.39±2.25; session 3, VEH: 98.46±1.26; CORT: 91.13±3.07; session 4, VEH: 99.24±0.43; CORT: 88.62±2.96; session 5, VEH: 99.23±0.77; CORT: 87.91±2.90) (t-test: *, p<0.05; **, p<0.01; ***, p<0.001).

need to integrate information of the cost and benefits of their choices, which in this particular case means they need to discern between grain-based and quinine-treated pellets. Limiting the satiation effect on animals within the sessions is a promising way to potentiate their appetitive and consummatory behaviour.

Satiation on CORT and VEH-treated animals was evaluated based on their consummatory behaviour. All mice consumed most of the rewards earned during the task, irrespective of the treatment and the session (for details see **Figure 13 C**). The proportion of consumed rewards was substantial in both conditions. As expected, CORT-treated animals ate less than animals from the control group, irrespective of the gambling session [sessions 1-5: $t_{72}=-3.3$, $p<0.01$; session 1: $t_{72}=-2.8$, $p<0.01$; session 2: $t_{72}=-2.0$, $p<0.05$; session 3: $t_{72}=-2.3$, $p<0.05$; session 4: $t_{72}=-3.7$, $p<0.001$; session 5: $t_{72}=-4.0$, $p<0.0001$] (**Figure 13 C**). These results suggest that the modifications of the gambling protocol have limited the intrasession satiation effect on animals, and further evaluation of the translational value of the A mGT is therefore justified.

1.2. Comparative study between human and mice gambling data

Objective: The objective of this section is evaluating the face validity and translational suitability of the A mGT to address DM under uncertainty. For that, behavioural human and mice data were directly compared using common analytical methodology (**Figure 14**).

In the human IGT and its rodent adaptations, optimal decisions leading to long-term benefits rely on the individuals' ability to refrain choosing large immediate rewards and instead to opt for lower but more frequent benefits. The majority of individuals from healthy human and animal populations succeed in developing an advantageous strategy through gambling tasks, but reduced reward responsiveness has also been evidenced in individuals recurrently settling on long-term disadvantageous choices (Bechara et al., 2002, 2000; Bechara and Damasio, 2002; Glicksohn et al., 2007; Pittaras et al., 2016; Rivalan et al., 2009; Steingroever et al., 2013; van Enkhuizen et al., 2014).

Translational research relies on interspecies data correspondence, yet analytical methods for gambling performance evaluation often differ for human and animal data (Bechara et al., 2000; Zeeb and Winstanley, 2013). Interspecies comparisons are in consequence indirect, even if animal gambling tasks are specially created to simulate human DM processes, and the face validity of the rodent tasks is often questioned.

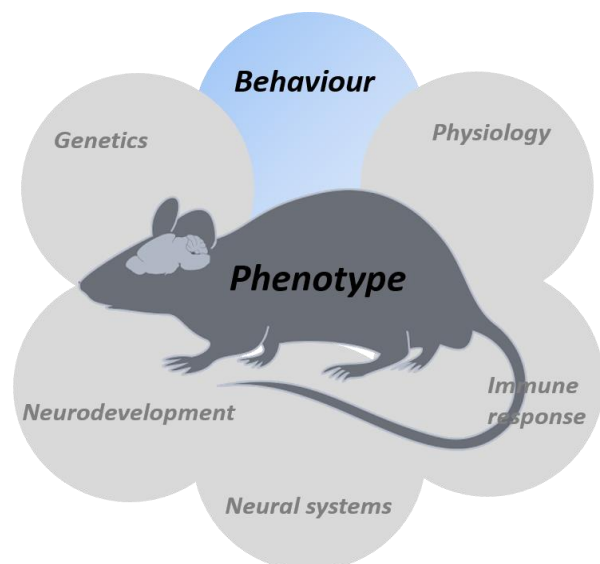


Figure 14. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour. This section aims at evaluating the translational value of a DM paradigm to reproduce human behaviour. The behaviour of humans and mice in physiological conditions during probabilistic value-based gambling tasks will be compared for that purpose.

In view of the relevance of the translational studies for identifying behavioural endophenotypes of pathological conditions, human and mice gambling data are directly compared using identical statistical methods.

A. Materials and Methods

Animals: Forty C57BL/6JRj mice were used for this study (mean weight (g) \pm SEM = 22.2 \pm 0.2).

Human participants: Forty healthy right-handed participants (mean age \pm SEM = 24.7 \pm 5.1; range 19–38) were involved in the study. None reported a previous medical history of psychiatric disorders, substance or alcohol abuse, neurological diseases, traumatic brain injury or stroke, and none reported taking any medication. After the participants received information regarding the task's objective, they gave written informed consent to take part in the study. They were informed that the monetary payment would be proportional to the global gain obtained in the task, but due to ethical considerations and independent to performance, all participants received 85€ at the end of the experiment. PhD D. Gabriel and collaborators from the University Hospital of Besançon (Clinical Psychiatry and CIC-1431, CHRU, Besançon, France) provided the data. The protocol was approved by the Committee of Protection of Persons (CPP-Est-11; authorization given by the General Health Administration (ANSM 2016-A00870-51 and NCT 02862821)).

Experimental protocols:

o Iowa Gambling Task (IGT)

An adapted electronic version of the IGT was used in this study. The protocol was already approved and operational at the beginning of this experiment (Giustiniani et al., 2015) (see **Figure 15**). The aim of the task was to maximise the monetary benefit through successive selections between four apparently identical decks. The differential decks' composition, monetary amounts and scheduled reward-penalties were predetermined identically to match the original IGT (Bechara, 1997; Bechara et al., 1994; Bechara and Damasio, 2005). Disadvantageous decks A^h and B^h yielded high immediate rewards but involved major economic losses on the long-run. Advantageous decks C^h and D^h provided frequent small wins and smaller long-term penalties, resulting in long-term gain. Decks also varied in their penalties' schedule: decks B^h and D^h featured infrequent losses, while decks A^h and C^h featured more frequent losses.

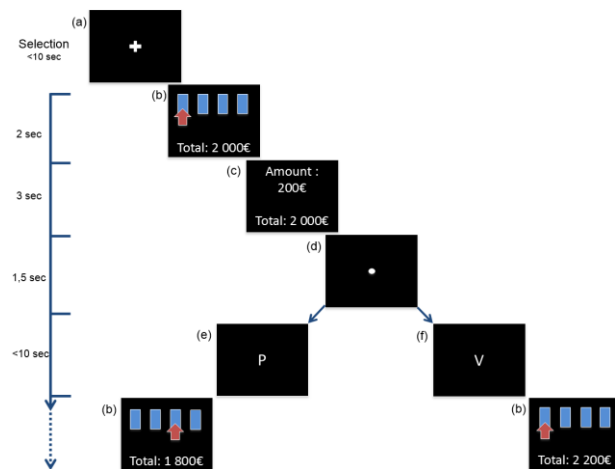


Figure 15. Design of the IGT experiment. (a) At the beginning of the trials, individuals had to fix a cross while making their choice by pressing a key. (b) The decision was followed by a feedback of the deck chosen and the total credit amount. (c) Immediately after, the monetary amount involved in the trial was displayed. (d) A fixation point appeared then in the centre of the screen to focus the sight, followed by a fixation letter announcing the result. Half of the individuals received the information that (e) the letter P meant loss and (f) the letter V meant gain, and the remaining individuals received the opposite information. Image from Giustiniani et al. 2015.

At the beginning of the task, participants had a loan of 2000€. Contrary to most IGT experiments, participants were not provided with any information regarding the presence of advantageous or disadvantageous decks and the number of trials, which avoided a somewhat partial advantage over the animals (Macphail, 1996, but see Rivalan et al., 2011). The number of trials was deliberately increased from 100 to 200, since a longer exploration phase typically relates from the absence of preliminary information (Balodis et al., 2006; Fernie and Tunney, 2006; Glicksohn et al., 2007). This adaptation potentiated strategy definition as reported by previous studies (Buelow et al., 2013; Bull et al., 2015).

- *Adapted mouse Gambling Task* ([^]mGT)

The adapted murine protocol, as described in the *First Chapter, Section 1* was used for this study.

Intra- and interspecies identification and comparison of decisional profiles:

A k-mean clustering method was used to identify interindividual differences within human and mice populations. This statistical method enables the automatic identification of the optimum partition of objects into a specific number of clusters, minimising the intracluster and maximising the intercluster variances (Timmerman et al., 2013). The k-mean method was chosen since it had already been used for interindividual behavioural assessment in mouse gambling tasks (Pittaras et al., 2016), but not in IGT studies. In accordance with Pittaras and collaborators, cluster identification was conducted using the mean percentage of advantageous choices made on the final 30% of the task (referred here as “endpoint performance”: last 60 and 30 choices, for humans and mice respectively), when performance was highly stable [advantageous choices vs chance level, humans: $t_{39} > 4.5$, $p < 0.01$; mice: $t_{39} > 2.6$, $p < 0.01$].

Intra- and interspecies comparison of behavioural dimensions:

For developing a decisional optimal strategy, i.e., maximizing the benefits and reducing the losses to favourably orient future choices, humans and rodents rely upon cognitive control mechanisms critical for optimisation of goal-directed behaviour. Choice strategy characterizing decisional profiles was thus assessed based on the following four behavioural dimensions:

- *Rigidity/stickiness*. Neurobiology literature commonly refers to rigidity as a tendency to persevere, or even as the inability to change habits or to modify behaviours once developed (Morris and Mansell, 2018; Stewin, 1983). Despite the fact that behavioural rigidity or stickiness is mostly understood as a synonym of task-switching difficulties, settling a rigid choice pattern in gambling tasks can be a successful way to maximize benefits during the exploitation phase. Here, rigidity/stickiness scores (%) were calculated in terms of the frequency that individuals chose the same option, without taking in consideration the switches they made (Pittaras et al., 2016).
- *Flexibility*. Behavioural flexibility can be understood as the ability of individuals to respond and adjust their behaviour to environmental stimuli (Coppens et al., 2010), i.e., their capacity to change their behaviour when they detect that initial responding patterns are no longer effective for a specific situation (Kashdan and Rottenberg, 2010). Here, flexibility scores (%) were measured in terms of the proportion of switches from one option to another, without

taking in consideration their direction (from advantageous to disadvantageous options and contrariwise) (Pittaras et al., 2016).

- *Lose-shift*. This behavioural dimension allows approaching negative outcome appreciation and refers to the rate at which individuals change their selection on the next trial when they are not rewarded. Lose-shift scores (%) were calculated as the proportion of switches following monetary losses or quinine pellets delivery.
- *Win-stay*. Complementary to lose-shift scores, win-stay scores (%) allow approaching hedonic appreciation and it refers to the rate at which individuals choose the same option on the next trial after being rewarded.

Each behavioural dimension was calculated at the beginning (first 40% of the task: first 80 and 40 choices in humans and mice, respectively) and at the end of the experiment (last 40% of the task: last 80 and 40 choices in humans and mice, respectively). These blocks will be referred here as “time course”.

B. Results

The results have been the objective of the scientific publication “*Modelling decision-making under uncertainty: a direct comparison study between human and mouse gambling data*”, ENPP, 2020, that can be found as [Annex III](#).

1. Overall gambling performance: mice learn faster than humans

Using a Bayesian analysis, different learning rates were revealed for mice and humans. In the chosen gambling paradigm, mice learnt faster than humans: after 100 trials, mice had better overall gambling performance [CI 95 % = 1.191 to 1.277]. However, when mice performance was compared to the last 100 human trials, both species showed similar performances [CI 95 % = 0.956 to 1.016]. These results were taken into consideration, but in order to circumvent variations in the gambling tasks’ designs and to directly compare the data sets, performance was analysed as a function of task progression. Mice and human trials were split into five blocks representing 20% of the total number of trials, i.e., 20 trials in a mice block and 40 trials in a human block (referred here as “blocks”).

Humans. The comparison of the blocks showed that humans learnt the task contingencies (performance different from 50% of advantageous choices) after completion of 40% of the task, presenting reward responsiveness from the third block onwards [blocks 1&2: $t_{39} < 2.5$, all $p_s > 0.08$; blocks 3&4: $t_{39} > 3.6$, all $p_s < 0.01$; block 5: $t_{39} = 4.9$, $p = 0.0001$]. Their performance improved gradually over time, evidencing a significant learning effect [main effect of block: $F_{4,156} = 15.4$, $p = 0.0000$] from the third block and till the end of the task [block 1 vs blocks 3-5: all $p_s < 0.0001$, post-hoc] (**Figure 16 A,C**).

Mice. Reward responsiveness was evidenced earlier in mice, since they oriented towards the favourable choices already after the first 20% of the task [block 1: $t_{39} = 1.4$, $p = 0.16$; blocks 2-5: $t_{39} > 5.7$, all $p_s = 0.0000$]. Their performance progressively improved over time [block: $F_{4,195} = 11.9$, $p = 0.0000$] from the second block and till the end of the task [block 1 vs block 2: $p < 0.05$; block 1 vs block 3: $p < 0.001$; block 1 vs blocks 4&5: all $p_s = 0.0000$, post-hoc]. In their respective gambling tasks, mice and human did not display a significant difference in the proportion of advantageous choices they made [main effect

of species: $F_{1,78}=3.1$, $p=0.80$], and they performed similarly along the task progression [species x blocks interaction: $F_{4,312}=0.06$, $p=0.99$]. See **Figure 16 B,C**.

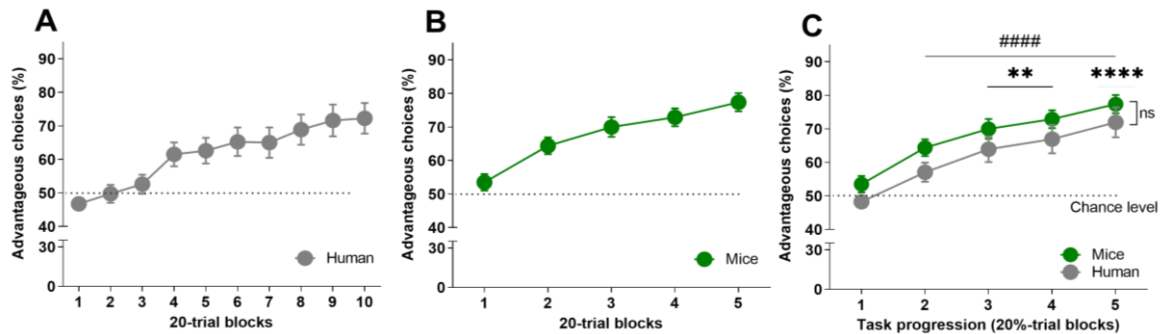


Figure 16. Gambling performance in human and mice populations. (A-B) Performances expressed as percentage of advantageous choices during task progression (blocks of 20 trials). After 100 trials, mice performed better than humans (Bayesian: CI 95%=1.191 to 1.277), but final performances were similar (Bayesian: CI 95%=0.956 to 1.016). **(C)** No significant (ns) differences between species when comparing performance as a function of task progression (20%-trial blocks). Human performance differed from chance level from the third block onwards, while mice performance was different from the second block (t-test: mice: ####, $p<0.0001$; human: **, $p<0.01$; ****, $p<0.0001$).

2. Comparable decisional profiles with similar proportions among the species

The intraspecies variability was studied by using a common analytical method that allows direct comparisons between human and mice data. Individuals were classified in three different decisional profiles following a k-mean clustering stratification from their endpoint performance: (1) good decision-makers (DMs), (2) intermediate DMs and (3) poor DMs.

Good DMs. Individuals from this profile displayed the optimal strategy, maximizing their benefits along the task. They represent 42.5% of the human population (mean (%) endpoint performance \pm SEM: 97.4 ± 0.8) and 40.0% of the mice population (91.3 ± 1.5) See **Figure 17 A₁,B₁**.

Poor DMs. These individuals did not develop a significant preference for neither the advantageous, nor the disadvantageous options, and scored around chance level along the entire gambling tasks. They represent 25.0% of humans (34.3 ± 5.2) and 22.5% of mice (54.1 ± 2.5). See **Figure 17 A₁,B₁**.

Intermediate DMs. This third decisional profile corresponds to individuals who developed a preference towards the advantageous options, though failed in identifying the optimal strategy. They represent 32.5% of humans (64.5 ± 2.4) and 37.5% of mice (73.8 ± 1.0). Subgroups' proportions in humans and mice were compared so as to evidence differences in decisional strategies among species. Remarkably, the proportions of individuals characterizing good, intermediate and poor DMs profiles are consistent between humans and mice [$\chi^2=0.011$, $p=0.99$; confirmed by Bayesian analysis]. See **Figure 17 A₁,B₁**.

Humans. The three DM subgroups learnt at different rates [blocks x cluster interaction: $F_{8,148}=16.2$, $p=0.0000$]. After completion of 40% of the task, good DMs performed above the chance level [blocks 1&2: $Z<2.6$, all $ps>0.05$; blocks 3-5: $Z>3.5$, all $ps<0.01$], while intermediate and poor DMs differed from 50% of advantageous choices only in the last 20% of the task [blocks 1-4: $Z<2.3$, all $ps>0.05$; intermediate, block 5: $Z=2.9$, $p<0.05$; poor, block 5: $Z=2.8$, $p<0.05$]. Good DMs performed

differently than poor DMs from the second block of the task onwards [UMW (Z), block 1: $Z > -1$, $p < 0.05$; block 2: $Z = -2.5$, $p < 0.05$; blocks 3-5: $Z < -4.0$, all $ps < 0.0001$], and differently than intermediate DMs in the last 60% of the task [blocks 1&2: $Z > -1.9$, all $ps > 0.05$; block 3: $Z = -3.7$, $p < 0.001$; blocks 4&5: $Z < -4.3$, all $ps < 0.0000$]. Intermediate and poor DMs performed differently from the third block onwards [blocks 1&2: $Z < 1.3$, all $ps > 0.05$; blocks 3&4: $Z > 2.5$, all $ps < 0.05$; block 5: $Z = 4.0$, $p < 0.00001$]. See **Figure 17 A₂**.

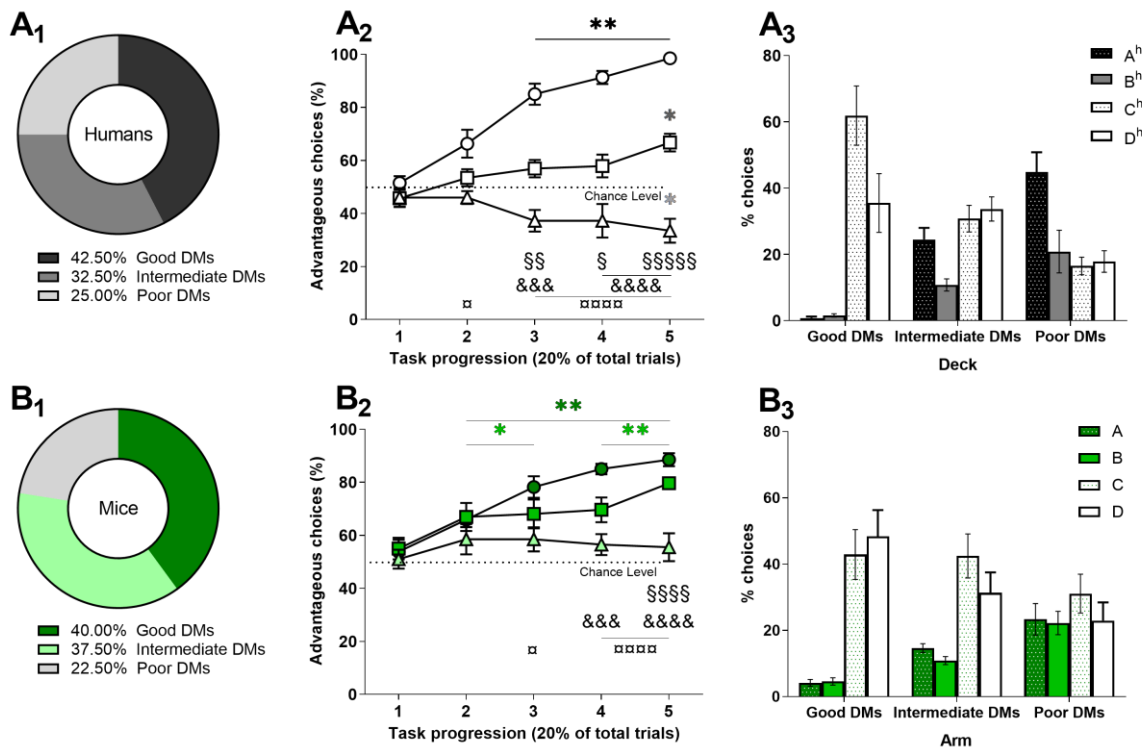


Figure 17. Comparable categories of good, intermediate and poor DMs with similar proportions among species. Proportions of (A₁) human and (B₁) mice individuals in good, intermediate and poor DM subpopulations (χ^2 , $p = 0.99$). Gambling performance in (A₂) humans and (B₂) mice during task progression. Group performance different from chance level (Wilcoxon: *, $p < 0.05$; **, $p < 0.01$). Clusters differences (good-circles; intermediate-squares; poor-triangles), Mann Whitney U: good vs intermediate: &, $p < 0.05$; &&, $p < 0.01$; &&&, $p < 0.001$; &&&&, $p < 0.0001$; good vs poor: §, intermediate vs poor: §. Mean percentage of choices for each option in the last 30% trials in (A₃) humans and (B₃) mice.

Mice. Like humans, the three mice subgroups learnt at different rates [blocks x cluster interaction: $F_{8,185} = 5.3$, $p = 0.0000$]. Good and intermediate DMs performed above the chance level after 20% of the task was completed [good DMs, block 1: $Z = 0.2$, $p = 1$; blocks 2-5: $Z > 3.2$, all $ps < 0.01$; intermediate DMs, block 1: $Z = 1.8$, $p = 0.39$; blocks 2&3: $Z > 2.6$, all $ps < 0.05$; blocks 4&5: $Z > 3.1$, all $ps < 0.01$], while poor DMs' performance never differed from 50% of advantageous choices [blocks 1-5: $Z < 1.4$, all $ps > 0.86$]. Good DMs performed differently than poor DMs from the third block onwards [block 1: $Z = -0.7$, $p = 1$; block 2: $Z = 1.7$, $p = 0.48$; block 3: $Z = 3.0$, $p < 0.05$; blocks 4&5: $Z > 3.9$, $p < 0.0001$], and differently than intermediate DMs later on [blocks 1-3: $Z = -2.0$, all $ps > 0.20$; block 4: $Z = -3.8$, $p < 0.001$; block 5: $Z = -4.0$, $p < 0.0001$]. Intermediate and poor DMs performed differently only during the last 20% of the task [blocks 1-4: $Z < 2.0$, all $ps > 0.24$; block 5: $Z = 3.9$, $p < 0.0001$]. See **Figure 17 B₂**.

Further comparisons were conducted in order to study how similarly subgroups of both species were behaving. Few differences were evidenced and they suggest a differential dispersion of

population's data (for further details see [Annex I: Supplementary results, Distribution of human and mice gambling data](#)). Among the intermediate DM individuals, mice made more advantageous choices than humans [$Z=-2.7$, $p<0.05$], while humans from the good DM subgroup performed better than mice of the same subgroup [$Z=3.1$, $p<0.01$]. Otherwise, humans made globally worse decisions than mice [$Z=-3.0$, $p<0.01$]. Apart from these differences, mice from the intermediate DM subgroup developed a weaker preference for the most advantageous option (D) than good DMs did [$p<0.05$], whilst humans from the same profile chose the best deck (D^h) as frequently as good DMs did [$p=0.99$] See [Figure 17 A₃, B₃](#). All statistical details of the human and mice subgroups' preferences can be found in [Annex I: Supplementary results, Gambling preferences in human and mice DM subgroups](#).

3. Relationship between gambling performance and the behavioural dimensions

To infer strategies mediating gambling performance, the dynamics of rigidity/stickiness, flexibility, lose-shift and win-stay behaviours along the task were examined:

a. Correlations between endpoint performance and behavioural dimensions ([Figure 18](#))

Humans. On one side, stickiness scores significantly correlate with endpoint IGT performance at the beginning [$r=0.428$, $p<0.05$] and at the end of the task [$r=0.489$, $p<0.01$]. On the other side, flexibility scores showed a negative correlation with endpoint performance at the beginning of the task

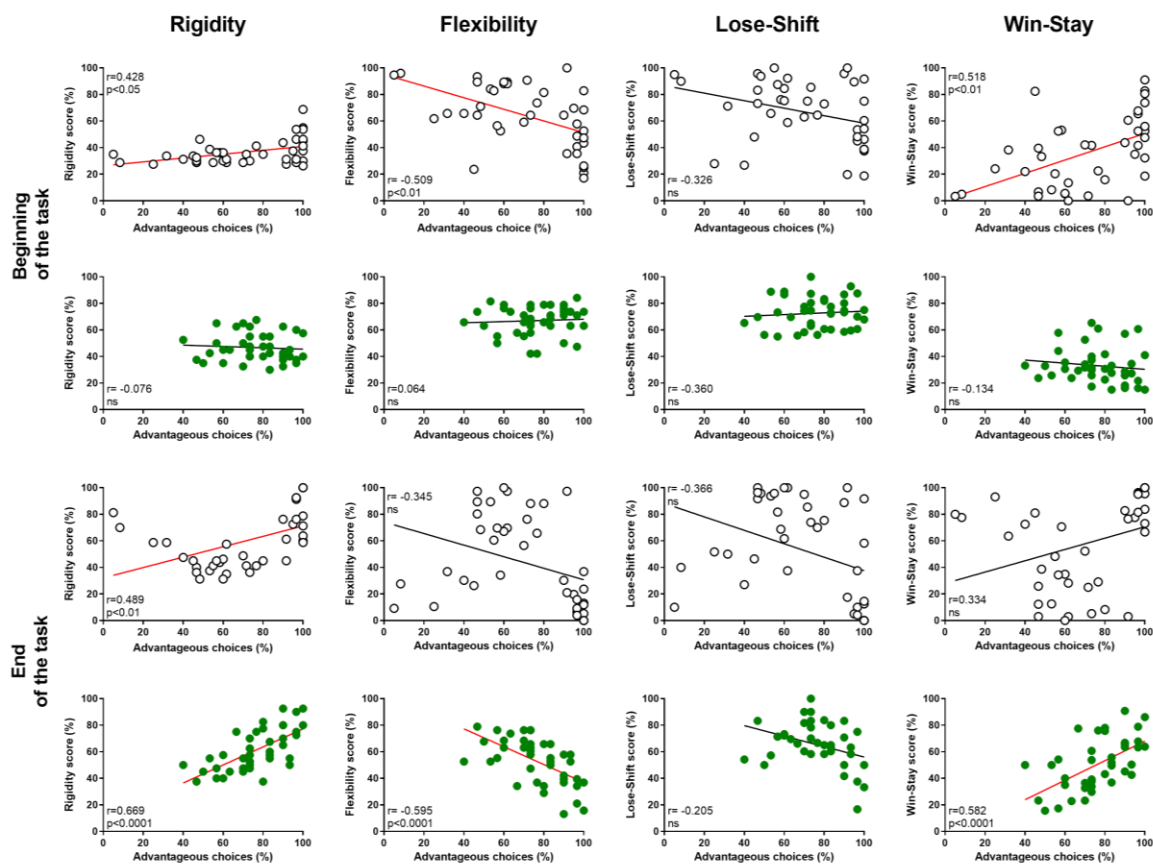


Figure 18. Correlations between endpoint gambling performance and behavioural dimensions of DM. Correlations between human IGT (white dots) or mice A_mGT (green dots) endpoint performances and behavioural dimensions of DM (from left to right: rigidity/stickiness, flexibility, lose-shift and win-stay scores) at the beginning (upper part of the figure) and at the end of the tasks (lower part of the figure). Significant correlations are red-coloured and non-significant correlations are black-coloured.

[$r=-0.509$, $p<0.01$], that mitigated at the end of the task [$r=-0.345$, $p=0.23$]. No significant correlation was found between lose-shift scores and endpoint performance, irrespective of the moment of the experiment [beginning: $r=-0.326$, end: $r=-0.366$, all $p>0.10$]. The win-stay choices however, significantly correlate with endpoint performance at the beginning of the task [beginning: $r=0.518$, $p<0.01$; end: $r=0.334$, $p=0.28$].

Mice. Like in humans, stickiness scores significantly correlate with endpoint performance at the end of the task [$r=0.669$, $p<0.0001$], but not at the beginning [$r=-0.076$, $p=1$]. On the contrary, flexibility scores negatively correlate with endpoint performance at the end [$r=-0.595$, $p<0.0001$], but not at the beginning of the task [$r=0.064$, $p=1$]. Like in the human population, no significant correlation was found between lose-shift choices irrespective of the moment of the experiment [beginning: $r=-0.360$, end: $r=-0.205$, all $p>0.10$]. However, win-stay choices at the end of the task correlate with endpoint Δ mGT performance [beginning: $r=-0.134$, $p=1$; end: $r=0.582$, $p<0.0001$].

b. Dynamics of the behavioural dimensions along the gambling tasks

A significant common effect of the time course was found for flexibility [main effect time course: $F_{1,78}=48.4$, $p=0.0000$], lose-shift [time course: $F_{1,78}=15.8$, $p<0.001$] and win-stay scores [time course: $F_{1,78}=51.9$, $p=0.0000$]. These three dimensions did not differ between humans and mice [main effect of species: flexibility, $F_{1,78}=1.9$; lose-shift, $F_{1,78}=4.0$; win-stay, $F_{1,78}=1.2$; all $p>0.19$] contrary to the stickiness scores [species x time course interaction: $F_{1,78}=4.6$, $p<0.05$]. Humans and mice more frequently chose the same option as the task progressed [humans: $p=0.0000$; mice: $p<0.0001$, post-hoc], but humans were significantly less rigid than mice at the beginning of the experiment [beginning: $p<0.05$; end: $p=1$, post-hoc]. See **Figure 19**.

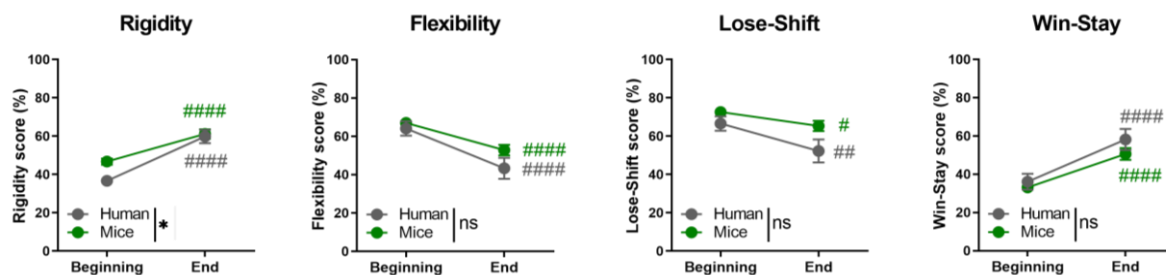


Figure 19. Dynamics of the behavioural dimensions during task progression. Similar behavioural strategies in human and mice populations through the gambling tasks. (From left to right) Significant progression of the rigidity/stickiness, flexibility, lose-shift and win-stay scores for both populations, between the beginning and the end of the task (RM-ANOVA: #, $p<0.05$; ##, $p<0.01$; ###, $p<0.001$; ####, $p<0.0001$). Mice were significantly more rigid in their choices than humans and the beginning of the task, but behaved alike at the end of the task (RM-ANOVA: *, $p<0.05$; ns, not significant).

At the subgroup level (see **Figure 20** and **Table 3** for statistical details), the interspecies comparison revealed that humans from the intermediate and poor DM profiles were significantly less rigid than the corresponding mice at the beginning of the task. However, this was not the case for good DMs. At the end of the task, only humans from the intermediate DM subgroup still differed from mice in this behavioural dimension. Poor DMs from both species were equally flexible irrespective of the moment of the task. Mice from the good DM subgroup behaved always more flexibly than humans of the same group. However, mice from the intermediate DM group were always less flexible than the correspondent humans. For lose-shift scores, only good DMs behaved differently at the beginning of the task, with mice changing option after a penalty more often than humans. Win-stay scores were

different between species at the beginning of the task in the intermediate and good DM subgroups, but not for poor DM individuals. At the end of the task, only mice from the good DM subgroup were more prone to switch from an option after a penalty than humans. However, humans from the good DM subgroup chose the same option after being rewarded more frequently than mice, oppositely to the intermediate DM individuals. No differences were found for the other subgroups.

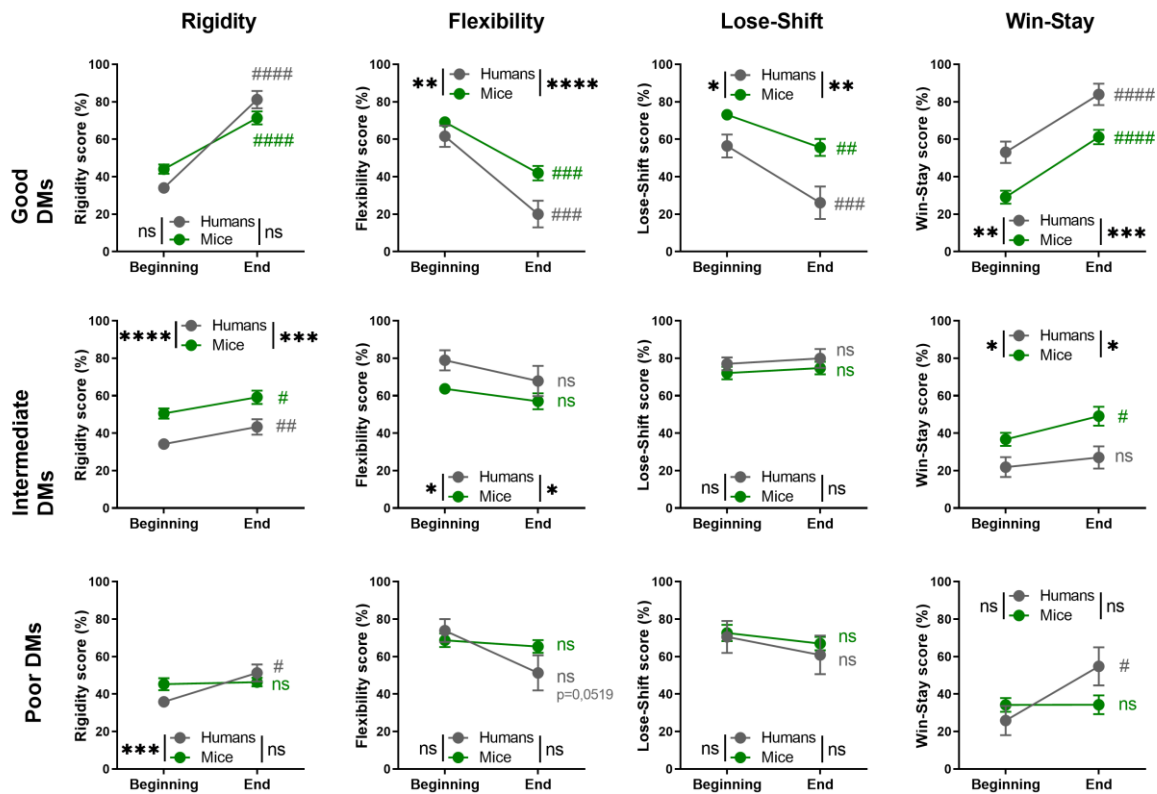


Figure 20. Differential dynamics of the behavioural dimensions during task progression among decisional profiles. Progression of the rigidity/stickiness, flexibility, lose-shift and win-stay scores for human and mice subpopulations of good, intermediate and poor DMs (Wilcoxon: #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$; ####, $p < 0.0001$; ns, not significant). The comparison of behavioural scores between species at the beginning and at the end of the tasks revealed significant differences between the three decisional profiles (Mann-Whitney U: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; ns, not significant). See **Table 3**.

1.4. Summary and discussion of the results

In this first chapter, specific modifications were implemented in a murine IGT version with the intention of limiting the impact of intrasession acute satiation on animals' behaviour, and therefore facilitating their appetitive behaviour. Then, the working hypothesis assessed that animals' motivation to complete the task could be potentiated and evaluated from their consummatory behaviour. This aspect is of fundamental importance for the following experiments addressing decisional processing in the CORT model, since chronic CORT administration has been demonstrated to impact feeding habits and related behaviours in mice (David et al., 2009; Dieterich et al., 2019). In accordance with the working hypothesis, the modifications of the murine gambling protocol $-^A mGT-$ ensured significant

| Behavioural dimension | Time course | Humans vs Mice comparison | | |
|-----------------------|-------------------------------|---------------------------|--------------------|--------------|
| | | DM subgroup | Statistics UMW (Z) | p value |
| Rigidity/Stickness | Beginning (first 40% task) | Good DMs | -0.8 | 0.44 |
| | | Intermediate DMs | -4.01 | <0.0000 **** |
| | | Poor DMs | -3.1 | <0.001 *** |
| | End (last 40% task) | Good DMs | 1.3 | 0.19 |
| | | Intermediate DMs | -3.5 | <0.001 *** |
| | | Poor DMs | 0.2 | 0.84 |
| Flexibility | Beginning (first 40% task) | Good DMs | -3.0 | <0.01 ** |
| | | Intermediate DMs | 2.3 | <0.05 * |
| | | Poor DMs | 0.53 | 0.60 |
| | End (last 40% task) | Good DMs | -3.8 | <0.0001 **** |
| | | Intermediate DMs | 2.2 | <0.05 * |
| | | Poor DMs | -0.98 | 0.32 |
| Lose-shift | Beginning (first 40% task) | Good DMs | -2.2 | <0.05 * |
| | | Intermediate DMs | 1.2 | 0.24 |
| | | Poor DMs | 0.5 | 0.60 |
| | End (last 40% task) | Good DMs | -2.8 | <0.01 ** |
| | | Intermediate DMs | 1.2 | 0.22 |
| | | Poor DMs | -0.6 | 0.55 |
| Win-stay | Beginning (first 40% task) | Good DMs | 2.6 | <0.01 ** |
| | | Intermediate DMs | -2.1 | <0.05 * |
| | | Poor DMs | -0.0 | 0.97 |
| | End (last 40% task) | Good DMs | 3.3 | <0.001 *** |
| | | Intermediate DMs | -2.1 | <0.05 * |
| | | Poor DMs | 1.1 | 0.28 |

Table 3. Statistical details of the comparisons of time-dependent behavioural dimensions for each decisional profile between humans and mice. Data related to Figure 20.

relative food-intake, thus limiting intrasession satiation in both control and CORT-treated animals. These results therefore indicate that the ^AmGT opens a window of opportunity to study value-based DM under uncertainty in physiological and pathological conditions.

The translational suitability and validity of the ^AmGT was evaluated. The results evidenced that for the selected gambling tasks, mice and humans perform similarly, with closely corresponding performance curves when compared as a function of task progression. Both species developed a long-term advantageous strategy and reached equivalent endpoint performances despite the design differences. Nevertheless, reward responsiveness appeared earlier in mice than in humans, a fact that can be attributable to the rewards' nature and that is considered a major limitation for rodent versions of gambling tasks. In animal tasks, inedible or unpleasant food is frequently used to model monetary losses, but food is a primary reinforcer, whilst money is a secondary reinforcer as its value depends on the individual and its situation. Another possible explanation is the different task schedule: splitting the murin task in daily sessions might have offered the individuals the opportunity to “re-calibrate” their emotional state with regard to the appreciation of gains and penalties, and to consolidate their knowledge, at least to certain extent, before each new gambling session. That might have given an advantage to mice compared to humans, which gambled in a one single-session. In any case, the good alignment of the human and mice gambling performance is highly relevant for the present study, especially considering the difficulty in controlling factors influencing the individuals' motivational state, such as hunger and satiation in animals (Brevers et al., 2013; van den Bos et al., 2014), or interest in money in humans.

To further substantiate the face validity of the $\hat{m}GT$, the interindividual variability in both species was studied using a common k-mean clustering approach applied to their endpoint performance. The results showed that when clustered into three different decisional profiles, subgroups' proportions of individuals were similar in both species, even if data dispersion was more pronounced in the human population. Consequently, the similarity between the subgroups' proportions between humans and mice strengthens the face validity of the animal model.

Finally, overall gambling performance in humans and mice apparently rely on comparable, but not identical decisional strategies. In fact, both humans and mice went through an exploration phase before allocating their choice towards the advantageous options (exploitation phase). They rigidified their choice behaviour as the task progressed, i.e., as the task contingencies were inferred. This took place alongside with a decrease in their choice flexibility that is indicative for all options being sufficiently sampled. With the task's progression, individuals fostered their behaviour when they were rewarded at the same time that they learnt to better cope with penalties. Specifically, the behavioural dynamics of good DMs was similar for both species, whereas individuals from the intermediate and poor decisional categories behaved slightly different. Although the limited behavioural domains studied here preclude in depth characterisation of the relationship between reward and penalty appreciation, these apparent discrepancies do not compromise the validity of the model. Overall, these results highlight similar patterns of choice behaviours across species for the selected tasks and support good face validity of the $\hat{m}GT$, along with the working hypothesis. Henceforth, the findings here described endorse the translational value of the animal model to address human pathology, and here specifically, to study the consequences of chronic exposure to GC on DM under uncertainty.

SECOND CHAPTER – Decision-making under enduring distress: identification and characterisation of behavioural changes

2.1. Pharmacodynamical validation: emergence of negative valence behaviours

Objective: The first objective of this chapter is to establish the chronic CORT paradigm in the laboratory to model the emergence and maintenance of chronic distress-induced phenotypes (**Figure 21**).

The emotional phenotype of mice resulting from chronic CORT administration has been extensively documented (Darcet et al., 2014; David et al., 2009; Dieterich et al., 2020, 2019; Gourley et al., 2008; Gourley and Taylor, 2009). Corticosterone-treated animals exhibit a behavioural spectrum reminiscent to human anxiety-depression symptomatology, including hyponeophagia and overappreciation of negative outcomes. They also show physiological adjustments such as the deterioration of the coat state, which has been interpreted as a consequence of poor grooming behaviour. Accordingly, these data support the use of chronic CORT administration as a tractable mean to address the effects of human emotional disturbances concurrent with either exo- or endogenous hypercortisolaemia. Since the use of animal models is of utmost importance to provide advances in basic clinical research on chronic distress-related disorders, a systematic evaluation of their various validities is mandatory.

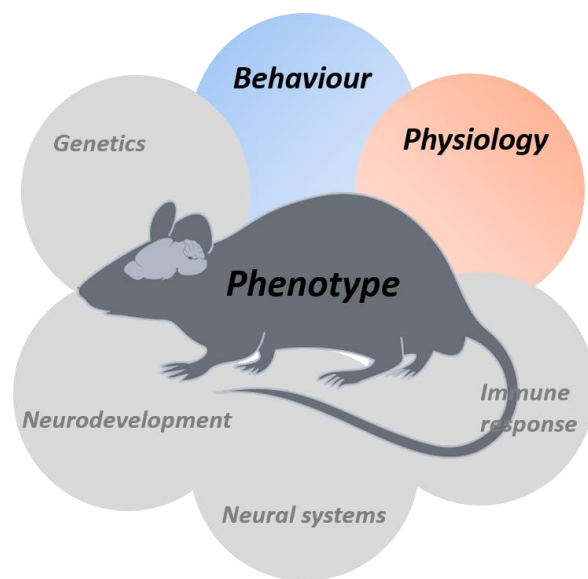


Figure 21. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour and physiology. This section aims at evaluating the efficiency of the CORT paradigm to provoke the emergence of negative valence behaviours reminiscent to human chronic distress-related symptomatology. For that, a set of physiological and behavioural evaluations will be performed in animals chronically treated with CORT. The characterized phenotype will be compared with the general phenotype of a healthy, non-treated mice population.

A. Materials and Methods

Animals: Sixty-one *C57BL/6JRj* mice and thirty-six *CRF-cre x Ai9* mice were CORT-treated during this doctoral study (CORT groups). In parallel, sixty-one *C57BL/6JRj* mice and twenty-nine *CRF-cre x Ai9* mice received vehicle in the drinking water (VEH groups).

The comparison of physiological and behavioural measurements between different mouse strains allows the detailed characterisation of CORT-induced alterations for ulterior experimental implementation. In the case that sufficient scientific evidence of grounded results exists, replications

of entire experiments are not recommended for ethical reasons. However, differences between mouse strains of shared genetic background have been reported (see for instance Dedic et al., 2018). Hence, the selection of the best tools for the evaluation of the treatment efficacy is an important step in projects comprising different animal strains, as it is the present case.

Experimental protocols:

- *CORT Enzyme-Linked ImmunoSorbent Assay (ELISA)*

To objectively the steady-state CORT concentration in mice plasma as an internal control of the treatment's efficacy, a DetectX Costicosterone Immunoassay kit (DetectX®, *Arbor Assays™*, Michigan, USA) was used.

Final trunk blood samples were collected from *C57BL/6JRj* mice after 12-13 weeks of differential treatment (VEH, n=40; CORT, n=40). In order to measure the homeostatic stress reactivity of the HPA axis, submandibular blood samples were also collected from a subset of mice (VEH, n=10; CORT, n=9) following a gentle restrain stress (Benedetti et al., 2012) directly before sacrifice. Samples were centrifuged at 2100 g for 15 min at 20 °C, and plasma was collected and stored at -80 °C until assayed.

From a provided CORT stock solution, standards were prepared to generate an assay curve for the samples to be read off (range of CORT concentration: from 10.000 to 78.128 pg/mL). Plasma samples for the ELISA were first left to warm up to room temperature and then treated with a dissociation agent for at least 5 min. Samples were then diluted with an assay buffer (1:100). Diluted and standards samples were pipetted into the plate's wells in duplicate before adding first, the CORT conjugated and second, the CORT antibody. To ensure adequate mixing of the reagents, the plate was sealed and gently shaken for 1 hour at room temperature. Surplus were discarded and wells were washed with wash-buffer before adding the substrate. After 30 min of incubation (without shaking) at room temperature, the immunoreaction was stopped. Finally, the absorbance generated from each well was determined using a plate reader at 450 nm.

- *Fur Coat State (FCS) evaluation*

The state of the fur coat is frequently degraded in rodents subjected to different stress procedures, especially when stress was sustained. Consequently, it has been interpreted as a result of poor frequency and extent of their grooming behaviour, i.e., their self-oriented practice of cleaning, consequence of which the state of the coat deteriorates and becomes bristled and grubby (Nollet et al., 2013). Scientific evidence has also reported adverse effects of chronic exposure to exogenous GCs on epidermal homeostasis (Pérez, 2011) and hair growth initiation (Stenn et al., 1993).

The coat's state was evaluated weekly from the beginning of the differential treatment. For this, seven body regions were rated (head, neck, abdomen, front legs, hind legs and tail) and a score between 0 and 1 was assigned to each one as described by Nollet et al., 2013 (0: good state; 0.5: coat moderately degraded; 1: bad state). A final score between 0 and 7 was calculated adding all scores.

- **Novelty Suppressed Feeding (NSF) test**

The NSF test is a paradigm evaluating hyponeophagia in rodents, i.e., the inhibition of feeding produced by a novel environment. During the test, animals face a conflict between two main motivations: the drive to eat and the fear of a novel environment where they have to expose themselves in order to eat. This test has been proven useful to assess anxiety-related behaviour in rodents and to have good predictive validity (David et al., 2009). Animals, which were under food restriction, were individually placed in an opaque cylindrical open-field apparatus (diameter: 47 cm; height: 60 cm), in the centre of which two grain-based pellets were located on a white piece of filter-paper (diameter: 10 cm). The latency of the animals to start eating, i.e., animals sitting on their haunches and biting the pellet with the help of their forepaws, was recorded and used as an index of anxiety-like behaviour since it is sensitive to classic anxiolytic drugs (Santarelli et al., 2001) (**Figure 22**). The test finished immediately after animals started to eat or when a maximum time of 15 min elapsed. Hereafter, animals were transferred to an individual home cage where food was available, and the total amount of food consumed during 5 min was measured. Other studies have used this parameter as a control measure for appetite change. Afterwards, animals were transferred to new home cages.

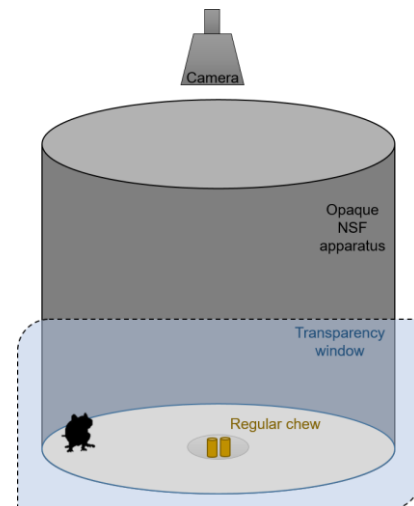


Figure 22. Novelty Suppressed Feeding test. Food deprived animals placed in a novel environment confront a conflict between start eating from the available food and the fear of expose themselves. Anxiety levels increase the latency of animals to start eating.

- **Forced Swim Test (FST)**

The FST is a behavioural paradigm originally developed by Porsolt and his colleagues (Porsolt et al., 1978) to screen the potential anti-depressant properties of drugs. It is based on the observation that when rodents are immersed in a recipient full of water with no escape, they display episodes of immobile floating after initial attempts to escape. Episodes of immobility progressively increase in frequency and duration. Immobility in the FST is interpreted as passive coping, i.e., a passive behavioural strategy to deal with difficulties, in line with the interpretation of de Kloet and collaborators (de Kloet and Molendijk, 2016).

Mice were individually placed in a beaker (width: 26 cm; depth: 18 cm) filled with warm water (31.5 ± 0.5 °C) and left to swim for 6 min (**Figure 23**). When the time elapsed, mice were removed from the water, dried in an individual cage next to a source of heat and placed back in their home cages. The overall time of immobility, as well as the first immobility latency (i.e., the time from the placement in the beaker to the first immobility episode) were recorded. Animals were considered immobile when they floated even if they accompanied the floating with gentle movements of one of their limbs or their tail.

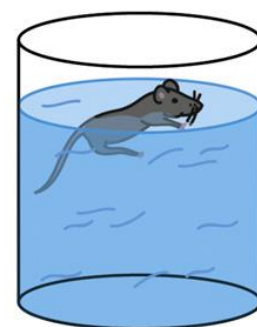


Figure 23. Forced Swim Test. Animals are placed in a beaker filled with water. During 6 min, the time spent in escape-directed behaviours and passive floating are registered as measures of depressive-like behaviour. Image from Franklin et al., 2012.

B. Results

1. Higher steady-state plasma hormone concentration and blunted HPA axis reactivity to acute stressor after chronic CORT administration

Plasma CORT concentrations after 12-13 weeks were measured as a control of the treatment's efficacy in *C57BL/6JRj* mice. As expected, terminal blood sample analyses showed significantly higher basal plasma CORT levels in CORT-treated animals (mean CORT concentration (ng/mL) \pm SEM; VEH: 28.18 \pm 4.97; CORT: 79.02 \pm 12.41) [$t_{78}=-3.8$, $p<0.001$]. They also showed a blunted HPA axis reactivity to a novel acute stress (restrain stress) (VEH: 224.02 \pm 35.21; CORT: 70.18 \pm 29.97) [post-stress plasma CORT concentration, UMW: $Z=2.9$, $p<0.01$], confirming the treatment's efficacy (**Figure 24**).

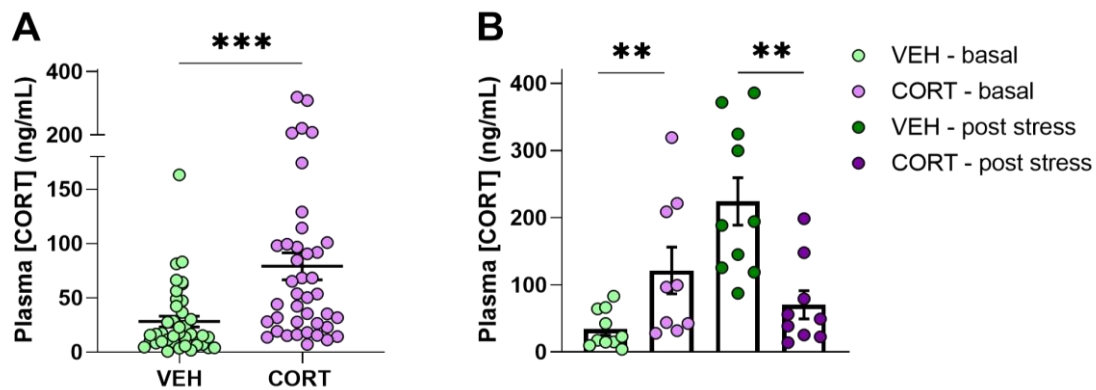


Figure 24. Higher steady-state plasma hormone concentration and blunted HPA axis reactivity to an acute stressor in mice chronically treated with CORT. (A) Basal plasma CORT concentrations of terminal blood samples (12-13 weeks of differential treatment) were confirmed significantly higher in CORT-treated animals in comparison to control animals (t -test: ***, $p<0.001$). **(B)** Blunted HPA axis reactivity to a novel acute stress in mice after chronic exposure to CORT (Mann Whitney U: **, $p<0.01$).

2. Variable degradation of the mice FCS following chronic CORT administration

Taking into consideration the effects of GC on epidermal homeostasis (Pérez, 2011) and hair growth initiation (Stenn et al., 1993), the weekly evaluation of the animals' FCS served informing not only about their self-oriented behaviour (Nollet et al., 2013), but also as an index of pharmacological efficacy (pharmacodynamics).

Along with the literature, the FCS of *C57BL/6JRj* CORT-treated mice ($n=61$) appeared significantly degraded after three weeks of treatment compared with the control group ($n=61$) [main effect of treatment: $F_{1,1279}=454.8$, $p<0.00$, $p\eta^2=0.26$; week: $F_{13,1279}=25.0$, $p<0.00$, $p\eta^2=0.20$; treatment x week interaction: $F_{13,1279}=14.3$, $p<0.00$, $p\eta^2=0.13$; from the third week of treatment: all $ps<0.01$, post-hoc] (see **Figure 25 A**). This result confirms the efficacy of the pharmacological treatment to induce an anxio-depressive-like phenotype in *C57BL/6JRj*-treated mice. The evaluation of the FCS of *CRF-cre x Ai9* animals revealed a strain-dependent pharmacological effect of the CORT treatment. Contrary to *C57BL/6JRj* mice, the fur coat of these genetically modified mice did not degrade after several weeks of CORT treatment (VEH, $n=29$; CORT, $n=36$) [treatment x week interaction: $F_{6,441}=1.4$, $p=0.21$, $p\eta^2=0.21$] (see **Figure 25 B**). This result will help guiding the physiological and emotional monitoring of *CRF-cre x Ai9* CORT-treated animals and subsequently, other complementary parameters should be included in this process.

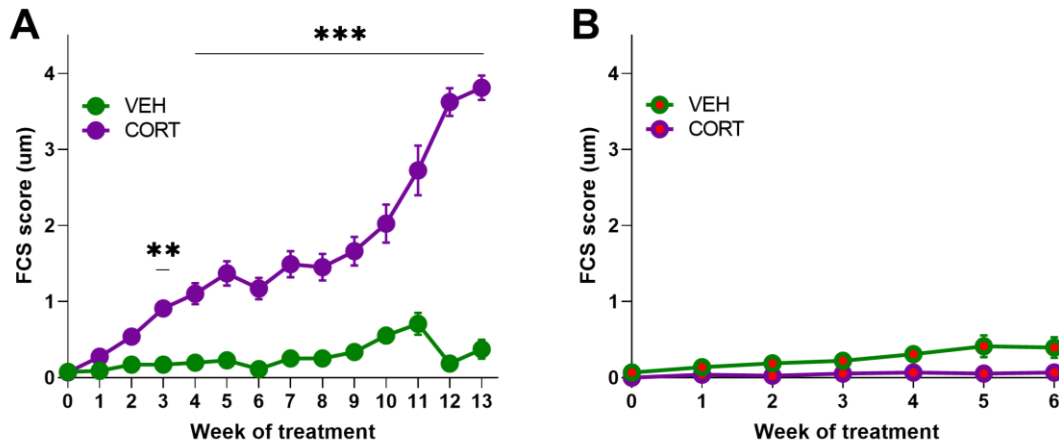


Figure 25. Evolution of the FCS of C57BL/6JRj and CRF-cre x Ai9 mice chronically treated with CORT. (A) CORT administration in C57BL/6JRj mice provokes significant FCS degradation from the third week of treatment (ANOVA: **, $p < 0.01$; ***, $p < 0.001$), whereas **(B)** no effect of the treatment is evidenced in CRF-cre x Ai9 mice.

3. Accentuated hyponeophagia in the NSF test after chronic CORT administration

The NSF test is a relevant paradigm to study hyponeophagia by presenting food-restricted animals to food in a novel environment. In this conflict-based test, addressing anxiety-related behaviour, animals presenting longer latencies to start eating are classified as anxious (Samuels and Hen, 2011). Along with the literature (David et al., 2009; Dieterich et al., 2019), C57BL/6JRj mice treated with CORT during 5-6 weeks displayed a typical anxiety-like behaviour in the NSF, i.e., they approached and started to eat from the presented food after a significantly longer latency period ($n=21$; mean latency to eat (s) \pm SEM: 537.27 ± 69.74) than VEH-treated animals ($n=21$; 85.63 ± 11.89) [UMW: $Z = -4.8$, $p < 0.0000$] [GBW, $\chi^2 = 28.0$, $p < 0.0001$]. However, the comparison of the food intake after the test, frequently used as an appetite control between groups, shows a significant effect of the treatment with CORT-treated animals eating less (mean food intake (g) \pm SEM: 0.04 ± 0.02) than VEH animals (0.09 ± 0.02) [$Z = 3.9$, $p < 0.0001$]. Since the weight of animals of both conditions was cautiously monitored daily, this difference can be attributed to CORT-induced metabolic effects preferentially rather than to hunger pressure. See **Figure 26 A,C**.

Within the evaluation of putative strain-dependent effects of the CORT treatment in the development of an anxious-like phenotype, a group of CRF-cre x Ai9 mice was also tested in the NSF paradigm. Firstly, the non-pathological measures of anxiety were compared showing that C57BL/6JRj and CRF-cre x Ai9 non-treated mice presented similar levels of hyponeophagia [UMW: $Z = -0.0$, $p = 0.98$]. After 5-6 weeks of treatment, an accentuated hyponeophagia was also evidenced in CORT-treated CRF-cre x Ai9 mice, with increased latencies to start eating from the available food ($n=36$; 428.57 ± 54.58) compared with control animals ($n=29$; 100.83 ± 16.21) [UMW: $Z = -4.5$, $p = 0.0000$] [GBW, $\chi^2 = 28.3$, $p < 0.0001$]. Concerning the measure of food intake after the test, CORT-treated animals consumed less (0.04 ± 0.00) than VEH animals (0.10 ± 0.01) [$Z = 4.1$, $p < 0.0001$] in agreement with the results obtained with C57BL/6JRj mice (see **Figure 26 D**). These results are therefore consistent and strengthen the evidence of the treatment's efficacy on exacerbating anxious-like behaviours in mice.

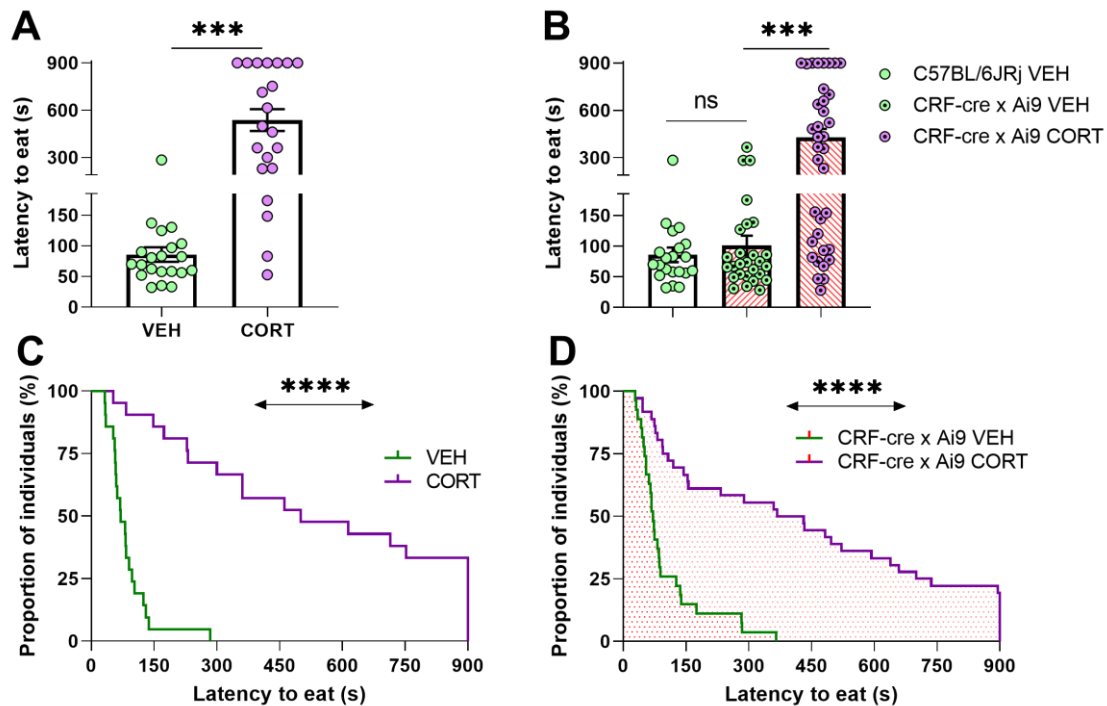


Figure 26. Five weeks of chronic CORT administration accentuates hyponeophagia in *C57BL/6JRj* and *CRF-cre x Ai9* mice. (A-B) In the NSF test and under a sustained food restriction protocol, animals chronically treated with CORT took longer to start eating from a food pellet placed in the centre of an arena (Mann Whitney U: ***, $p < 0.001$). (B) No differences for control animals were found between mouse strains (Mann Whitney U: ns, not significant). (C-D) Chronic administration of CORT significantly affects appetitive behaviour, as shown by the differences in the proportions of individuals starting to eat in the NSF test, irrespective of the mouse strain (Gehan-Breslow-Wilcoxon: ****, $p < 0.0001$).

4. A more passive coping style in the FST after chronic CORT exposure

Coping strategies in the face of distressing, uncertain conditions were measured in a FST. The switch from an active to a passive coping style (i.e., the acquisition of immobility after initial attempts to escape by swimming, struggling and climbing) has been directly associated to a complementary GR and MR mediated action of GCs in the behavioural adaptation to stress-coping (de Kloet et al., 2018; de Kloet and Molendijk, 2016; Molendijk and de Kloet, 2019).

After 9 weeks of treatment, *C57BL/6JRj* CORT-treated mice ($n=39$) displayed a more passive coping style when facing uncertainty in the FST, with a significantly longer immobility duration

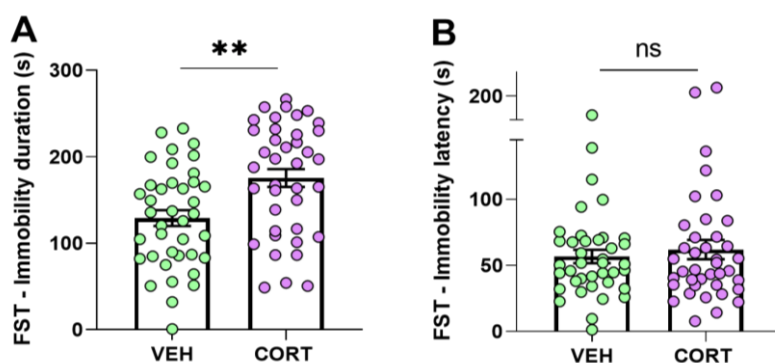


Figure 27. Chronic CORT administration significantly influences the coping style of mice in the FST. (A) Mice chronically treated with CORT spent more time immobile than control animals during the 6 min of test (t-test: **, $p < 0.01$). (B) However, the time spent actively swimming (active coping) before the first immobility event (immobility latency) was similar in mice across conditions (t-test: ns, not significant).

(immobility duration (s) \pm SEM: 175.43 \pm 10.36) compared with VEH animals (n=39) (129.07 \pm 9.21) [$t_{76}=-3.3$, $p<0.01$] (**Figure 27 A**). Nevertheless, the latency to acquire immobility for the first time did not differ between VEH (immobility latency (s) \pm SEM: 56.74 \pm 5.11) and CORT-treated animals (61.96 \pm 7.26) [$t_{76}=-0.6$, $p=0.56$] (**Figure 27 B**). These results further support the efficacy of the pharmacological treatment to impact coping strategies in *C57BL/6JRj*-treated animals, which display behavioral adjustments reminiscent to human anxio-depressive symptomatology.

2.2. Behavioural performance throughout the mouse Gambling Task

The results here described have been the objective of the scientific publication “*Chronic exposure to glucocorticoids induces suboptimal decision-making in mice*”, *ENPP, 2021*, that can be found as [Annex IV](#).

Objective: The second objective of this chapter is to study how chronic CORT administration affects animals’ performance in the ^AmGT in order to identify behavioural components of DM potentially impacted by distressful conditions (**Figure 28**).

In adapted gambling tasks, rodents have been shown to efficiently explore and sample from the different available options prior to establish a choice strategy upon associative and operant conditioning and through reinforcement learning. Besides, a high interindividual variability has been demonstrated and proposed to be shared with humans (see *First Chapter, Section 2*; Daniel et al., 2017; de Visser et al., 2011; Pittaras et al., 2016; Rivalan et al., 2013; Steingroever et al., 2013). In this line, and since the formation of and learning from action-outcome contingencies is required for the development of a long-term advantageous strategy in the ^AmGT, chronic CORT administration was expected to lead animals towards suboptimal DM performance. This working hypothesis is grounded on scientific evidence of disrupted cognitive processing during learning associations after chronic CORT exposure (Darcet et al., 2014; David et al., 2009; Dieterich et al., 2020; Gourley et al., 2008; Gourley and Taylor, 2009).

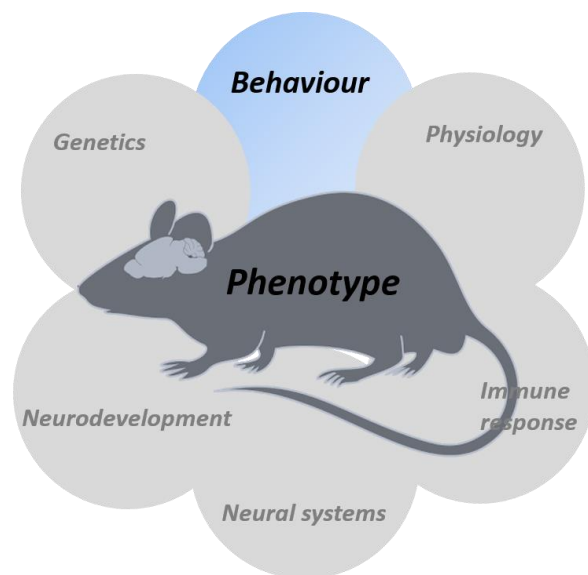


Figure 28. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour. This section aims at investigating the effects of sustained distress on the quality of the decisional processes. For that, animals chronically treated with CORT will be evaluated in the translationally validated gambling task for mice. The effect of sustained distressed on interindividual variability will be also addressed. The behaviour of distressed mice will be systematically compared with that of a healthy, non-treated mice population.

A. Materials and Methods

Animals: Eighty *C57BL/6JRj* mice were used for this study (free-feeding weight: mean \pm SEM (g) = 26.20 \pm 0.26). Mice started being treated seven weeks before the beginning of the behavioural assessment. Half the individuals received CORT, the remaining 40 animals received vehicle. Six mice displaying immediate spatial preference among options (choice proportion different from the expected in absence of spatial preference, thus 25% of choices for each of the 4 available options; χ^2 , $p < 0.05$) were discarded from the subsequent Δ mGT analyses (VEH, $n=39$; CORT, $n=35$).

Experimental protocol: The Δ mGT protocol described in *First Chapter, Section 1* was used for this study.

B. Results

Chronic-CORT administration delays reward responsiveness in the adapted mouse gambling task

In order to evaluate the effect of long-lasting elevated circulating GC on DM under uncertainty, overall behavioural performances of VEH and CORT-treated animals were compared. After 7 weeks of differential treatment, mice from both conditions showed a progressive increase in their Δ mGT performance over 5 sessions [main effect of session: $F_{4,288}=31.8$, $p=0.0000$, $\eta^2=0.31$], but no general difference was found between groups [treatment: $F_{1,72}=2.4$, $p=0.13$, $\eta^2=0.03$; session \times treatment interaction: $F_{4,288}=0.78$, $p=0.5$, $\eta^2=0.01$]. However, further analyses revealed that whereas VEH mice showed reward responsiveness from the second session onwards [% advantageous choices vs chance, session 1: $t_{38}=1.2$, $p > 0.05$, sessions 2-5: $t_{38} > 2.5$, all p s = 0.0000], CORT-treated mice required 20 more trials to allocate their responses towards the advantageous options [sessions 1&2: $t_{34} < 2.7$, all p s > 0.05 , sessions 3-5: $t_{34} > 4.8$, all p s = 0.0000]. See **Figure 29**.

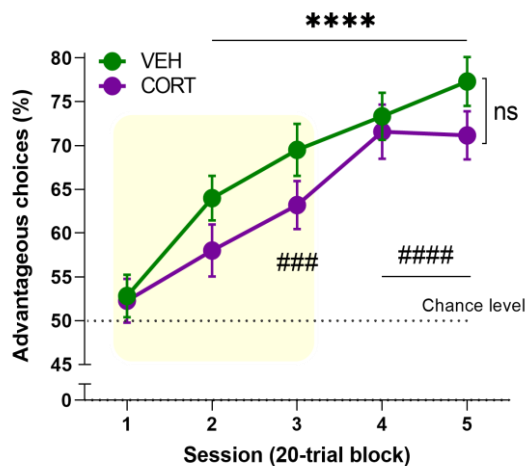


Figure 29. Sustained GC exposure delays the onset of optimal gambling performance in the Δ mGT. Behavioural Δ mGT performance in CORT-treated animals is characterized by a longer exploration phase in comparison with control individuals. In fact, reward responsiveness in CORT-treated animals was revealed after 40 choices, in the third gambling session (t-test: ###, $p < 0.001$; ####, $p = 0.0000$), whereas VEH-treated animals already chose preferentially the advantageous options after 20 choices, in the second session (t-test: ****, $p = 0.0000$). This difference in the emergence of reward responsiveness reveals a damaging effect of the pharmacological treatment on DM even if overall performances in both conditions are not significantly different (RM-ANOVA: ns, not significant). This negligible difference was suspected to be attributable to a veiled effect caused from behavioural interindividual variability.

To better characterize the influence of the CORT treatment on DM processes, scores obtained from the emotional evaluation (FCS and FST scores) were individually compared to Δ mGT final performance (percentage of advantageous choices in the last Δ mGT session). Mice FCS scores do not correlate with final Δ mGT performance [$r=0.0205$, $p=0.90$], and neither do their FST scores [total time of immobility: $r=-0.1491$, $p=0.31$; immobility latency: $r=-0.0505$, $p=0.67$]. These complementary results suggest that the physio-emotional adjustments here studied and the animals' coping style do not primarily influence groups' overall DM performance.

2.3. Behavioural interindividual variability on decisional profiles in the adapted mouse Gambling Task

Objective: The objective of this section is to study the effects of chronic CORT administration on behavioural interindividual variability through the identification and comparison of decisional profiles in CORT-treated and control animals (**Figure 30**).

Rodents' interindividual DM variability has been addressed in previous studies (see *First Chapter, Section 2*; Daniel et al., 2017; Pittaras et al., 2016), which showed that a majority of healthy individuals improve their gambling performance along the tasks. Smaller groups of individuals, however, fail to develop a long-term advantageous strategy (named *bad*, *risky* or *poor* decision-makers), or establish intermediate strategies that translate to less overall benefit. Investigating how and to which extent chronic GC exposure impacts this interindividual variability will help identifying the DM components that are at the origin of the behavioural adjustments. It will also help elucidating which stages of DM are more vulnerable to chronic distress. Hence, this type of comparative approach offers further experimental insight to substantiate the face validity of the CORT model for chronic distress-related disorders investigation.

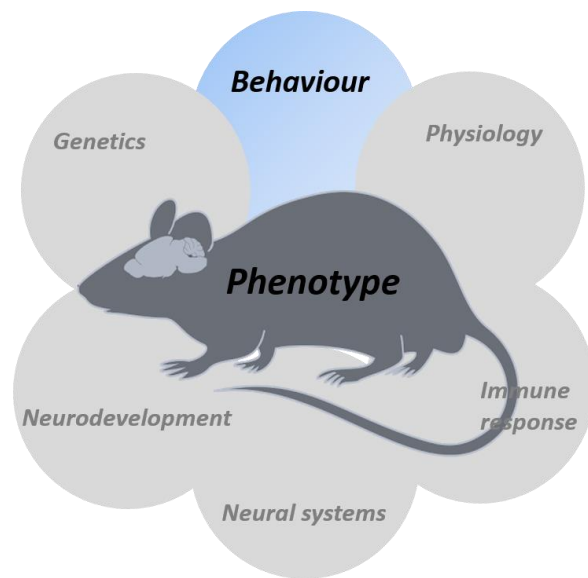


Figure 30. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour. This section aims at investigating the effects of sustained distress on choice strategy. The behavioural interindividual variability of a population of mice chronically treated with CORT will be compared with that of a healthy, non-treated mice population.

A. Materials and Methods

Animals: See *Second Chapter, Section 2*.

Decisional profiles identification: Individuals across pharmacological conditions were clustered into three different groups according to their distinct preference for the advantageous options in the last ^AmGT session:

- Good DMs: more than 70% preference for the advantageous options.
- Intermediate DMs: between 70% and 50% preference for the advantageous options.
- Poor DMs: 50% or less preference for the advantageous options.

B. Results

Chronic CORT administration increases the propensity to suboptimal decision-making performance

With the aim of exploring whether chronic CORT exposure could be considered a vulnerabilization factor to suboptimal DM, three different choice preference-based decisional profiles were compared across conditions. The majority of individuals displayed the optimal strategy (good DMs) (see **Figure 31**). They represent 82.05% of VEH and only 62.86% of CORT animals (session 5, mean percentage of advantageous choices \pm SEM: 82.78 ± 1.25). Individuals from the intermediate DM profile developed a delayed preference towards the advantageous options, without reaching the optimal strategy. They constitute only 5.13% of VEH whereas 28.57% of CORT mice (60.00 ± 1.38). Poor DMs failed to develop an option preference and correspond to 12.82% of VEH and 8.57% of CORT animals (39.38 ± 2.58). The distribution of CORT and VEH mice in the three subgroups was compared, highlighting a significant difference [X^2 , $p < 0.0001$] that is mainly accounted for by the intermediate DMs (**Figure 31**).

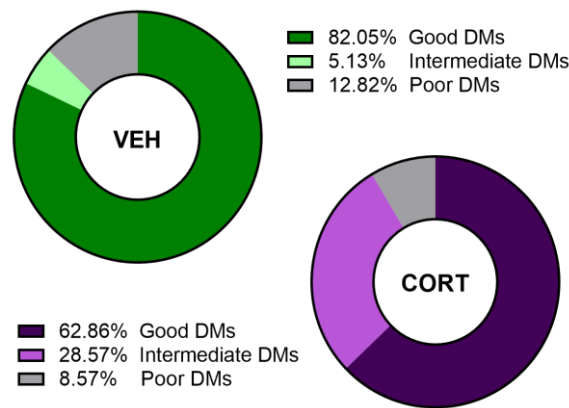


Figure 31. Chronic exposure to CORT increases the propensity for suboptimal DM in the ^AmGT. Different frequency distribution of VEH and CORT-treated individuals in good ($n=54$), intermediate ($n=12$) and poor ($n=8$) decisional clusters, with increased proportion of CORT animals among the intermediate DMs.

Decision-making subgroups learnt at different rates [main effect of cluster: $F_{2,71} = 21.0$, $p = 0.0000$; $p\eta^2 = 0.37$; session \times cluster interaction: $F_{8,284} = 6.1$, $p = 0.0000$, $p\eta^2 = 0.15$] (**Figure 32 A**). Good DMs ($n=54$) required 20 trials to converge towards the advantageous options [percentage of advantageous choices vs chance, session 1: $t_{53} = 2.1$, $p > 0.05$, sessions 2-5: $t_{53} > 6.2$, all $p_s < 0.0000$], while intermediate DMs ($n=12$) required 80 trials [session 1-4: $Z < 2.05$, $p > 0.05$, session 5: $Z = 7.3$, $p < 0.0001$]. Unlike the other two categories, poor DMs ($n=8$) never exhibited a preference [session 1-5: $Z < 2.4$, all $p_s > 0.05$]. In the last session of the ^AmGT, good DMs performed differently than intermediate DMs [$p < 0.05$, post-hoc], and the latter differently than poor DMs [$p < 0.05$, post-hoc]. Good and poor DMs performed differently from the fourth session [session 4: $p < 0.05$, session 5: $p < 0.0001$, post-hoc]. Further analyses revealed that the CORT treatment delayed reward responsiveness within the good DMs cluster: CORT-treated animals ($n=22$) performed differently than chance from the third session

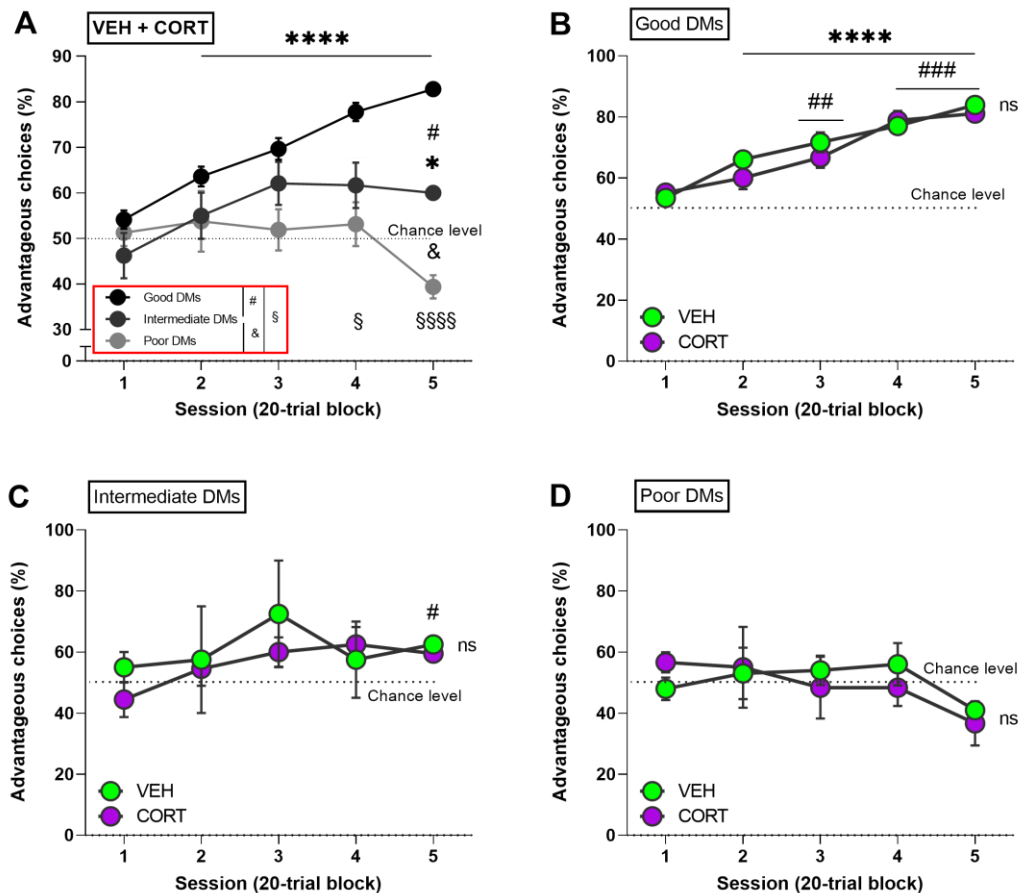


Figure 32. Three different decisional strategies lead to differential final performance in the ^AmGT. A k-mean clustering method applied to final overall ^AmGT performance (all individuals including VEH and CORT-treated animals) was used to identify three different decisional profiles. **(A)** The three subgroups learnt at different rates. Good DMs (black) improved their performance already in the second session (*t*-test: ****, *p*=0.0000), while intermediate DMs (dark grey) significantly improved only in the last session (Wilcoxon: *, *p*<0.05). Individuals from these two categories behaved differently in the last session (Mann Whitney U: #, *p*<0.05). Poor DMs (light grey), however, never allocated their response towards the advantageous options and performed differently than good DMs from the fourth session (Mann Whitney U: §, *p*<0.05; §§§§, *p*=0.0000), and than intermediate DMs in the last session (Mann Whitney U: &, *p*<0.05). CORT and VEH-treated animals within the same subgroup performed always alike (Mann Whitney U: ns, not significant). **(B)** Reward responsiveness in VEH-treated animals from the good DM cluster was significant from the second session (Wilcoxon: ****, *p*=0.0000), whereas CORT-treated animals differed from chance level from the third session onwards (Wilcoxon: ##, *p*<0.01; ###, *p*<0.001). **(C)** CORT-treated animals from the intermediate profile differed from chance level (50%) in the last session (Wilcoxon: #, *p*<0.05), **(D)** whereas poor DMs never differed from chance level, independent of the pharmacological condition.

onwards [sessions 1&2: $Z < 2.4$, $p > 0.08$, sessions 3-5: $Z > 3.5$, all p s < 0.01], while VEH animals ($n = 32$) converge towards advantageous choices already from the second session [sessions 1: $t_{31} = 1.2$, $p = 0.24$, sessions 2-5: $t_{31} > 6.1$, all p s = 0.0000] (**Figure 32 B**).

As a mean to obtain better insight into the origin of these decisional strategies, the physiological scores from individuals conforming the three DM clusters were compared. Good, intermediate and poor DMs did not differ neither in their FCS scores [VEH, $F_{2,36} = 1.7$, $p = 0.20$, $p\eta^2 = 0.08$; CORT, $F_{2,32} = 0.2$, $p = 0.85$, $p\eta^2 = 0.01$], nor in their FST scores [main effect of clusters: immobility duration,

$F_{2,70}=0.2$, $p=0.80$, $\rho\eta^2=0.01$; immobility latency, $F_{2,70}=0.10$, $p=0.90$, $\rho\eta^2=0.00$] (see **Figure 33**). These results confirm those suggesting that the physio-emotional adjustments here addressed and the animals' coping style do not explain DM performance in the ^AmGT.

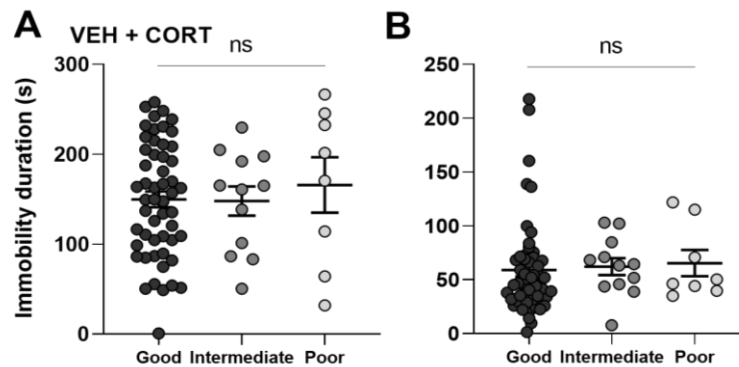


Figure 33. Behavioural interindividual variability in the ^AmGT does not relate to differential coping style. Decisional profiles identified in the ^AmGT displayed similar coping styles (ANOVA: ns, not significant) when they were tested in the FST. Good, intermediate and poor DMs spent the same amount of time immobile and have similar latencies for their first immobility event. These results further substantiate the evidence from Second Chapter, Section 2 indicating that coping styles do not explain decisional strategies.

2.4. Dynamics of choice strategy in the adapted mouse Gambling Task

Objective: The objective of this section is to study how chronic CORT administration affects the behavioural dimensions addressing choice strategy, in order to better understand its impact on cognitive mechanisms necessary for optimization of benefits in the ^AmGT (**Figure 34**).

In healthy mice, reward responsiveness and the course-dependent increase in advantageous choices in the ^AmGT have been demonstrated to hinge on the emergence of a preference (increased stickiness/rigidity) and a progressive decrease in the exploration of options (decreased flexibility). Individuals also become more sensitive to reward (increased win-stay scores) and learn to cope with penalties (decreased lose-shift scores) as the task progresses (see First Chapter, Section 2). Following the demonstration of suboptimal DM performance in animals chronically treated with CORT (see Second Chapter, Section 3), it is appropriate to

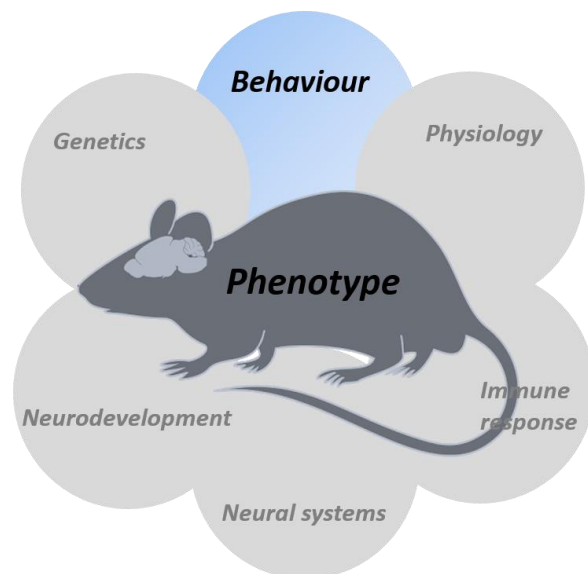


Figure 34. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour. This section further investigates the effects of sustained distress on choice strategy. For that, the behavioural interindividual variability of a population of mice chronically treated with CORT will be studied through multiple behavioural domains that inform of outcome-based learning and outcome responsiveness.

interrogate whether the pharmacological treatment interferes with the behavioural domains reflecting choice strategy in order to elucidate their implication in decisional alterations. The effects of chronic exposure to GC on these behavioural domains will therefore inform about the vulnerability of various cognitive components within DM processing.

A. Materials and Methods

Animals: See *Second Chapter, Section 2*.

Behavioural dimensions: To infer choice strategies mediating Δ mGT performance, the evolution of the four behavioural dimensions (stickiness/rigidity, flexibility, lose-shift and win-stay) was studied as previously described (see *First Chapter, Section 2*). The scores of these behavioural domains were systematically compared with final Δ mGT performance (percentage of advantageous choices in the last Δ mGT session).

B. Results

1. Chronic CORT administration features overappreciation of negative outcome

Consistent to precedent data, stickiness [main effect of block: $F_{1,72}=54.8$, $p=0.0000$, $p\eta^2=0.43$] and flexibility [block: $F_{1,72}=35.4$, $p=0.0000$, $p\eta^2=0.33$] scores changed along the task and no effect of the pharmacological treatment was evidenced [main effect of treatment, stickiness: $F_{1,72}=0.6$, $p=0.43$, $p\eta^2=0.01$; flexibility: $F_{1,72}=3.4$, $p=0.07$, $p\eta^2=0.05$] (**Figure 35 A,B**). Final stickiness [$r=0.669$, $p=0.0000$] and flexibility scores [$r=-0.585$, $p=0.0000$] significantly correlate with final Δ mGT performance, confirming their role in the development of a long-term advantageous strategy.

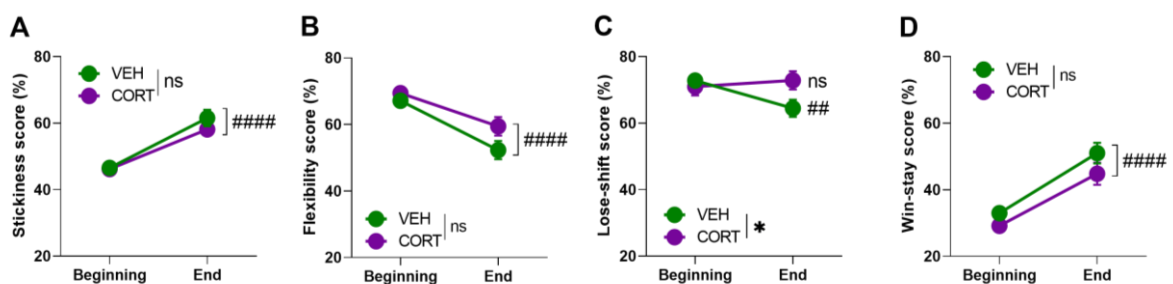


Figure 35. Sustained GC exposure affects hedonic appreciation through overappreciation of negative outcome. The evaluation of the behavioural dimensions along the Δ mGT was studied in order to evaluate the influence of chronic CORT administration in the emergence of reward responsiveness and the development of an optimal DM strategy. **(A)** Irrespective of the treatment (RM-ANOVA: ns, not significant), mice became more rigid and **(B)** less flexible (RM-ANOVA: #####, $p=0.0000$) in their choices as the task progressed. However, **(C)** while VEH-treated animals learnt to cope with penalties (RM-ANOVA: ##, $p<0.01$), CORT mice continued to frequently change option after a negative outcome at the end of the task (RM-ANOVA: ns). Final lose-shift scores significantly differed across conditions (RM-ANOVA: *, $p<0.05$). **(D)** Moreover, animals more frequently selected the same option after being rewarded with the task progression, irrespective of the pharmacological condition (RM-ANOVA: #####, $p=0.0000$).

Remarkably, a significant effect of the treatment in interaction with the time course was evidenced for lose-shift scores [time course x treatment interaction: $F_{1,72}=4.7$, $p<0.05$; $p\eta^2=0.06$]. At

the beginning of the task, all animals were prone to change option after a negative outcome (mean percentage \pm SEM of lose-shift, VEH: 72.83 \pm 1.89; CORT: 70.97 \pm 2.63). At the end of the task, however, animals from the control group were significantly less prone to change after a penalty (64.49 \pm 2.59) than CORT-treated animals (72.85 \pm 2.75) [$p < 0.01$, post-hoc] (**Figure 35 C**). It is also worth noting that whereas lose-shift scores do not correlate with final Δ mGT performance in CORT animals [$r = -0.050$, $p = 0.77$], a trend was evidenced for the VEH condition [$r = -0.307$, $p = 0.058$]. Concerning win-stay scores, all animals more frequently chose the same option after being rewarded as the task progressed [main effect of time course: $F_{1,72} = 52.8$, $p = 0.0000$, $p\eta^2 = 0.42$], irrespective of the treatment [treatment: $F_{1,72} = 2.6$, $p = 0.11$, $p\eta^2 = 0.03$] (**Figure 35 D**). As expected, final win-stay scores significantly correlate with final Δ mGT performance [$r = 0.614$, $p < 0.0000$], along with precedent data.

2. Chronic CORT administration does not differentially impact choice strategy

Concerning interindividual variability, the behavioral domains were contrasted across the identified decisional profiles. On the one hand, good DMs progressively developed and relied on a more rigid and less flexible strategy than intermediate and poor DMs [main effect of cluster, stickiness: $F_{2,71} = 7.0$, $p < 0.01$, $p\eta^2 = 0.16$; flexibility: $F_{2,71} = 4.3$, $p < 0.05$, $p\eta^2 = 0.11$]; time course x cluster interaction, stickiness: $F_{2,71} = 13.5$, $p < 0.0001$, $p\eta^2 = 0.27$; flexibility: $F_{2,71} = 9.6$, $p < 0.001$, $p\eta^2 = 0.21$]. On the other hand, intermediate and poor DMs were equally rigid and flexible in their choices [$p = 1$, post-hoc] (**Figure 36 A,B**). Correspondingly, only final stickiness [$r = 0.551$, $p < 0.0001$] and flexibility [$r = -0.587$, $p = 0.0000$] scores of good DMs significantly correlate with final Δ mGT performance, since only these individuals succeeded in optimizing their gains.

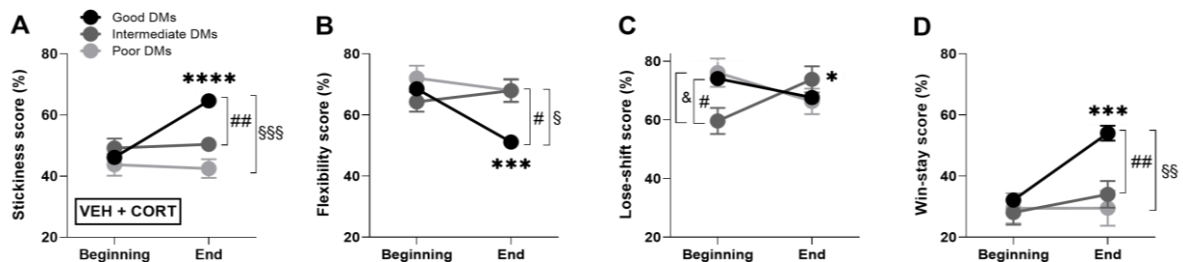


Figure 36. Characterization of the behavioural domains of the three decisional profiles. The three DM subgroups showed pronounced differences with regards to the behavioural dimensions of (A) stickiness, (B) flexibility and hedonic appreciation including (C) penalties and (D) rewards (RM-ANOVA: beginning vs end: *, $p < 0.05$; **, $p < 0.001$; ***, $p = 0.0000$; good vs intermediate DMs: #, $p < 0.05$; ##, $p < 0.01$; good vs poor DMs: \$, $p < 0.05$; \$\$, $p < 0.01$; \$\$\$, $p < 0.001$; intermediate vs poor DMs: &, $p < 0.05$). However, when comparing CORT and VEH-treated animals within decisional profiles, no differences were found irrespective of the cluster and the time course (see **Table 4** for statistical details).

In terms of the outcome responsiveness, the three DM subgroups behaved differently along the task [main effect of cluster, lose-shift: $F_{2,71} = 0.7$, $p = 0.50$, $p\eta^2 = 0.02$; win-stay: $F_{2,71} = 7.7$, $p < 0.001$, $p\eta^2 = 0.18$; time course x cluster interaction, lose-shift: $F_{2,71} = 6.0$, $p < 0.01$, $p\eta^2 = 0.14$; win-stay: $F_{2,71} = 7.6$, $p < 0.01$, $p\eta^2 = 0.18$]. Initially, good and poor DMs changed option after a penalty more frequently than intermediate DMs [$p < 0.05$, post-hoc]. The latter, however, significantly increased the lose-shift-based strategy along the task [$p < 0.05$, post-hoc] till final lose-shift scores did not differ between subgroups [$p > 0.26$, post-hoc] (**Figure 36 C**). Besides, good DMs more frequently chose the same option after being rewarded as the task progressed [$p < 0.001$, post-hoc], becoming significantly different from

intermediate and poor DMs [$p < 0.01$, post-hoc] (**Figure 36 D**). As for rigidity and flexibility scores, only final lose-shift [$r = -0.447$, $p < 0.001$] and win-stay scores [$r = 0.614$, $p = 0.0000$] from good DMs correlate with final A mGT performance. No effect of the CORT treatment was evidenced for the behavioural dimensions within DM clusters, irrespective of the moment of the task (see **Table 4**).

| Behavioural dimension | Time course | Vehicle vs CORT treated mice comparison | | |
|-----------------------|-------------------------------|---|------------------|---------|
| | | DM subgroup | Statistics U (Z) | p value |
| Stickiness | Beginning (first 40% task) | Good DMs | 0.12 | 1 |
| | | Intermediate DMs | 0.11 | 1 |
| | | Poor DMs | 0.61 | 1 |
| | End (last 40% task) | Good DMs | 0.28 | 1 |
| | | Intermediate DMs | -1.19 | 1 |
| | | Poor DMs | 1.20 | 1 |
| Flexibility | Beginning (first 40% task) | Good DMs | -0.88 | 1 |
| | | Intermediate DMs | -0.22 | 1 |
| | | Poor DMs | -1.65 | 0.59 |
| | End (last 40% task) | Good DMs | -1.08 | 1 |
| | | Intermediate DMs | -0.43 | 1 |
| | | Poor DMs | -0.75 | 1 |
| Lose-shift | Beginning (first 40% task) | Good DMs | -0.48 | 1 |
| | | Intermediate DMs | 0.21 | 1 |
| | | Poor DMs | -1.64 | 0.61 |
| | End (last 40% task) | Good DMs | -2.01 | 0.27 |
| | | Intermediate DMs | 0.00 | 1 |
| | | Poor DMs | -0.75 | 1 |
| Win-stay | Beginning (first 40% task) | Good DMs | 0.03 | 1 |
| | | Intermediate DMs | 0.21 | 1 |
| | | Poor DMs | 1.64 | 0.61 |
| | End (last 40% task) | Good DMs | 0.54 | 1 |
| | | Intermediate DMs | 0.64 | 1 |
| | | Poor DMs | 0.75 | 1 |

Table 4. Statistical details of the comparisons of time-dependent behavioural dimensions for each decisional profile between VEH and CORT-treated mice. Data related to Figure 36.

2.5. Summary and discussion of the results

In this second chapter, the efficacy of chronic CORT administration to induce an anxiety-depressive-like phenotype in mice has been confirmed by using various evaluation paradigms. Firstly, animals chronically treated with CORT display a significant deterioration of their FCS that might reflect the detrimental effects of chronic GC exposure on hair proliferation and epidermal homeostasis. However, it being an indication of disrupted self-oriented behaviour is the most common interpretation. This kind of self-negligence impacting physical appearance and reminiscent to human *avolition* (i.e., the inability to perform self-directed, purposeful activities), could also be indicative of a motivational loss in CORT-treated animals. This could have directly impacted their ability to initiate grooming behaviours. Secondly, CORT-treated animals presented an accentuated hyponeophagia in the NSF test, attesting the emergence of an anxious-like state in these animals. However, increased feeding latencies in the NSF test could also result from a reduction of spontaneous behaviour of

motivational basis, reminiscent to human *abulia* (i.e., lack of will or initiative). Finally, CORT-treated animals displayed a more passive coping style in the FST, a behaviour considered typical of the depressive-like repertoire and that suggests an overappreciation of negative stimuli. Furthermore, the strain-dependent discrepancies evidenced in CORT-induced FCS degradation suggest the existence of physiological variability among mouse strains sharing the same genetic background. This informative result needs to be further explored, yet it is taken into consideration in ongoing investigations (*Involvement of mPFC CRF expression in DM under chronic distress*). Thus, the obtained results strengthen the suitability of the CORT model to assess chronic-stress related physio-emotional dysfunction.

The effect of chronic GC exposure on DM was investigated using the ^AmGT protocol. Interestingly, several weeks of chronic CORT administration delay reward responsiveness, hence the encoding of contingencies required to settle on advantageous choices towards optimal DM. While animals from both conditions reached similar final performance through progressively increasing their preference for the advantageous options, CORT-treated animals required more trials to learn the task contingencies. In other words, chronic CORT exposure lengthens exploration and delays the onset of optimal DM performance.

The results also highlighted a significant effect of the CORT treatment on the interindividual variability in the ^AmGT. The distribution of CORT-treated individuals into three common decisional profiles, namely good, intermediate and poor DMs, has unveiled significant intra-group dispersion in terms of their physiological condition. An enhanced proportion of individuals failed to maximize their benefits after chronic CORT administration.

Optimal ^AmGT performance in control animals relies on an explorative phase during which the strategy is sensitive to the outcome of previous choices. Hence, rewards and losses guide animals' behaviours in a not far off lose-shift/win-stay-based strategy. The analyses addressing behavioural domains through the ^AmGT suggest a CORT-induced hedonic overappreciation of negative outputs (lose-shift scores), with impaired learning to cope with losses in animals upon chronic CORT, consistent with FST scores. The hedonic impact of rewards (wins-stay scores) across the ^AmGT does though not directly account for differential DM performance. Altogether, these results suggest that CORT-induced suboptimal ^AmGT performance could originate from a dysregulated negative rather than from a positive valence system.

Combined, the results of this second chapter suggest that prolonged elevated circulating GC compromise adaptive behaviour for optimal DM under uncertainty by exacerbating the general appreciation of negative events, along with clinical data (Foland-Ross and Gotlib, 2012). This overappreciation of losses in treated animals, in combination with the phenotypical signs indicative of lack of motivation, further support the suitability of the CORT model on cognitive translational investigation.

THIRD CHAPTER – Impact of chronic exposure to glucocorticoids on emotional-cognitive modulators of decision-making.

Some of the results described here have been the objective of the scientific publication “Chronic exposure to glucocorticoids induces suboptimal decision-making in mice”, ENPP, 2021, that can be found as [Annex IV](#).

3.1. Behavioural study on hedonic appreciation.

Objective: The first objective of this chapter is evaluating the effect of chronic CORT administration on hedonic appreciation mostly related to positive valence systems, aiming to obtain insight into the role of positive food reinforcers on DM processing. Therefore, the emergence of an amotivational state in depressive-like individuals reminiscent to human anhedonia is to be addressed (**Figure 37**).

Although not the only one, pleasure is a central modulating factor of goal-directed behaviour and general mental well-being. Reward processing includes the establishment of a stimulus-reward association that leads to a development of interest, desire and anticipation for the latter (Kring and Barch, 2014; Rizvi et al., 2016). Consequently, disruptions in reward processing have been suggested as a core feature for impaired associative learning in many psychiatric conditions, including depression (Der-Avakian and Markou, 2012; Griffiths et al., 2014) and schizophrenia (Horan et al., 2006). Anhedonia is the reduced ability to feel pleasure or lack of reactivity to positive experience from reward (Husain and Roiser, 2018; Pizzagalli, 2014). Yet, even though anhedonia is a core feature of reward deficits, it is not the only possible factor affecting hedonic appreciation. It therefore does not adequately capture all multifaceted reward-related deficits observed in neuropsychiatric conditions, and specifically in mood disorders (Der-Avakian and Markou, 2012). Reward anticipation, which involves momentary motivational arousal in healthy conditions, effort evaluation to obtain a reward and motivation in a broad picture are, among others, processes involved in hedonic appreciation that may lead to different goal-directed behavioural output.

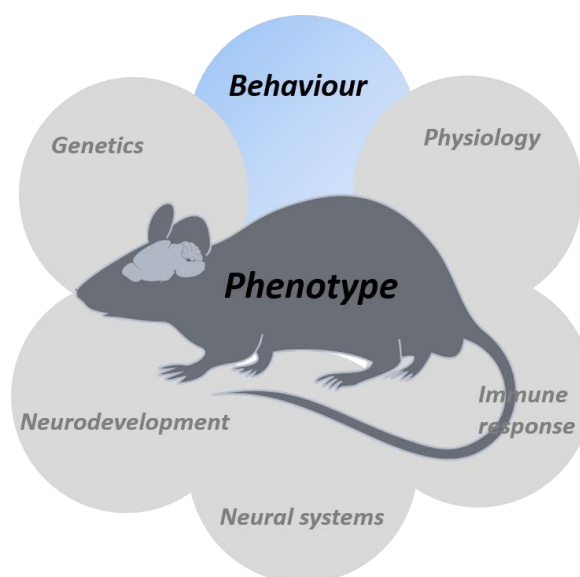


Figure 37. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. **Behaviour.** This section aims at investigating the effects of sustained distress on hedonic appreciation, which is a motivational component of decisional processes. Understanding how chronic CORT exposure affects reward valuation and processing in mice is a useful strategy to investigate the origins of suboptimal DM during the value-based gambling protocol, since it uses food as outcome reinforcer.

Chronic CORT administration in mice has been demonstrated to efficiently induce negative valence behaviours mostly accounted for by an anxious-like state (see results from the *Second Chapter*; Dieterich et al., 2019). Yet, results from other studies are controversial concerning the impairment of positive valence behaviours (Dieterich et al., 2019; Olausson et al., 2013). Nevertheless, the study of reward appreciation in rodents is of translational relevance, especially given the nature of preclinical paradigms to address instrumental behaviour.

A. Materials and Methods

Animals: Hedonic appreciation was evaluated in 80 *C57BL/6JRj* mice (free-feeding weight: mean \pm SEM (g) = 26.20 \pm 0.26) and 65 *CRF-cre x Ai9* mice (free-feeding weight: mean \pm SEM (g) = 26.82 \pm 2.35). Half the animals were CORT-treated, the other half received VEH in the drinking water.

Experimental protocols:

○ *Sucrose Preference Test (SPT)*

Hedonic appreciation was firstly addressed by studying the preference over water of 2.5% and 0.8% sucrose solutions (D(+)-Saccharose, *Carl Roth*, Karlsruhe, Germany). The basis of the SPT is founded upon animal's natural preference for sweetness, and assuming that this preference is a function of the experience evoked by the sweet solution (Berridge, 2004, 1996). This paradigm allows investigating hedonic aspects of motivated behaviours, also referred as 'liking phase' (Husain and Roiser, 2018), through consummatory behaviours that have frequently been interpreted in the scientific literature as reminiscent to human anhedonia.

The SPT lasted four consecutive nights. On the first night or *forced sucrose phase*, the drinking beverage (CORT/VEH) was substituted by the sucrose solution inside the home cages. Mice were subsequently tested in a two-bottle paradigm: they had exclusive access over night to two identical bottles, one containing drinking water, the other the sucrose solution (adapted from Lutz, 2013) (**Figure 38**). Every morning, bottles were removed and weighted, and animals were relocated in their home cages. The sucrose preference was calculated as the percentage of sucrose solution consumption relative to the total liquid intake, during the last session.



Figure 38. Sucrose Preference Test. Animals are left to choose between drinking from a bottle of water or a bottle containing a sucrose solution. Reductions in the natural animal preference of the sweet solution are indicative of amotivated-anhedonic states. Image from Franklin et al., 2012.

○ *Free Feeding Task (FFT)*

This behavioural paradigm, modified from Vichaya et al., 2014, allows further investigation of the contributions of positive valence systems to DM by focusing on hedonic appreciation evaluation. Animals under food deprivation are free to eat from two different sorts of nourishments, including a sweet option appealing to the animal's natural preference for sweetness (**Figure 39**). This paradigm is particularly interesting, since it allows approaching several aspects of motivated behaviours highly



Figure 39. Free Feeding Task. Food deprived mice are free to eat from two different palatable rewards. This task allows approaching different stages within the decisional process: the choice itself informs about the valuation of options, while the latency of animals to start eating and the total intake during 5 min are measures of the appetitive and consummatory motivational dimensions.

informative for DM processing interpretation. Subjective valuation of the presented options is addressed by the choice itself (i.e., food reward primarily chosen for consumption), while appetitive behaviour can be evaluated from the latency until animals are starting to eat. Subsequently, hedonic appreciation can be approached through animals' consummatory behaviour. In addition, cognitive aspects of behavioural flexibility and risk-taking can be inferred from paradigm variations of food neophobia evaluation (i.e., presentation of at least one unfamiliar sort of food). Mice were placed in a transparent cage where two recipients with food pellets were exposed, one with 10 grain-based pellets and the other one with 10 chocolate pellets. The nature of the first pellet chosen, as well as the latency of this first choice i.e., the latency to start eating, and the total number of pellets consumed were noted. The test finished when all pieces of the first

choice were eaten, or after a maximal test duration of 5 minutes. Animals which failed to choose within the assigned time were considered as *undecided*. Animal's reward appreciation was evaluated after 6-8 weeks of VEH/CORT treatment, before and after presentation of the food options in their home cages.

B. Results

1. Chronic CORT exposure does not alter hedonic appreciation in the SPT

Hedonic appreciation in mice was studied through their consummatory behaviour in the SPT, demonstrating that all *C57BL/6J* individuals expressed a strong preference for the sucrose solution compared to water, independent of the sucrose concentration [consumption of sucrose solution vs

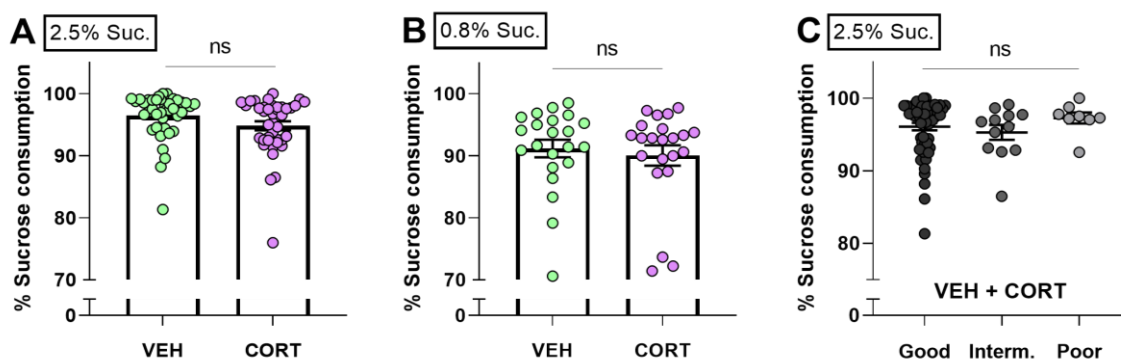


Figure 40. Chronic CORT administration in mice does not influence hedonic appreciation through consummatory behaviour in the SPT. (A) Mice chronically treated with CORT showed a significant preference for the sweet solution compared to water [2.5%, $n=40$, % of sucrose vs 50% solution; t -test: $t_{39}=61.6$, $p<0.00$], as control animals [2.5%, VEH, $n=40$; t -test: $t_{39}=79.3$, $p<0.00$], (B) and irrespective of the sucrose concentration [0.8%, CORT, $n=22$; Wilcoxon: $Z=4.1$ $p<0.0001$; VEH, $n=22$; Wilcoxon: $Z=4.1$, $p<0.0001$]. (C) Individuals conforming the three decisional profiles identified in the A mGT did not differ in preference for the 2.5% sucrose solution over water (ANOVA: ns, not significant).

50%: all $p < 0.0001$]. No differences were found between CORT-treated mice and individuals from the control groups concerning the effect of the pharmacological treatment, indicating intact hedonic aspects of consummatory behaviour after chronic CORT exposure, independently of the concentration of the sucrose solution [2.5% sucrose solution: $t_{78}=1.8$, $p=0.08$; 0.8% sucrose solution, UMW: $Z=0.5$, $p=0.60$] (see **Figure 40 A,B**). Hence, the SPT did not evidence altered reward processing or hedonic underappreciation in mice after chronic CORT exposure.

In order to investigate if differential A mGT performance could be interpreted as a function of reward appreciation, the SPT scores of the subset of animals that were tested in the A mGT (VEH, $n=39$; CORT, $n=35$) was studied in further detail. Sucrose preference scores were compared to final A mGT performance (percentage of advantageous choices in the last A mGT session), and no correlation was found for these parameters [$r=-0.0022$, $p=0.99$]. Accordingly, individuals conforming the different decisional profiles did not differ in their sucrose solution consumption [2.5% sucrose solution: $F_{2,71}=0.7$, $p=0.49$, $\eta^2=0.02$] (**Figure 40 C**).

2. Chronic CORT administration emphasizes food neophobia

Mice reward appreciation was further assessed by using a FFT paradigm after 6-8 weeks of differential treatment. In order to simultaneously address food neophobia, mice were exposed to grain-based pellets before the first behavioural testing, but not to the high-palatable reward (chocolate pellets).

Concerning *C57BL/6JRj* mice (VEH, $n=40$; CORT, $n=40$), the results showed that the majority of individuals chose preferably the already encountered grain-based option (57.5%), while only 12.5% of them chose chocolate pellets. The remaining 30.0% of the animals, the so-called *undecided*, failed to choose between the presented rewards within the 5 testing minutes. Interestingly, 72.5% of VEH and 42.5% of CORT-treated animals chose the grain-based reward. Individuals that chose the, unfamiliar, chocolate reward represented 22.5% of the control animals as opposed to only 2.5% of the CORT-treated mice. Noteworthy, 55.0% of CORT-treated animals did not select an option, while only 5.0% of VEH-treated animals failed to choose between options before the end of the task. The distribution of CORT and VEH-treated mice between the three possible classes was compared, highlighting a significant difference [χ^2 , $p=0.0000$] which is mainly accounted for by the chocolate reward and undecided choices (see **Figure 41**).

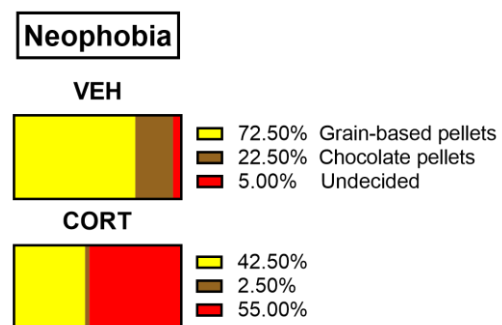


Figure 41. Chronic exposure to CORT emphasizes neophobia in the FFT. Different frequency distribution of VEH and CORT-treated individuals in their choice between familiar (grain-based pellets, VEH: $n=29$; CORT: $n=17$) and unfamiliar food rewards (chocolate pellets, VEH: $n=9$; CORT: $n=1$). Chronic CORT administration affects appetitive behaviour through increment of the proportion of animals failing to choose between rewards within the test duration (undecided, VEH: $n=2$; CORT, $n=22$).

Along the same line, the latency of the first choice was compared between the different conditions, showing that VEH-treated ($n=38$) and CORT-treated ($n=18$) animals, if they chose, required a similar amount of time to select their first pellet (latency of the first choice (s) \pm SEM, VEH:

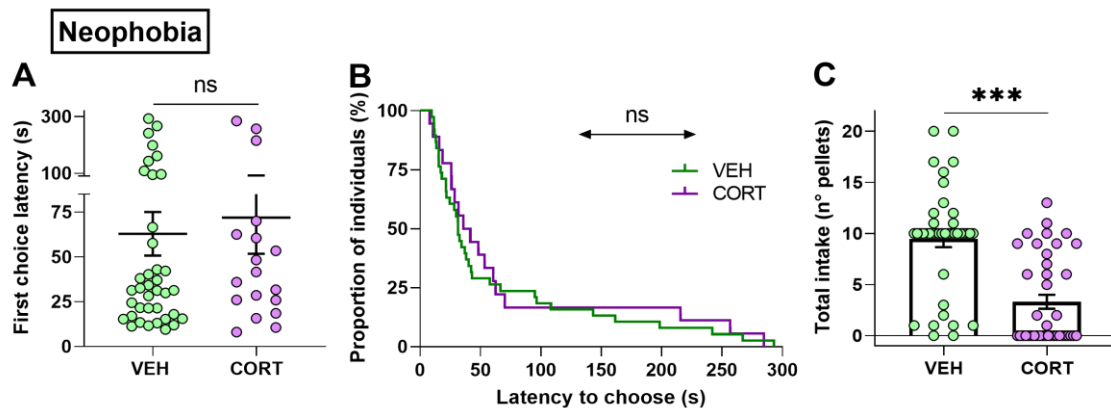


Figure 42. Similar latencies to choose but significantly reduced food intake in mice chronically treated with CORT during the neophobia approach of the FFT. (A) Among individuals that ate during the FFT (data from undecided individuals not shown here), VEH and CORT-treated animals required the same amount of time to make a choice and to start eating (*t*-test: ns, not significant). (B) Appetitive behaviour of animals that ate (independently of their choice) was not affected by the CORT treatment, as shown by the negligible difference in proportions of individuals starting to eat in the FFT test (Gehan-Breslow-Wilcoxon: ns). (C) CORT-treated mice eat significantly less than control animals during the FFT (*t*-test: ***, $p < 0.001$).

62.90±12.17; CORT: 71.93±20.22) [$t_{54} = -0.4$, $p = 0.69$] [GBW, $\chi^2 = 0.5$, $p = 0.50$] (Figure 42 A,B). Additionally, the total number of eaten pellets was compared between conditions, evidencing a significantly reduced intake in the CORT condition (reward intake ± SEM, VEH: 9.48±0.81; CORT: 3.33±0.68) [$t_{78} = 5.8$, $p = 0.0000$] (Figure 42 C). Combined, these results are in accord with previous data (see Second Chapter, Section 2), showing altered appetitive and consummatory behaviour after chronic exposure to CORT, which point to disrupted reward appreciation with CORT-induced exacerbation of the natural food neophobia of rodents.

3. Chronic CORT administration does not obstruct reward valuation but reduces self-initiated behaviour in the FFT



Figure 43. Chronic exposure to CORT does not affect reward valuation, but impinges appetitive behaviour in the FFT. Different frequency distribution of VEH and CORT-treated individuals in their choice between grain-based pellets (VEH: $n = 25$; CORT: $n = 21$) and chocolate pellets (VEH: $n = 15$; CORT: $n = 15$). Chronic CORT administration affects appetitive behaviour, as evidenced through an increment of the undecided class (undecided, VEH: $n = 0$; CORT, $n = 4$).

The same C57BL/6J mice (VEH, $n = 40$; CORT, $n = 40$) were re-tested in the FFT paradigm a week after the first behavioural assessment. During this period, chocolate pellets were presented in their home cages during three consecutive days in order to counter neophobic behaviour. During re-testing, the majority of individuals continued to primarily choose the grain-based option (57.5%). However, the choice of chocolate pellets increased to 37.5%. Noteworthy, indecision represented only 5.0% of the animals. Individuals opting for the non-chocolate reward represented 62.5% of VEH and 52.5% of CORT-treated animals. The chocolate reward was chosen by 37.5% of VEH and CORT-treated mice. Notably, the

undecided group was only comprised by CORT-treated animals. As previously, the distribution of individuals of the CORT and VEH conditions into the three possible classes was compared. In this re-testing phase, CORT-treated animals chose the chocolate reward as frequently as control animals, but the distribution of the *undecided* group was significantly different compared with the results of the neophobic testing [χ^2 , $p < 0.05$] (see **Figure 43**).

Regarding the latency of the first choice, individuals from both conditions required less time to select their first reward compared with the first testing session [FFT-1 “neophobia” vs FFT-2 “re-testing”, VEH: $t_{37} = 4.5$, $p < 0.0001$; CORT: $t_{17} = 3.2$, $p < 0.01$] [GBW, VEH: $\chi^2 = 47.8$, $p < 0.0001$; CORT: $\chi^2 = 16.3$, $p < 0.0001$] (**Figure 44 A,B**). When compared, CORT-treated animals ($n = 36$) required longer than VEH-treated animals ($n = 40$) to select their first reward, although the difference is not significant (latency of the first choice (s) \pm SEM, VEH: 9.64 ± 1.14 ; CORT: 25.02 ± 8.89) [$t_{74} = -1.8$, $p = 0.07$] [GBW, $\chi^2 = 1.3$, $p = 0.25$] (**Figure 44 C,D**). Furthermore, the total food intake over the task continued being low in CORT-treated animals (reward intake \pm SEM, VEH: 14.58 ± 0.74 ; CORT: 11.70 ± 1.01) [$t_{78} = 2.3$, $p < 0.05$] (**Figure 44 E**).

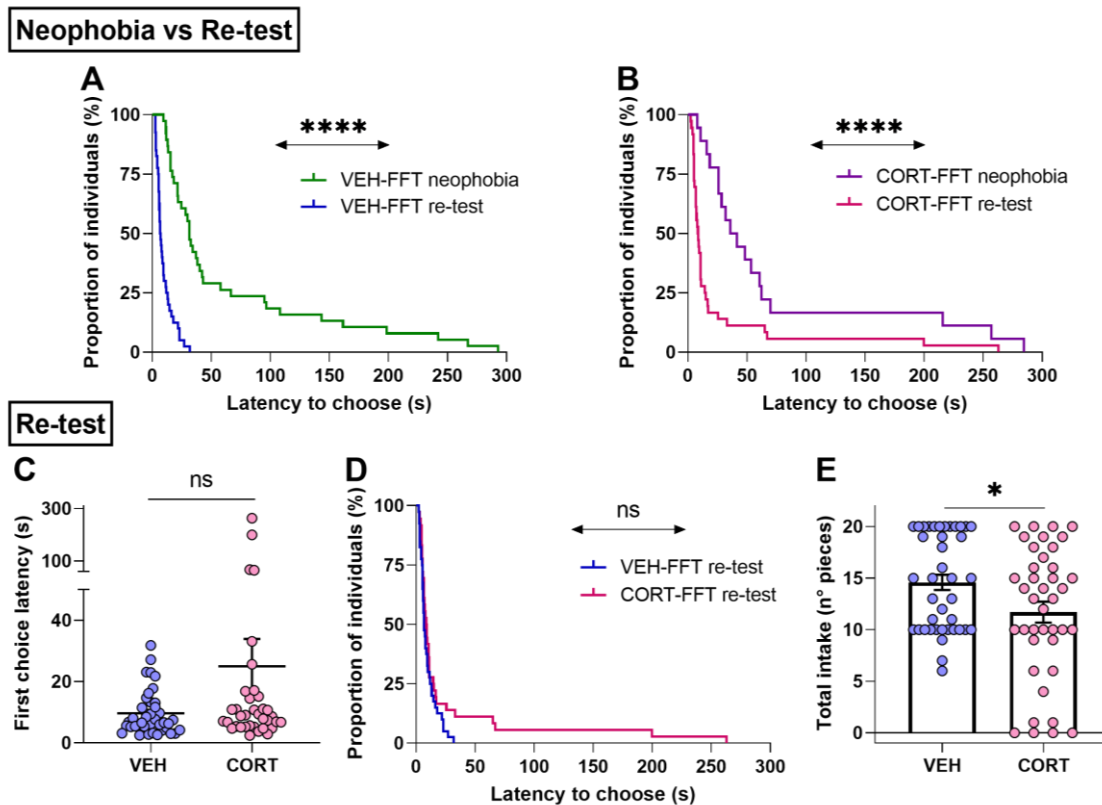


Figure 44. Similar latencies to choose, but significantly reduced food intake in mice chronically treated with CORT during the FFT re-testing. (A) Animals from the control group started eating significantly earlier in the FFT-re-testing (Gehan-Breslow-Wilcoxon: ****, $p < 0.0001$) (B) as well as CORT-treated animals (****, $p < 0.0001$), indicating countering of neophobia. (C) In the FFT re-testing, VEH and CORT-treated animals required the same amount of time to choose between the two options and start eating (data from undecided individuals excluded) (t-test: ns, not significant). (D) Considering animals that ate during the re-testing FFT, VEH and CORT individuals displayed similar appetitive behaviour (independently of their choice), as shown by the lack of difference in the proportion of individuals starting to eat in the FFT (Gehan-Breslow-Wilcoxon: ns). (E) Effect of the CORT treatment on mice consummatory behaviour, with treated animals eating significantly less than control animals (t-test: *, $p < 0.05$).

Within the evaluation of conjectural strain-dependent effects of the CORT treatment on reward processing, a group of *CRF-cre x Ai9* mice (VEH, $n=29$; CORT, $n=36$) was tested in the FFT after reward presentation in the home cage (animals were tested only once). As for *C57BL/6JRj* mice, the majority of individuals chose the grain-based option in the first place (47.7%), while 40.0% of the animals chose chocolate pellets. *Undecided* represented 12.3% of *CRF-cre x Ai9* animals. Individuals opting for the grain-based reward represented 37.9% of VEH and 55.6% of CORT-treated animals. The chocolate reward was chosen by 55.2% of the control group, but only 27.8% of CORT-treated mice. Finally, 6.9% of VEH and 16.7% of CORT mice formed the *undecided* group. Contrary to *C57BL/6JRj* mice, no significant difference was found in the distribution of CORT and VEH individuals into the three classes, but CORT-treated animals tended to choose the chocolate option less frequently than control animals [χ^2 , $p=0.07$] (Figure 45).

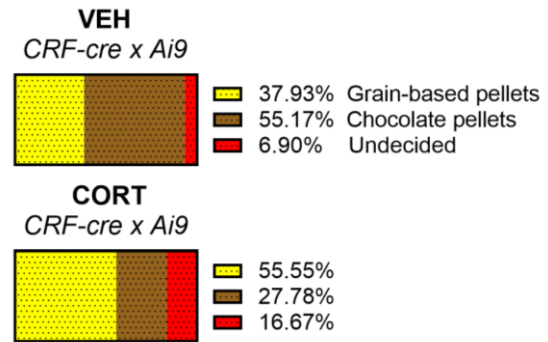


Figure 45. Chronic CORT administration does not affect reward valuation of *CRF-cre x Ai9* mice in the FFT. No differences were found in the frequency distribution of VEH and CORT-treated individuals concerning the choice between grain-based pellets (VEH: $n=11$; CORT: $n=20$) and chocolate pellets (VEH: $n=16$; CORT: $n=10$). Similar proportion of undecided individuals in VEH ($n=2$) and CORT ($n=6$) conditions.

Regarding the latency of the first choice and contrary to *C57BL/6JRj* mice, CORT-treated mice ($n=30$) took significantly longer than control mice ($n=27$) (latency of the first choice (s) \pm SEM, VEH: 44.33 ± 11.18 ; CORT: 91.86 ± 13.95) [$t_{63}=-2.7$, $p<0.01$] [GBW, $\chi^2=9.0$, $p<0.01$] (Figure 46 A,B). This difference between mouse strains could potentially be accounted for by the differences in the experimental design (*C57BL/6JRj*: re-test after presentation of reward vs *CRF-cre x Ai9*: first test after reward presentation). Concerning the total food intake over the task, CORT-treated animals also

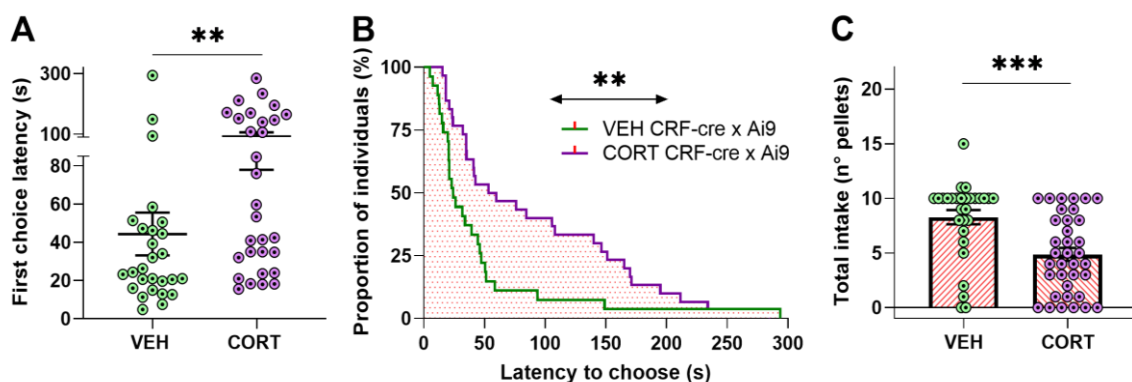


Figure 46. Different latencies to choose and significantly reduced food intake in *CRF-cre x Ai9* mice chronically treated with CORT during the FFT. (A) CORT-treated mice required longer than control animals to choose between the two options and start eating (data excluded from undecided individuals) (t -test: **, $p<0.01$). (B) Among the animals that made a choice during the 5 minutes of testing, CORT individuals showed reduced appetitive behaviour (independently of their choice), as shown by the difference in the proportion of individuals starting to eat in the FFT (Gehan-Breslow-Wilcoxon: **, $p<0.01$). (C) CORT-induced reduction of consummatory behaviour, with treated mice eating significantly less than control animals during the FFT (t -test: ***, $p<0.001$).

consumed less than the control group (reward intake \pm SEM, VEH: 8.28 ± 0.66 ; CORT: 4.86 ± 0.59) [$t_{63}=3.9$, $p < 0.001$] (**Figure 46 C**).

Combined, these results do not evidence an impairment of reward valuation in mice following chronic CORT administration when food rewards are familiar, but they do indicate an obstruction of self-initiated behaviour reminiscent to human abulia. Moreover, the reduction of reward intake in CORT-treated animals shows an impact of the pharmacological treatment on consummatory behaviour, in line with the results obtained for the NSF test (see *Second Chapter, Section 1*), but contrary to those obtained for the SPT. Despite the difference in their latency to choose, which is significant between conditions in *CRF-cre x Ai9* mice but not in *C57BL/6J* mice, the results obtained for both mouse strains are consistent and strengthen the evidence for the pharmacological treatment as a useful mean to address reward processing under chronic distress.

4. Reward valuation and consummatory behaviour in the FFT do not explain mice gambling performance

In order to better understand the differences obtained between consummatory scores in the SPT and FFT, the total consumption of sucrose solution (2.5%) and the total intake during the FFT re-

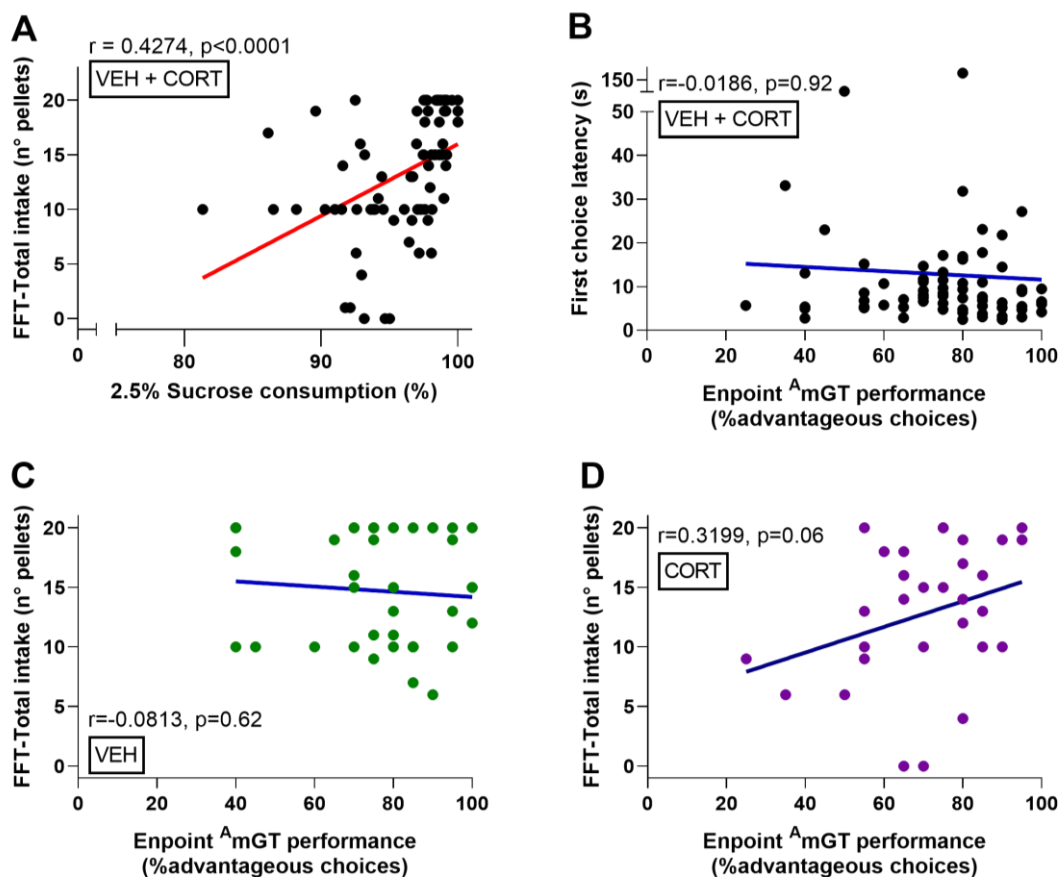


Figure 47. Reward valuation and consummatory behaviour do not correlate with ^AmGT performance. (A) Consummatory SPT and FFT scores significantly correlate between them, indicating a CORT-induced effect on reward appreciation not discernible only by sucrose preference (see results from SPT). (B) Mice final performance in the ^AmGT does not neither correlate with the latency to choose and start eating, (C) nor with the total intake in the re-test phase of the FFT. (D) However, CORT-treated animals performing worse in the ^AmGT tend to eat less in the FFT.

test phase were directly compared. A significant correlation between SPT and FFT scores was obtained [$r=0.4274$, $p<0.0001$], indicating that chronic CORT administration might affect consummatory behaviour in mice, and suggesting that SPT assessment at 2.5% might not be powerful enough to reveal this effect (**Figure 47 A**).

Following the same scheme as previously, re-testing FFT scores were compared to the final Δ mGT performance. No correlation was found between the endpoint gambling performance and the latency to choose in the FFT [$r=-0.0186$, $p=0.92$] (**Figure 47 B**). Contrary to the control group [$r=-0.0813$, $p=0.62$], a trend was evidenced for CORT-treated animals in terms of total food intake [$r=0.3199$, $p=0.06$], with mice that consumed less in the FFT scoring worse in the Δ mGT (**Figure 47 C,D**). This trend was confirmed by comparing the total intake in the re-testing FFT between decisional profiles, with poor DMs tending to eat less than good and intermediate DMs within the CORT condition [$H_{2,35}=5.5$, $p=0.07$]. In order to determine if animals' choice behaviour in the FFT could be a defining factor for differential Δ mGT strategies, the distribution of individuals into the three FFT classes was studied for each DM profile. Good, intermediate and poor DMs chose similarly in the FFT [χ^2 , $p=0.39$]. Collectively, these data suggest that Δ mGT performance does not directly depend on appetitive behaviour, but might be influenced by consummatory behaviour in situations of sustained distress.

3.2. Behavioural study on spatial working memory.

Objective: The second objective aims at investigating the effect of chronic CORT exposure in spatial WM, a cognitive dimension potentially required in outcome-related associative learning necessary for adaptive goal-directed-based DM in the Δ mGT (**Figure 48**).

It has been demonstrated in rodents that spatial WM is required for solving maze tasks where food rewards are to be located. As a temporally limited capability to record and use environmental information to orientate in space, spatial WM enables rodents to remember the food location through mental constructs of spatial relations between environmental cues. There is a scientific debate about whether WM plays a strong role in DM processing (Bagneux et al., 2013; Hinson et al., 2002; Jameson et al., 2004; Turnbull et al., 2005), and available data suggests that WM and gambling performance may experience asymmetric relationships, i.e., poor gambling

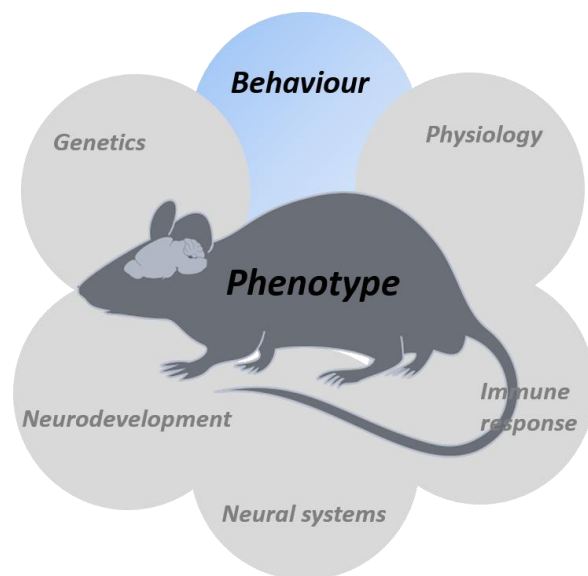


Figure 48. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour. This section aims at investigating the effects of sustained distress on spatial WM, which is a cognitive dimension required for associative learning processes. For that, mice chronically treated with CORT will be tested in a food-rewarding spatial WM task and their ability to succeed will be compared with that of a healthy, non-treated mice population. Understanding how chronic CORT exposure affects learning through this mnemonic dimension will help interpreting the observed delay in reward responsiveness in CORT-treated mice during the adapted gambling task.

performance could occur without impaired WM implication, but impaired WM could entail suboptimal DM (Bechara et al., 2000; Bechara and Damasio, 2005). Along the same line, data on the relationship between human WM impairments and depressive disorders are incongruous (Bourke et al., 2012; Gärtner et al., 2018), as deficits in WM are not always reported in animal models of anxiety-depression (Darcet et al., 2016a). Since here available options in the ^AmGT appear associated to different localisations, WM could potentially play a role in the retention of spatial information, and therefore in the elaboration of a decisional strategy. Similarly, the retention of information regarding contingencies associated to each ^AmGT option, as well as their consolidation and or extinction, would certainly be necessary for decisional strategy development. Thus, the working hypothesis of this section advocates that if chronic exposure to GC results in spatial WM impairments, those would lead to poorer decisional performance in the ^AmGT.

A. Materials and Methods

Animals: Spatial WM capability of forty-four *C57BL/6J* mice was tested in a *delayed spatial Win Shift Task* (dWST). At the beginning of the testing period, mice had been VEH- (n=22) or CORT-treated (n=22) for five weeks.

Experimental protocol: The dWST took place on an 8-arm radial maze (identical opaque equidistant arms, length: 37.0 cm; width: 5.7 cm; and a central start-point zone; *Panlab*, LE760/2) over 5 consecutive daily sessions.

Habituation. This first phase before dWST training lasted 3 days. During the first habituation session, animals were placed in the start-point zone and were allowed to explore the maze (all arms open) for 10 min. In the second habituation session, food rewards were scattered around the maze (all arms open) and mice had 10 min to explore and eat. In the third and last habituation session, one pellet was disposed at the end of each arm inside a food cup and invisible from an arm's entry. Again, animals had 10 min to explore, locate and eat the rewards.

Training. After habituation, mice were trained for 5 consecutive daily sessions before dWST testing, to visit all 8 arms of the maze. For this, mice were placed on the start-point of the maze, inside an opaque cylindrical structure that prevented visual assessment of the maze. After 5 sec, the cylinder was removed and all 8 arms opened. Mice were allowed to collect 1 food reward at the end of each arm, while reentries into a previously visited arm were considered an error. The session stopped when all 8 pellets had been collected, or after 15 min had elapsed.

Delayed spatial WST testing. The day following the last training session mice started being tested in the dWST, which lasted 5 consecutive days. Each daily session consisted of a *study* and a *test* phase. Specifically, in *study phase* mice were placed on the start-point of the maze inside the cylindrical structure, and when the cylinder was removed half the arms opened (randomly distributed across mice and sessions) and mice had to collect the available reinforcer in each of the 4 arms. Mice were then brought back to their home cage. After 3 min, they were placed back on the start-point of the maze for the *test* phase, during which all 8 arms were opened but only those previously closed during the *study phase* were baited. Reentries in previously visited arms were considered an error. The session lasted until the last pellet was collected, or 15 min had elapsed.

B. Results

1. Chronic exposure to CORT does not obstruct mice training in a radial maze-based task

The evolution of mice training-performance prior to dWST testing was analysed with the purpose of ensuring that, at the start of spatial WM testing, all animals held similar knowledge to continue proceeding. As expected, the time that animals needed to visit all 8 arms of the maze significantly decreased through the training, without difference between animals from both conditions [main effect of session: $F_{1,40}=15.3$, $p<0.001$, $\rho\eta^2=0.28$; main effect of treatment: $F_{1,40}=0.4$, $p=0.51$, $\rho\eta^2=0.01$; session x treatment interaction: $F_{1,40}=0.1$, $p=0.74$, $\rho\eta^2=0.003$]. In contrast, animals did not significantly reduce the number of re-entries during the training, regardless of their treatment [main effect of session: $F_{1,41}=0.8$, $p=0.37$, $\rho\eta^2=0.02$; main effect of treatment: $F_{1,41}=1.2$, $p=0.27$, $\rho\eta^2=0.03$; session x treatment interaction: $F_{1,41}=1.4$, $p=0.24$, $\rho\eta^2=0.03$]. Despite that, the results confirmed that at the end of the training all animals held similar knowledge to unbiasedly proceed to spatial WM testing (Figure 49 A,B).

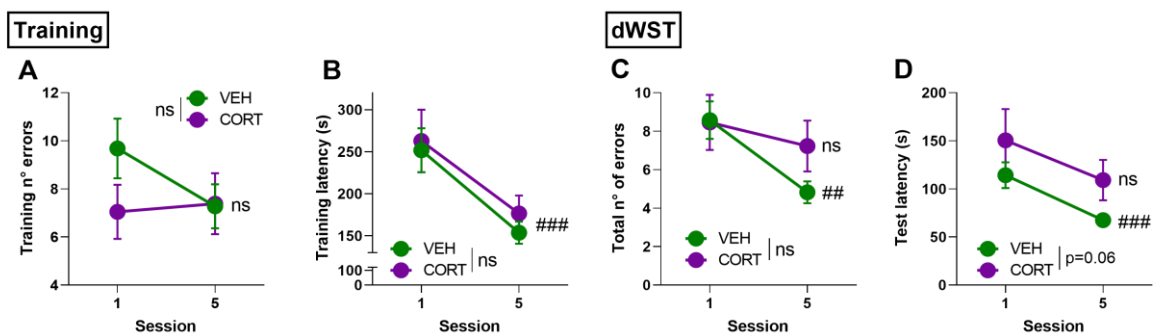


Figure 49. Chronic CORT administration hampers spatial WM in mice. (A,B) Mice learnt to find food reward through training in an 8-arm radial maze independent of their treatment. The evaluation of spatial WM capabilities in the dWST revealed a general learning process, mainly accounted for by VEH-treated animals rather than CORT mice, as shown by, (C), a decreased in the total number of errors and, (D), the time required to finish the task (RM-ANOVA: ##, $p<0.01$; ###, $p<0.001$; ns, not significant).

2. Chronic CORT administration hampers spatial WM in the dWST

Through the investigation of this cognitive dimension results showed that chronic CORT did not impact spatial WM at the population level, but that CORT-treated animals tended to take longer to complete the task [main effect of treatment, total number of errors: $F_{1,41}=1.8$, $p=0.19$, $\rho\eta^2=0.04$; task latency: $F_{1,41}=3.7$, $p=0.06$, $\rho\eta^2=0.08$]. However, a learning process was clearly present since the performance globally improved during the task [session, total errors: $F_{1,41}=4.8$, $p<0.05$, $\rho\eta^2=0.10$; test latency: $F_{1,41}=5.5$, $p<0.05$, $\rho\eta^2=0.12$]. More in-depth analysis demonstrated that this learning effect was accounted for by VEH-treated animals only [total errors: $Z=2.8$, $p<0.01$; test latency: $Z=3.3$, $p<0.001$], while CORT mice did not improve during the task [total errors: $Z=0.5$, $p=0.64$; test latency: $Z=1.2$, $p=0.23$] (Figure 49 C,D).

3. Differential gambling performance is not directly attributable to spatial WM capabilities

Individuals conforming the three DM categories were characterized in terms of their spatial WM capabilities. The three subpopulations were found to differ in their dWST performance [cluster x session interaction, total number of errors: $F_{2,40}=7.5$, $p<0.01$, $\rho\eta^2=0.27$; task latency: $F_{2,40}=5.0$, $p<0.05$,

$p\eta^2=0.20$]. Poor DMs significantly improved during the task [total number of errors: $p<0.001$; task latency: $p<0.01$, post-hoc], unlike good [total number of errors: $p=0.30$; task latency: $p=0.32$, post-hoc] and intermediate DMs, the latter group even making more mistakes at the end of the task than at its beginning [total number of errors: $p<0.05$; task latency: $p=0.19$, post-hoc]. Ultimately, only intermediate and poor DMs were different in terms of total number of errors [session 5, intermediate vs poor: $p<0.01$; good vs intermediate: $p=0.12$; good vs poor: $p=0.50$, post-hoc], but not in task latency [session 5, intermediate vs poor: $p=0.20$; good vs intermediate: $p=0.36$; good vs poor: $p=0.66$, post-hoc].

When contrasted, final dWST scores do not correlate with endpoint A_mGT performance [total number of errors: $r=0.0008$, $p=1$; task latency: $r=-0.0262$, $p=0.87$], which questions the influence of spatial WM on DM processing during the here studied gambling task.

3.3. Evaluation of the motivational dimension of effort allocation

Objective: The third objective of this chapter is to investigate how chronic CORT exposure impacts animals' motivational state through instrumental responding, in order to better understand its role as potential modulator of DM processing under uncertainty (**Figure 50**).

Motivation is defined as “a reason or reasons for acting or behaving in a particular way” (Husain and Roiser, 2018), and is often understood as a measure of the amount of energy or other resources that an individual is willing to invest towards achieving a valued outcome (Berridge, 2004). Motivation is therefore considered a critical modulator of reward-related behaviour, which may impact DM processing in gambling tasks. Individuals' motivational state modulates their engagement in rewarding behaviours by initiating them in a first moment (*appetitive behaviour*), and then by maintaining them through persistent effort (Husain and Roiser, 2018; Salamone et al., 2018).

Loss of motivation in goal-directed behaviour has been described as a symptom of many psychiatric disorders (Hartmann et al., 2015; Husain and Roiser, 2018; Pedersen et al., 2009) including depression (Treadway et al., 2012; Treadway and Zald, 2011). Actually, depressed patients, even if able to experience pleasure *per se*, are usually reluctant to expend effort in exchange for rewarding experiences. In combination with energy-related deficiencies or *anergia*, or even with *fatigue* (i.e., weariness to

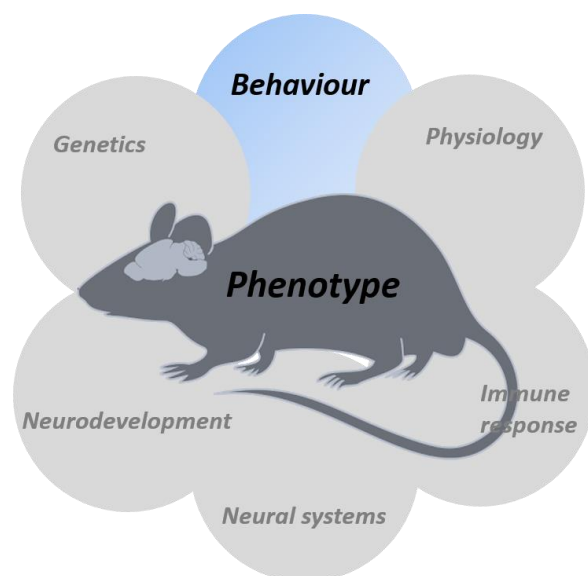


Figure 50. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour. This section aims at exploring the effects of sustained distress on effort allocation for food reward, which is considered a motivational component potentially influencing decisional processes. Understanding how chronic CORT exposure impacts effort valuation and processing will help comprehending the origin of suboptimal DM in CORT-treated mice during the adapted gambling task.

partake in physical and mental activity), over-estimation of the effort necessary for obtaining rewards (altered *effort valuation*) and reduced reward anticipation could be at the origin of motivational deficits in this clinical population, potentially leading to a suboptimal integration of cost/benefit information (Treadway and Zald, 2011). Preclinical studies have succeeded in modelling depressive-like motivational deficits via effort-related operant tasks, and in particular chronic CORT administration has been demonstrated to interfere rodents' instrumental conditioned responding (i.e., sustained effort) to food reward (Dieterich et al., 2020, 2019; Gourley et al., 2008; Olausson et al., 2013). However, differences in the experimental design, including duration of the treatment, use of nose-poke vs lever-press tasks and different rewarding foods, prevent from accurately discerning between motivation-related aspects as intolerance for increasing efforts, satiety and reward appreciation. Here efforts have been made to harmonize parameters already approached (treatment duration, food reward nature, satiation effect) in order to accordingly measure motivational components potentially influencing DM in CORT-treated animals.

A. Materials and Methods

Animals: Forty-two *C57BL/6JRj* mice were used for this study (free-feeding weight: mean \pm SEM (g) = 26.57 \pm 1.89). The behavioural testing started at the seventh week of treatment (VEH, n=21; CORT, n=21).

Experimental protocol: To address effort valuation as component of animals' motivational state, an operant protocol of Progressive Ratio (PR) schedule of reinforcement was used (adapted from Sharma et al., 2012). For this purpose, a habituation period and an operant training were necessary before testing.

Habituation. In order to habituate animals to the device, they were introduced during 10 min for 3 consecutive days in the operant chambers. During this time, mice could explore an empty and illuminated food receptacle in one side, and two unilluminated identical holes in the opposite side of the chamber. The third day they could find food in the food receptacle.

Training. Following the habituation sessions, mice were trained to nose-poke in one of the holes on a Fixed Ratio (FR)-1 schedule of reinforcement, where a single nose-poke elicited the delivery of a food pellet in the receptacle. Only the lit hole was designated as "active" and triggered the reward delivery. The allocation of the active and inactive holes was random for each mouse when no spatial preference was evidenced during the habituation period. When a mouse displayed a spatial preference (twice as frequent visits to a hole respective to the other), the active hole was selected against it. Five-second timeout to the FR-1 was given after a nose-poke, for both, right or wrong choices. During the timeout, additional nose-pokes did not result in reward delivery, giving mice the time to consume the food. Each training session lasted an hour, or until a maximum of 50 rewards were delivered. Animals were considered to acquire a food-maintained operant responding (*acquisition criteria*) when they (1) exhibited a discrimination index of $\geq 3:1$ for the active versus inactive responses, (2) obtaining ≥ 20 rewards per session (3) over three consecutive sessions. When these acquisition criteria were fulfilled, the schedule was increased to FR-5 for which five active nose-pokes triggered the delivery of one food reinforcer. The FR-5 training lasted 3 consecutive days.

Effort-related choice testing. After completion of the operant training, mice continued in a PR schedule of reinforcement. The ratio schedule for the PR testing was calculated as previously described

(Richardson and Roberts, 1996) using the following formula: $[5e^{(R*0.2)}] - 5$, where R is the number of rewards already earned plus 1. The ratios to obtain a food reward are: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95 and so on. The last ratio completed by an animal is its breakpoint (BP). A PR session lasted an hour or ended after 10 min of inactivity (absence of visits to the holes or the food receptacle). The PR reinforcement testing finished when the number of rewards earned in a session deviated $\leq 10\%$ for 3 consecutive days.

B. Results

1. Chronic CORT exposure delays acquisition of action-outcome contingency in the FR-1 schedule of reinforcement

The treatment's effect on instrumental acquisition, i.e., the formation of an action (nose-poke) – outcome (reward delivery) associative contingency, was evaluated from the resulting FR-1 data. The comparison revealed that chronic CORT administration resulted in animals requiring more sessions to comply with the acquisition criteria (mean of total number of FR-1 sessions \pm SEM, VEH: 4.62 ± 0.51 ; CORT: 6.37 ± 0.96) [UMW: $Z = -2.0$, $p < 0.05$], which is in agreement with previous studies (see for instance Dieterich et al., 2019) (**Figure 51 A**). To complement this result, the total number of correct responses (equivalent to the number of obtained rewards) made by animals across the three first FR-1 sessions was also compared across the different conditions. A RM-ANOVA revealed a learning effect across the sessions [main effect of session: $F_{2,80} = 65.7$, $p = 0.0000$, $p\eta^2 = 0.62$] and a significant reduced number of correct responses resulting from the pharmacological treatment (mean number of correct responses \pm SEM, VEH: 110.33 ± 7.42 ; CORT: 72.24 ± 7.23) [treatment: $F_{1,40} = 13.5$, $p < 0.001$, $p\eta^2 = 0.25$]. Contrary to expected, a significant interaction between factors was not revealed [session x treatment: $F_{2,80} = 0.69$, $p = 0.50$, $p\eta^2 = 0.02$] (**Figure 51 B**). Combined, these results suggest that chronic CORT exposure impairs the formation of action-outcome contingencies in operant tasks.

2. Chronic CORT administration in mice decreases effort allocation in an effort-related choice task

Preceding the PR reinforcement testing to study effort allocation as an evaluative element of the animals' motivational state, mice were evaluated in a transitional FR-5 schedule of reinforcement. The quantity of obtained rewards was found to be influenced by the pharmacological treatment [main effect of treatment: $F_{1,40} = 26.3$, $p = 0.0000$, $p\eta^2 = 0.40$], with CORT-treated animals (mean of total number of rewards \pm SEM: 96.86 ± 8.45) obtaining a smaller amount of food than control animals (144.95 ± 4.06) (**Figure 51 C**), but without significant effect of the session [session: $F_{2,80} = 1.7$, $p = 0.18$, $p\eta^2 = 0.04$; session x treatment interaction: $F_{2,80} = 0.01$, $p = 0.99$, $p\eta^2 = 0.00$]. CORT and VEH-treated animals also differed in the percentage of correct responses across the FR-5 reinforcement phase [treatment: $F_{1,40} = 9.7$, $p < 0.01$, $p\eta^2 = 0.19$], though both groups responded almost always correctly (mean percentage of correct responses \pm SEM, VEH: 93.84 ± 0.98 ; CORT: 89.67 ± 0.92) [session: $F_{2,80} = 1.1$, $p = 0.34$, $p\eta^2 = 0.03$; session x treatment interaction: $F_{2,80} = 0.6$, $p = 0.55$, $p\eta^2 = 0.01$] (**Figure 51 D**). However, these differences need to be carefully interpreted and are not to be directly attributed to the animals' motivational state, since intrasession satiation could have had a behavioural impact (a maximum delivery of 50 rewards).

The explicit study of motivation to expend effort for a food reinforcer was assessed through the operant nose-poke-based PR reinforcement test, which revealed a significant deleterious effect of the pharmacological treatment, which again is in line with previous studies (Dieterich et al., 2019;

Olausson et al., 2013). Animals were tested daily on the PR reinforcement schedule until they stabilized their performances, i.e., they reached their BP, and no difference between pharmacological conditions was found for the number of required sessions (mean number of sessions \pm SEM, VEH: 5.79 ± 0.57 ; CORT: 8.15 ± 1.45) [UMW, $Z = -0.6$, $p = 0.57$] (**Figure 51 E**). Congruently with the scientific literature, these results showed that mice chronically treated with CORT had a lower BP in the PR paradigm (mean BP \pm SEM, CORT: 85.33 ± 13.62) compared with animals from the control group (VEH: 139.57 ± 14.96) [$Z = 3.2$, $p < 0.01$] (**Figure 51 F**). In other words, chronic CORT administration lessened the animals' motivation to expend effort for food rewards, in agreement with previous findings on altered reward and effort valuation (see for instance Gourley et al., 2008).

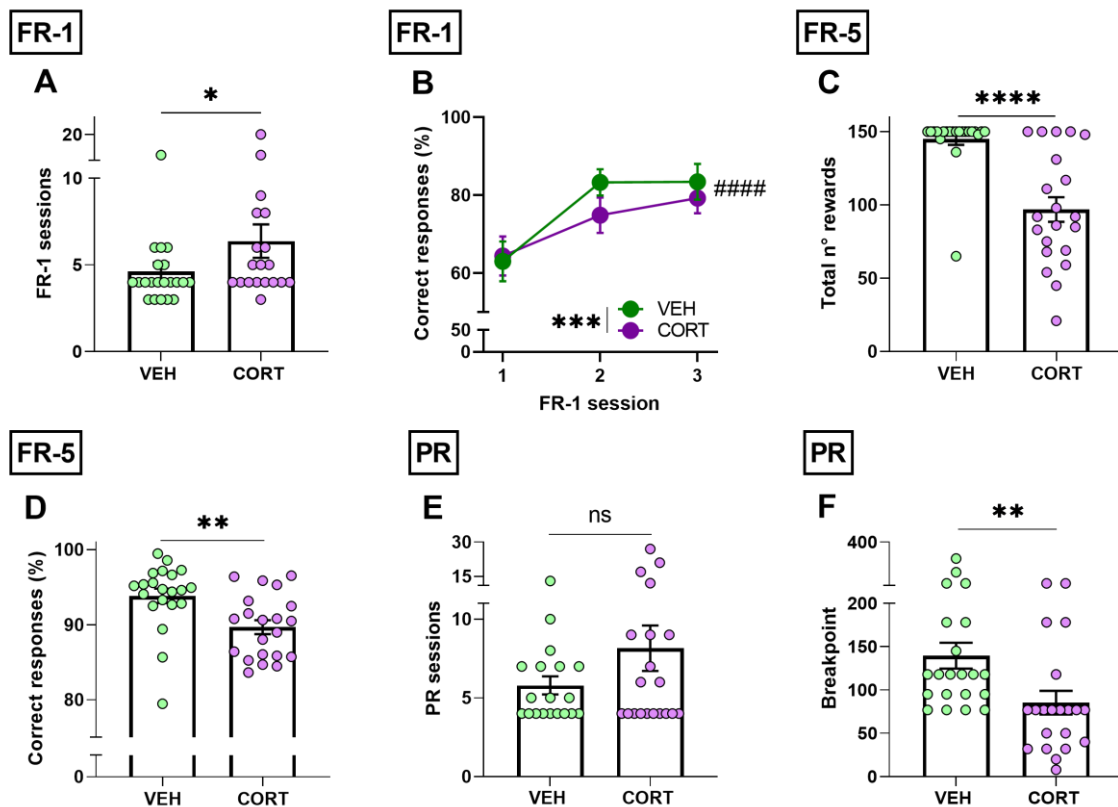


Figure 51. Reduced instrumental conditioned responding in mice after chronic CORT administration. (A) Compared with control animals, CORT-treated mice required more sessions to reach the acquisition criteria in the FR-1 reinforcement phase, indicating a detrimental effect of the pharmacological treatment in action-outcome association (Mann Whitney U: *, $p < 0.05$). **(B)** Animals of both conditions improved the proportion of correct responses during the three first FR-1 sessions (RM-ANOVA: ####, $p = 0.0000$), but the overall performance of CORT-treated animals was less accurate than in control animals (RM-ANOVA: ***, $p < 0.001$). **(C)** During the transitional FR-5 phase, CORT-treated animals obtained significantly less food rewards than control animals (RM-ANOVA: ****, $p = 0.0000$), and **(D)** even if they predominantly answer correctly, their performance was worse than in VEH animals (RM-ANOVA: **, $p < 0.01$). **(E)** All animals, irrespective of their condition, required a similar number of sessions to reach their BP in the PR testing (Mann Whitney U: ns, not significant). **(F)** However, CORT-treated animals significantly allocated less effort to obtain food reward, indicating a deleterious effect of the pharmacological treatment on reward and effort valuation and processing (Mann Whitney U: **, $p < 0.01$).

3.4. Evaluation of behavioural flexibility.

Objective: The fourth objective of this chapter is to evaluate the effect of chronic CORT exposure on behavioural flexibility to obtain a better insight about its influence in reward responsiveness leading to suboptimal decision-making in the CORT model (**Figure 52**).

Cognitive flexibility refers to mental processes required for adjusting behaviour to novel situations, enabling individuals to respond disengaging from previous tasks for reconfiguring new and adapted responses. *Behavioural flexibility* refers to the ability to adaptively modify behaviour in a dynamic environment. Both constructs, cognitive and behavioural flexibility, are closely intertwined and manifest themselves through high-level executive processes enabling *mental set-shifting* (i.e., the ability to switch back and forth between multiple tasks), *information updating and monitoring* via WM processes, and *prepotent responses inhibition* (i.e., the inhibition of the automatic behavioural responses associated to immediate reinforcement) (Dajani and Uddin, 2015; Seu et al., 2009; Uddin, 2021). Two main types of behavioural flexibility can be discerned: *strategy shifting* and *reversal learning* (RL). The former refers to the ability to actively suppress a previously learnt rule and acquiring a new strategy by shifting across modality to the opposite rule. For example, shifting response from visual to tactile stimuli. Reversal learning however, requires the acquisition of a new strategy by shifting within modality to the opposite contingency, e.g., by shifting from one visual stimulus to another visual but different stimulus. Even if strategy shifting and RL are usually dissociated in studies on behavioural flexibility, they have been described as being hierarchically organized and therefore are not completely independent (Young and Shapiro, 2009).

Cognitive and behavioural flexibility are affected in a wide range of clinical conditions, including attention-deficit/hyperactivity disorder (ADHD), schizophrenia and mood disorders, and in high heterogeneous deficit patterns (Uddin, 2021). To study these flexibilities and their relationship with a wide variety of stressors, several animal tasks have been developed. Impaired behavioural flexibility has been described in animal models of chronic stress on strategy shifting and RL tasks (Dieterich et al., 2019; Hurtubise and Howland, 2017), but these effects seem to be highly dependent upon type, intensity and duration of the stressors (Hurtubise and Howland, 2017). As per motivation-based effort-allocation testing, efforts have here been made to harmonize parameters (treatment

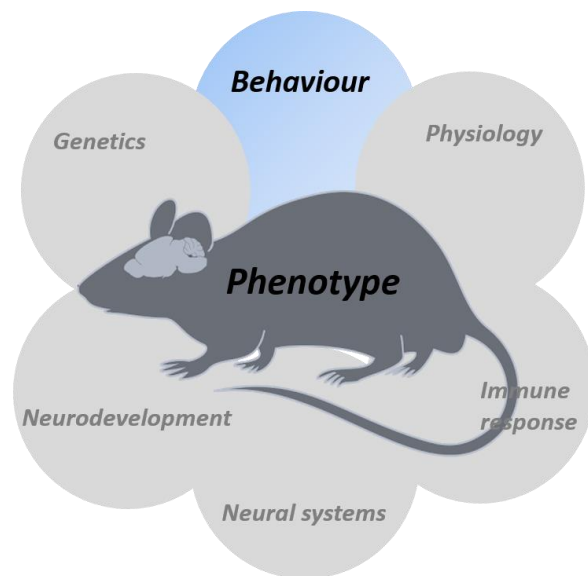


Figure 52. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour. This section aims at exploring the effects of sustained distress on behavioural flexibility, which is a cognitive component required for behavioural adjustment and learning in versatile environments. The study of the effects of chronic CORT exposure on behavioural flexibility will be useful to better understand the origins of the observed delayed reward responsiveness during the gambling task in CORT-treated animals.

duration, food reward nature, satiety effect) in order to accordingly measure behavioural flexibility in CORT-treated animals, as potential modulator of DM processing during the ^AmGT.

A. Materials and Methods

Animals: Thirty *C57BL/6JRj* mice were used for this study (free-feeding weight: mean \pm SEM (g) = 25.86 \pm 2.54). Half the individuals were CORT-treated (n=15) and the other half constituted the control group (VEH, n=15).

Experimental protocol: Following the PR reinforcement schedule testing (*Third Chapter, Section 3*), mice rested one day before their behavioural flexibility was evaluated using a RL task. Animals were exposed to a FR-5 schedule similar to the operant training for two consecutive days and, from then onwards, the active and inactive holes were reversed: the unilluminated hole was the one rewarding the animals after 5 consecutive visits (shift of modality to the opposite contingency). The RL testing lasted 2 days with 2 consecutive sessions per day separated with an intersession time of 10 min (a total of 4 testing sessions). Each session lasted an hour or until a maximum of 50 rewards were delivered. The relative number of correct answers for each RL session was calculated as a measure of animal behavioural flexibility (referred here as RL performance). Individual learning rates (i.e., the ratio between performances of consecutive RL sessions) were calculated and used to further study behavioural adaptability to the contingency shift. Subgroups of individuals based on differential learning rate were identified by k-mean clustering.

B. Results

1. Chronic CORT administration delays behavioural adaptation to changing environmental contingency in a reversal learning task

In order to figure out if CORT-induced suboptimal ^AmGT performance could result from impaired behavioural flexibility, and since the ability to behavioural adjustment is crucial for gain optimization, mice were tested in a RL task. In order to obtain reward animals learnt to shift from visiting the lit to the unlit hole as the task progressed, thus improving their RL performance [main effect of session: $F_{3,84}=56.2$, $p=0.0000$, $p\eta^2=0.67$]. However, no general difference was found between pharmacological conditions [treatment: $F_{1,28}=0.1$, $p=0.77$, $p\eta^2=0.00$] but an interaction between factors [session x treatment interaction: $F_{3,84}=3.1$, $p<0.05$, $p\eta^2=0.10$] (**Figure 53 A**).

Individuals from both conditions significantly increased the proportion of correct responses from the second RL session, and from that moment onwards their performances stabilized [VEH, session 1 vs 2-4: all $ps<0.0001$; CORT, session 1 vs 2: $p<0.001$; session 1 v 3&4: all $ps<0.0001$; post-hoc] (**Figure 53 A**).

To further disentangle these differences, individual learning rates between the first two RL sessions were calculated. Besides three individuals with significantly higher learning ratios (VEH, n=2: learning rates of 17.01 and 6.17; CORT, n=1: learning rate of 9.08), mice clustered into two groups, named here *high-flexible* and *low-flexible*, characterized by differently adjusting their behaviour to the new task contingency. Interestingly, the *high-flexible* subgroup (46.67%) more than doubled its performance (mean learning ratio \pm SEM: 2.29 \pm 0.53) and comprises mainly VEH-treated animals (VEH:

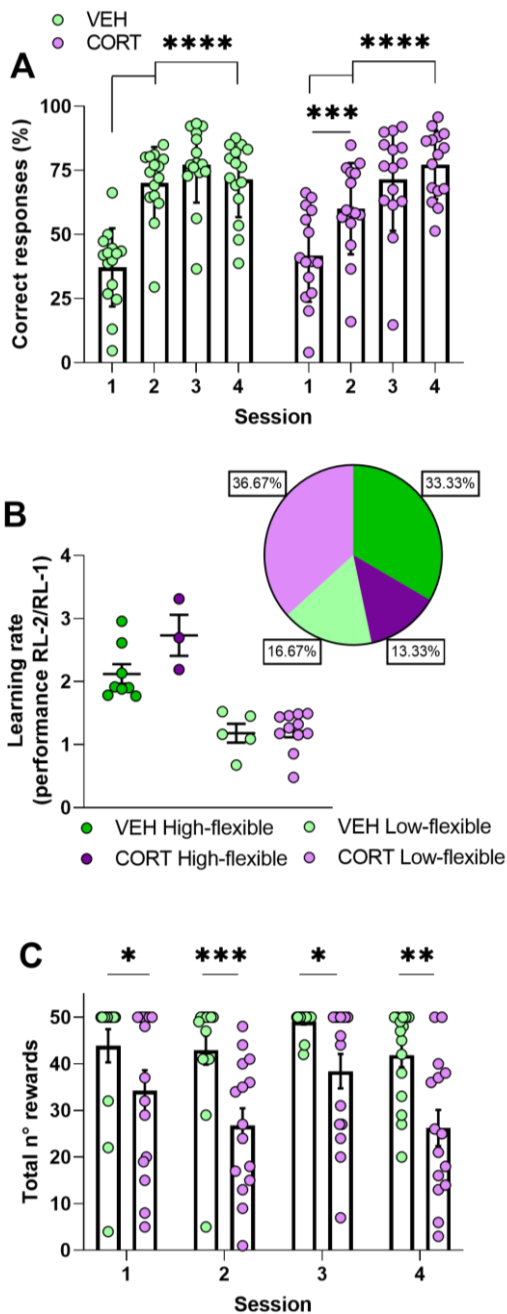


Figure 53. In mice, chronic exposure to CORT induces behavioural inflexibility in a RL task. (A) CORT and VEH-treated animals adaptively change their behaviour to the new environmental contingencies in the RL task, significantly increasing the proportion of correct responses from the second RL session (RM-ANOVA post-hoc: ***, $p < 0.001$; ****, $p = 0.0000$). **(B)** According to their learning rate (ratio of correct responses between the first two RL sessions), individuals clustered in two groups named here high-flexible and low-flexible, with differential ability to adjust their behaviour to the new task contingency (extreme high flexible not included in the representation of data dispersion, $n = 3$). The high-flexible cluster is conformed mostly by control animals, whereas low-flexible individuals are predominantly from the CORT condition, suggesting a deleterious effect of the pharmacological treatment on behavioural flexibility in the RL task. **(C)** Mice chronically treated with CORT obtained fewer rewards (total number of earned rewards \pm SEM, CORT, session 1: 34.20 ± 4.40 ; session, 2: 26.80 ± 3.66 ; session 3: 38.40 ± 3.70 ; session 4: 26.20 ± 3.87) than control individuals (VEH, session 1: 43.84 ± 3.56 ; session 2: 42.93 ± 3.113 ; session 3: 49.07 ± 0.64 ; session 4: 41.87 ± 2.59) irrespective of the session (Mann Whitney U: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

$n = 10$, 71.43%; CORT: $n = 4$, 28.57% -extreme flexible individuals included). Individuals from the low-flexible subgroup (53.33%) however, only improved their performance by 20% (1.20 ± 0.31) and are predominantly CORT-treated animals (VEH: $n = 5$, 31.25%; CORT: $n = 11$, 68.75%). The distribution of VEH and CORT animals across the two subgroups shows to be significantly different [χ^2 , $p < 0.05$] (see **Figure 53 B**).

Combined, these results show that chronic CORT exposure delays adaptive behavioural change in face of new environmental contingencies in a RL task, thus potentially compromising early stage of learning processes. This is in agreement with previous results (see *Third Chapter, Sections 2 & 3*).

2. Chronic CORT administration affects appetitive behaviour in the RL task

Since the selected RL task evaluating behavioural flexibility uses food pellets as reward, motivational aspects influencing performance shall be considered. For this purpose, the total amount of food rewards earned during this task was also compared between conditions. Comparisons showed that control animals were always more rewarded than CORT-treated animals, irrespective of the RL session [UMW, session 1: $Z = 2.0$, $p < 0.05$; session 2: $Z = 3.4$, $p < 0.001$; session 3: $Z = 2.4$, $p < 0.05$; session 4: $Z = 2.8$, $p < 0.01$]. Even if greater differences corresponding to the second and fourth RL sessions might, at

least in part, reflect satiation in CORT-treated animals in line with previous results (see *First Chapter, Section 1*), the significant differences found for the first and third RL sessions suggest that the appetitive behavioural component of motivation for allocating effort for food reward is lessened in the pathological condition (see **Figure 53 C** for details). Again, this is in agreement with the results described in the *Third Chapter, Section 3*, and with the literature (Dieterich et al., 2019).

3.5. Study on psychomotor learning.

Objective: The objective of this section is to evaluate the effect of chronic CORT exposure on psychomotor learning using a DLS-dependent motor task that does not rely on reward appreciation (**Figure 54**).

Psychomotor learning processes are understood as learning processes that implicate a close relationship between cognitive functions and physical movement. Patients of various clinical conditions, including severe forms of depression, show psychomotor deficits frequently referred as *psychomotor retardation*. These are characterized by a slowing-down of mental processes in parallel with a reduction of physical movements. To address this symptomatology in rodents, rotarod tasks dependent on dorsostriatal networks integrity have been developed (Dang et al., 2006; Le Merrer et al., 2013; Lovinger, 2010; Yin et al., 2009), since these brain networks are known to participate in motor performance consolidation (Shiotsuki et al., 2010) via motor habit formation. Given the contribution of habit formation on instrumental behaviour, and since maze-based tasks partially rely on motor capabilities, psychomotor learning is here addressed. The aim is to primarily investigate if chronic CORT-induced suboptimal ^AmGT performance can result from psychomotor deficits in relation to the intrinsic effort-related dimension of the task.

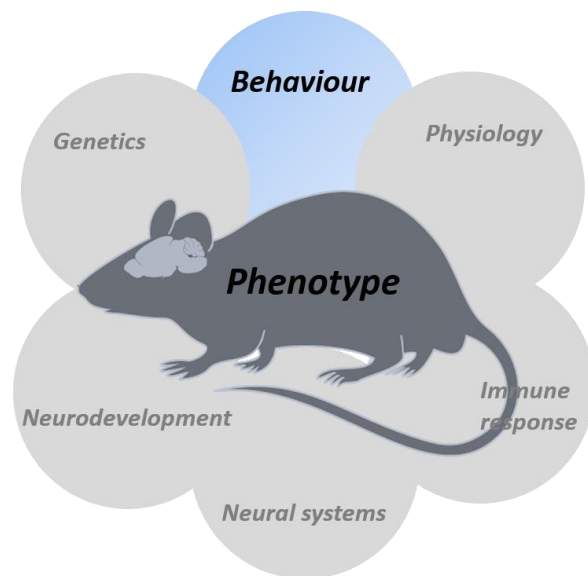


Figure 54. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour. This section aims at investigating the effects of sustained distress on psychomotor learning. The ability to learn and improve physical movements can be truncated in distressful situations and lead to maladaptive behaviour. Investigating how chronic CORT exposure affects psychomotor learning in mice will therefore help understanding and interpreting the origins of suboptimal DM during the value-based gambling task in CORT-treated animals.

A. Materials and Methods

Animals: Eighty *C57BL/6J* mice were tested in a motor learning task (MLT) after 11 weeks of differential treatment (VEH, n=40; CORT, n=40; free-feeding weight: mean \pm SEM (g) = 26.20 \pm 0.26).

Experimental protocol: Psychomotor learning skills were evaluated using a rotarod task (adapted from Le Merrer et al., 2013). Mice were trained to run on a 3 cm diameter automatized rotor (Mouse RotaRod NG, *Ugo Basile*[®] SRL, Gemonio, Italy), accelerating from 5 to 40 rpm in 5 min. Testing lasted 4 consecutive days after a first *habituation* session where animals learnt to stay running on the rotor at constant speed of 5 rpm, for at least 90 sec. During the 4 following sessions, mice were tested for 3 consecutive trials, with an inter-trial interval of 60 sec. Mice were placed on the rotor in a constant 5 rpm-speed rotation for a minute, and then the accelerating program was launched. Trials ended when they fell off the rotor or after 10 min.

B. Results

1. Altered motor performance in CORT-treated mice in a rotarod-based MLT

The aim was to study whether psychomotor performance sub-serving execution and exploitation of the ^AmGT was impacted by chronic CORT exposure, and as a measure to circumvent putative effects of motivational-based facilitation of striatal responses by food that might occur in maze-based tasks. For that, mice were tested in a rotarod task after 11 weeks of treatment. The results show that animals improved their performance along the task, i.e., they learnt to stay longer on the accelerating rotor [main effect of session: $F_{3,636}=38.5$, $p=0.0000$, $\eta^2=0.15$], but chronic CORT administration impaired their general performance [treatment: $F_{1,212}=12.7$, $p<0.001$; $\eta^2=0.06$]. Nevertheless, no significant interaction was found between factors [session x treatment interaction: $F_{3,636}=0.9$, $p=0.45$, $\eta^2=0.00$]. When comparing individual sessions, CORT animals stayed on the rotor for a shorter time (mean time on the rotor (s) \pm SEM, session 1: 221.77 \pm 8.70; session 2: 277.39 \pm 13.53; session 3: 302.99 \pm 13.40) than animals from the control group in the first three sessions (session 1:

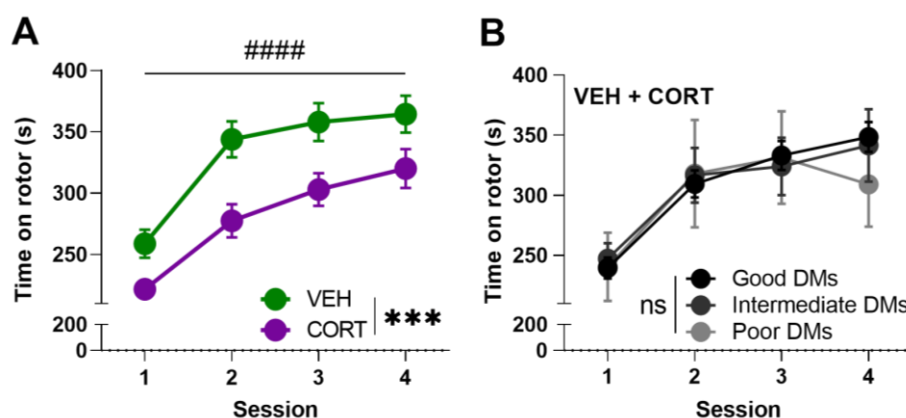


Figure 55. Reduced motor performance in a rotarod-based task in mice chronically treated with CORT. (A) All animals, irrespective of the treatment, improved their motor performance along the MLT (RM-ANOVA: ####, $p=0.0000$), but CORT-treated animals fell off the rotor earlier than control animals (RM-ANOVA: ***, $p<0.001$). **(B)** Individuals from the three decisional profiles similarly performed in the MLT (RM-ANOVA: ns, not significant).

258.80±11.36; session 2: 343.77±14.76; session 3: 357.92±15.54), a difference that was reduced at the end of the task (session 4, VEH: 364.40±15.08; CORT: 320.06±15.75), thus suggesting a motor alteration in the pathological condition (**Figure 55 A**).

2. Similar psychomotor performance in individuals from different decisional profiles in the ^AmGT

Animals of the present experiment were previously tested in the ^AmGT, allowing the psychomotor characterization of the three DM profiles. Good, intermediate and poor DMs did not behave differently during the rotarod task [main effect of cluster: $F_{2,211}=0.1$, $p=0.93$; $\eta^2=0.00$] (**Figure 55 B**) and motor scores do not correlate with final ^AmGT performance [$r=0.0497$, $p=0.47$]. Combined, these results suggest that the CORT treatment might interfere in early motor capabilities, but differential ^AmGT performance does not seem to directly rely on psychomotor skills.

3.6. Summary and discussion of the results

In this third chapter, various cognitive dimensions have been studied in animals chronically treated with CORT. The objective was to identify if these various cognitive dimensions potentially act as behavioural modulators of ^AmGT performance.

The results show that chronic CORT exposure generally has a detrimental effect on motivational components subserving goal-directed behaviour and specifically, on appetitive and consummatory aspects involved in reward and effort valuation, and subsequent learning processes.

Mice cognitive evaluation was here based mostly on food rewarding tasks (with exception of the rotarod task), thus hedonic appreciation of the reinforcers needed to be addressed. In its study, altered appetitive behaviour observed in animals from the pathological condition, as shown for instance by the increased presence of *undecided* individuals in the FFT, indicates a pharmacological effect on the animals' ability to initiate behavioural actions. However, the results concerning consummatory behaviours towards rewarding experience partially dissent from the initial working hypothesis, which expected changes in reward valuation. Indeed, animals of both conditions share similar SPT scores, and do not differentiate in their choice of reward in the FFT, but the overall food intake in the latter is lower in CORT-treated animals. Thus, chronic GC exposure may affect hedonic appreciation of rewards through restraining initiation of actions, rather than through obstruction of reward valuation, in a manner reminiscent to human abulia (Husain and Roiser, 2018).

The study of positive valence-based motivated behaviours in rodents is valuable for translational research in reinforcement learning, but unfortunately literature is incongruent with regards to findings concerning the CORT model. As chronic CORT administration induces a negative valence phenotype (see *Second Chapter, Section 1*; David et al., 2009), not all aspects of hedonic appreciation, including reward valuation, addressed through the SPT and the FFT here found were to be altered. Most importantly for the present doctoral study, individual differences in mice preference for sweetness do not relate to their ^AmGT performance, contrary to previous studies that showed different SPT scores in healthy mice displaying different gambling strategies (Pittaras et al., 2016). These differences might result from protocol differences, and since various sucrose concentrations are used in these experiments, data variability might arise due to ceiling effects on palatability. Nevertheless, the trend observed between gambling performance and consummatory scores in the

FFT (i.e., total intake) in CORT-treated animals suggests that hedonic appreciation could, to a certain degree, account for differential DM. A likely cause is a modulation of the reinforcement of actions following option selection and/or the reward prediction error. However, results are not conclusive and further investigations are necessary in order to disentangle the contribution of hedonic impact on Δ mGT performance.

Interestingly, the FFT paradigm, originally chosen to evaluate hedonic appreciation and particularly reward valuation, allowed addressing other behavioural aspects potentially influencing DM through the Δ mGT. Chronic CORT administration was found to exacerbate food neophobia in mice, but most importantly it prompted indecision. Animals treated with CORT were more frequently in a state of major hesitation or difficulty in initiating their choice between food rewards when one option was unfamiliar to them. This result adds value to data suggesting that chronic CORT exposure yields mice especially vulnerable to action-outcome encoding and further consolidation in food reward-based tasks, and in accord with previous results of this manuscript (*Second Chapter, Section 2*).

Based on food reward-based operant tasks such as PR schedule of reinforcement and probabilistic RL paradigms, previous studies have affirmed that positive valence behaviours are impaired in CORT-treated animals (Dieterich et al., 2019). The working hypotheses of the present study do not directly confront this statement, but hedonic appreciation was differently approached. The here operant-based experimental design enabled revealing the slower learning rates and delayed formation of action-outcome associations in individuals chronically exposed to high circulating levels of GC, in accord with the prolonged exploration phase exposed in the Δ mGT (see *Second Chapter, Section 2*). Instrumental acquisition was impaired after chronic CORT administration, as treated mice required longer training to reach the acquisition criteria, i.e., to sufficiently reinforce their response for food reward. A CORT-induced dysfunctional amotivated state in animals was further substantiated by the evaluation of effort allocation. It revealed a significant reduction in their effort for food reward in the PR scheduled paradigm, in line with the literature (Dieterich et al., 2019). Despite the fact that they were food deprived and the effect of satiation had been limited, instrumental conditioned responding was reduced in CORT-treated individuals with respect to control animals, and their internal drive to complete a food reward-based task was curtailed. In combination with RL-related inflexibility, altered reward/effort valuation, processing and integration point to chronic CORT exposure as a mean to obstruct action-outcome encoding over cue-processing, yielding to poor operational learning. Thus, in tasks such as the Δ mGT where success depends on reward responsiveness through reinforcement learning, reduced learning capabilities may likely hamper the integration of task contingencies necessary to gain maximization.

In the same line, CORT-induced suboptimal spatial WM performance seems to relate more to the learning rate than to the memory load. Hence, spatial WM deficits might contribute to poorer early exploration in the Δ mGT from impending integration of task contingencies, in line with preclinical (Bagneux et al., 2013; Hinson et al., 2002; Jameson et al., 2004; Turnbull et al., 2005) and clinical reports (Bourke et al., 2012). However, chronic CORT does not affect learning but impacts motor performance in the rotarod task, the only task that does not rely on motivational food-based cues, thus evoking clinical psychomotor retardation (Bennabi et al., 2013). Even if they do not directly predict differential DM, these spatial WM and motor deficits could somehow compromise the transition from goal-directed to habit-based behaviour in the Δ mGT, thus from the exploration to the exploitation phase. Optimal gambling performance benefits from reward responsiveness after an effective

exploration of options, during which reward and effort values are integrated. Maintaining and invigorating advantageous course of actions are therefore essential for successful environmental exploitation.

Overall the results of this third chapter are largely in agreement with initial working hypotheses, denoting that optimal DM under uncertainty is likely compromised after prolonged GC exposure through alteration of action-outcome encoding in goal-directed behaviours. This is in accord with clinical data (Darcet et al., 2014; Salamone et al., 2018), and suggest that appetitive and consummatory aspects of motivational-related DM components are key players in the modulation of this function. Consequently, the presented results support the suitability of chronic CORT administration on translational investigations addressing motivational-based learning impairments typical across multiple clinical conditions.

FOURTH CHAPTER – Neurobiological correlates of sustained distress-induced suboptimal decision-making

Part of the following results have been presented in the scientific publication “*Chronic exposure to glucocorticoids induces suboptimal decision-making in mice*”, ENPP, 2021 (see [Annex IV](#)).

4.1. Differential contributions to decision-making of the glucocorticoid receptor, mineralocorticoid receptor and corticotropin-releasing factor neuropeptide protein expression in telencephalic brain regions

Objective: The first objective of this chapter is to evaluate the effect of chronic CORT administration on the expression of the two main brain GC receptors, GR and MR, as well as on the expression of the neuropeptide CRF. Three relevant brain areas for reinforcement learning, emotional processing and stress-related symptomatology are investigated: the mPFC, the DLS and the VH (**Figure 56**).

The regulatory role of GCs on HPA axis activity (Smith and Vale, 2006) has indicated imbalances in the expression of GR and MR as biomarkers of depressive states (de Kloet et al., 2018; Zhe et al., 2008). The HPA axis activation is a tightly controlled process, with GRs playing a prominent role. Since MRs have a high affinity for GCs, they mainly participate in regulating HPA basal-tone. Glucocorticoid-receptors, however, serve primarily in the GC-mediated negative feedback after distress exposure of various responsive brain regions (Smith and Vale, 2006). The study of the GR/MR expression ratio is therefore an informative strategy to address responsiveness and neural adjustment following chronic distress (de Kloet et al., 2018; Pariante and Lightman, 2008; Zhe et al., 2008).

Since several studies have linked the CRF activity to the emergence of anxiety-depressive symptomatology, examining its involvement in stress-induced cognitive dysfunction is also of interest for the present project (Arborelius et al., 1999; Binder and Nemeroff, 2010; de Kloet et al., 2005; Gillespie and Nemeroff, 2005; Uribe-Mariño et al., 2016; Zobel et al., 2000). The CRF is the major

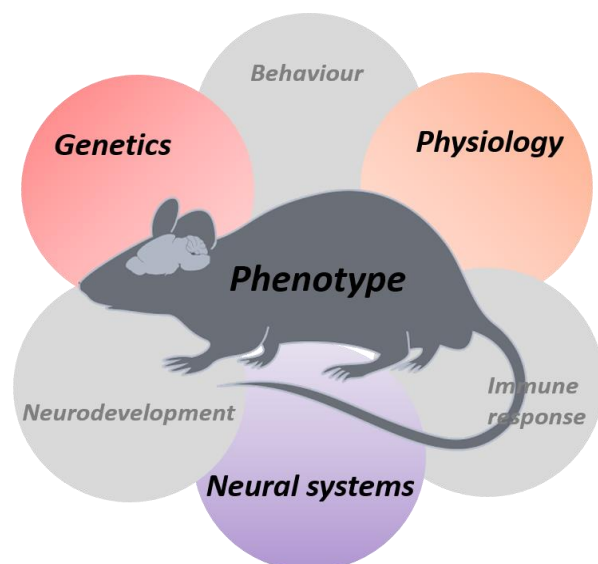


Figure 56. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Physiology, neural systems and genetics. This section aims at investigating the effects of sustained distress on the expression of the brain GC receptors and the CRF neuropeptide in three cerebral areas relevant for DM and stress-related symptomatology. The expression of GR, MR and CRF will be calculated in the mPFC, DLS and VH of mice chronically treated with CORT. The objective is to understand how the brain physiologically adjusts to sustained distress. The levels of expression will be systematically reported to those of a healthy, non-treated mice population. The correspondence between protein expression and interindividual behavioural variability will be also explored.

activator of the HPA axis and leads the neuroendocrine stress-response. As a neuropeptide, it also modulates extra-hypothalamic, cortical and subcortical neuronal activity. In addition, CORT and CRF synergetic effects have been confirmed in stress-induced memory alterations in preclinical studies (Chen et al., 2016).

Despite the complex network underlying DM and anxiety-depressive emotional disturbances, the three brain regions targeted in this study were chosen based on their contribution to decisional processes and for their sensitivity to stress. The mPFC and the DLS are respectively described as modulators of goal-directed and habit-based learning processes (Daw et al., 2005; Schwabe and Wolf, 2009). The VH in turn is involved in stress and emotional processing, exerting strong regulatory control on the HPA axis (Fanselow, 2010).

A. Materials and Methods

Animals: After being VEH- or CORT-treated for 12 weeks, the 80 *C57BL/6JRj* mice (free-feeding weight: mean \pm SEM (g) = 26.20 ± 0.26) that were tested in the ^AmGT (*Second Chapter, Section 2*) were sacrificed for protein quantification by Western Blotting.

Experimental protocol: Animals were sacrificed by rapid cervical dislocation in the central hours of the light cycle (from 2:00 p.m.). Brains were removed, snap-frozen and stored at $-80\text{ }^{\circ}\text{C}$ until processed. Bilateral samples from the mPFC (from 2.3 to 1.3 mm anterior to Bregma -aB), the DLS (from 1.1 to 0.1 mm aB) and the VH (from 2.8 to 3.8 mm posterior to Bregma -pB) were obtained from 1 mm-thick coronal sections using a cryostat (see **Figure 57**). Samples were individually sonicated in lysis buffer RIPA (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.5% sodium desoxycholate) supplemented with protease inhibitors (*Roche Diagnostics*, Meylan, France), centrifuged at 10000 g for 10 min at $4\text{ }^{\circ}\text{C}$ and stored at $-80\text{ }^{\circ}\text{C}$. Protein concentrations were estimated using a Bradford protein assay according to the manufacturer's recommendations (*Bio-Rad*, Marnes-la-Coquette, France) and 30 μg of each sample were solved in Laemmli buffer (*Bio-Rad*) and separated by a mixed 7.5-12% SDS polyacrylamide gel electrophoresis (GR and MR, and CRF respectively). After migration and transfer to PVDF membranes (*GE Healthcare*, England), blots were saturated in TBS-Tween 20 buffer (0.5% mM Tris-HCl, 45 mM NaCl, 0.05% Tween 20, pH 7.4) with 5% non-fat milk solution for an hour. Membranes were incubated with primary antibodies overnight at $4\text{ }^{\circ}\text{C}$ and with conjugated secondary antibodies for 2 hours at room temperature (see **Table 5**). Membranes were then exposed to chemiluminescent substrate (ECL Western Blotting Substrate, *PierceTM*) followed by film exposure (Hyperfilm ECL, *GE Healthcare*) or by using ChemiDoc XRS+ with image lab software (*Bio-Rad*). Membranes were reprobated with anti- β -actin, which served as a loading control and allowed normalization for sample comparison. For each antibody, protein extracts of different mouse organs

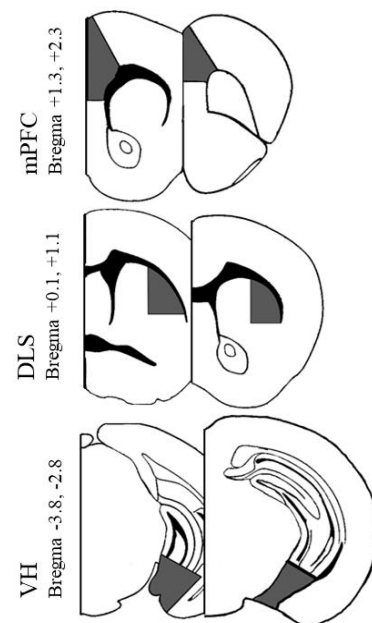


Figure 57. Schematic representation of the sampled brain regions' location for protein quantification. From top to bottom, mPFC, DLS and VH (grey coloured) were bilaterally sampled from 1 mm-thick coronal sections using a cryostat at $-13\text{ }^{\circ}\text{C}$, before being processed for protein quantification by Western Blotting.

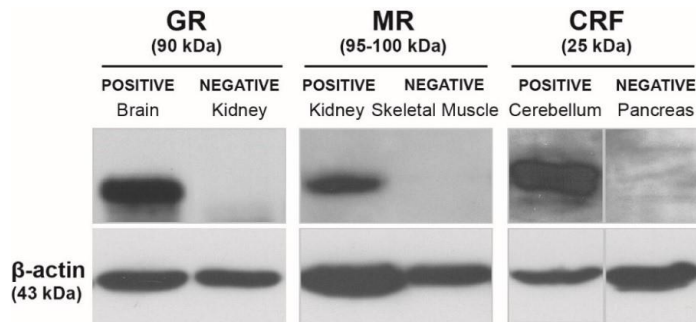


Figure 58. Western blot positive and negative controls for GR, MR and CRF antibodies. Different organs were identically treated as the targeted brain regions during protein extraction and quantification, and then used as positive and negative controls for GR, MR and CRF antibodies.

were used: brain, kidney and cerebellum as GR, MR and CRF positive controls respectively; kidney, skeletal muscle and pancreas as negative controls (see **Figure 58**).

All quantifications were carried out blind to the experimental conditions using *ImageJ* software (National Institutes of Health, Bethesda, MD, USA) (see **Annex II: Image Analysis, Western blotting**). Due to technical issues (e.g., film development problems) some samples were not included in the final analyses,

so that final samples sizes were: mPFC, GR/MR n=72, CRF n=53; DLS, GR/MR n=70, CRF n=55; VH, GR/MR n=66, CRF n=57.

B. Results

1. Downregulation of GR expression in the mPFC following chronic CORT administration

Immunoreactive signals for GR and MR appeared on membranes at 90 and 95-100 kDa respectively. After quantification, the mPFC GR/MR ratio value of CORT animals (mean \pm SEM: 1.55 ± 0.37) was found significantly decreased compared with control animals (2.30 ± 0.55) [UMW: $Z=2.2$, $p<0.05$]. To disentangle the origin of this difference, GR and MR levels were compared separately. While MR levels did not differ between conditions [$Z=0.1$, $p=0.91$], GR levels were significantly decreased in CORT-treated animals [$Z=2.1$, $p<0.05$]. This was not the case for the other two brain regions under investigation, whose GR/MR ratios did not differ between conditions [VH: $Z=1.2$; DLS: $Z=1.0$, all $ps>0.05$]. Besides, the regulatory role of the VH on the HPA activity is unmistakable when observing **Figure 59 A,B**, with GR/MR ratios more than 10 time higher than in the mPFC and the DLS.

Further comparisons between GR/MR ratios were performed in order to expose interindividual variability. Nonetheless, individuals conforming the three DM clusters, i.e., good, intermediate and poor DMs, do not differ in their GR/MR ratio irrespective of the brain region [cluster, mPFC: $H_{2,68}=1.3$; DLS: $H_{2,65}=0.3$; HV: $H_{2,61}=0.6$, all $ps>0.05$]. This suggests that DM performance does not primarily depend on homeostatic HPA dysregulation at the GR level.

2. CRF expression levels in the mPFC of CORT-treated mice negatively correlate with Δ mGT performance

When focusing in the expression levels of the key player CRF, no significant differences were found between conditions irrespective of the brain area [UMW, mPFC: $Z=0.9$; DLS: $Z=-1.1$; HV: $Z=0.0$, all $ps>0.05$] (**Figure 59 C**). At the subgroup level, individuals conforming the decisional profiles did not either differ in their CRF levels [cluster, mPFC: $H_{2,49}=1.0$; DLS: $H_{2,52}=0.3$; HV: $H_{2,52}=4.0$, all $ps>0.05$].

Most interestingly, a significant correlation between the mPFC CRF levels and final Δ mGT performance of CORT-treated animals was observed [$r=-0.5166$, $p<0.05$] (**Figure 59 D**), suggesting that

vulnerability to suboptimal DM induced by chronic CORT relates more to CRF signaling deregulation than to GR *per se* (Figure 59 E).

GR/MR ratios and CRF expression levels in the mPFC, DLS and VH were systematically compared to all behavioural scores (FST, SPT, FFT, dWST, MLT) obtained for the same cohort of animals that were evaluated in the ^AmGT. No significant correlations were found between behavioural and neurobiological scores (data not shown).

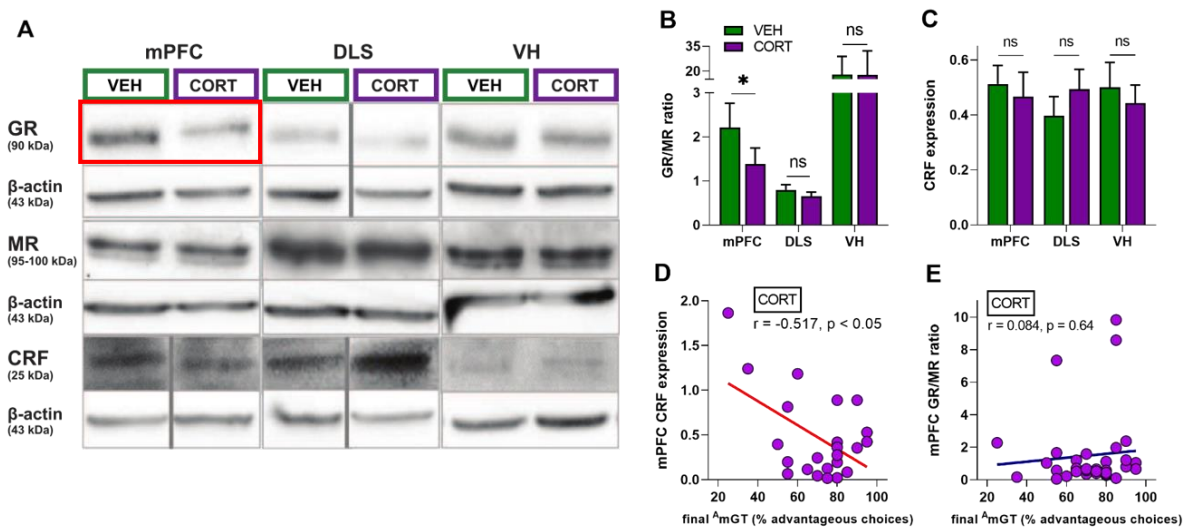


Figure 59. HPA axis imbalance in mice after chronic CORT administration. (A,B) Protein quantification by Western Blotting revealed a GR downregulation in the mPFC of CORT-treated animals that significantly diminished their GR/MR ratio value respect to control animals (Mann Whitney U: *, $p < 0.05$). The protein quantification did not reveal a differential expression due to the treatment in the VH, nor in the DLS (Mann Whitney U: ns, not significant). **(C)** Concerning the CRF, chronic CORT administration did not affect its expression in the brain regions investigated (Mann Whitney U: ns). **(D)** However, lower CRF levels in the mPFC correlate with better final ^AmGT performance in CORT-treated, but not in VEH-treated mice. **(E)** GR/MR ratio levels in the mPFC do not correlate with final gambling performance in CORT-treated animals.

4.2. Neurobiological underpinnings of effort-allocation decay in the Progressive Ratio task

Objective: The objective of this section is to identify neural networks underlying sustained functional connectivity relevant for effort-allocation during the PR testing in physiological and enduring stress conditions. This will help to disentangle the intricate relationship between motivation and DM (Figure 60).

Chronic CORT administration simultaneously compromises effort and reward processing, two major components of motivation that impact appetitive and consummatory behaviours in mice (see *Third Chapter, Sections 1 & 3*). Six to 8 weeks of chronic CORT administration significantly decrease effort allocation that mice are willing to expend for a food reward (grain-based pellet) in a PR schedule of reinforcement task. Therefore, impaired motivation could be a major cognitive component leading to suboptimal DM in gambling tasks.

Several stress-sensitive brain regions and networks are thought to be involved in anxiety-depression symptomatology as well as in gambling-related DM, such as the mPFC and the amygdala (de Visser et al., 2011). However, the convoluted relationship between the depressive phenotype resulting from long-lasting exposure to circulating GC and motivation remains elusive. To disentangle the neural underpinnings of this relationship and DM, the expression of the transcription factor FosB (together with cFos, products of the expression of immediate early genes from the Fos family and markers for chronic neuronal activation) was measured in the relevant brain areas after behavioural PR testing, defined in the following:

(i) the PL and (ii) IL cortices were studied for their role in instrumental acquisition and appetitive behaviours, for their contribution in reward anticipation and processing, and for their participation in the stress response (Akana et al., 2001; Capuzzo and Floresco, 2020; Delatour and Gisquet-Verrier, 2000).

(iii) the OFC was investigated for its direct participation in effort modulation, for its contribution in subjective outcome valuation, and for participating in the integration of contextual salient information (Gourley et al., 2010; Stalnaker et al., 2009).

(iv) the study of NAc activation (differentiating core and shell sub-parts) allowed approaching multiple motivational aspects including reward processing (Floresco, 2015; Piantadosi et al., 2017).

(v) the IC was investigated for its relevance in interoceptive information and effort-based processing, and for its involvement on the representation of anticipatory cues (Gogolla, 2017; Nieuwenhuys, 2012).

(vi) the PVN was of interest for its participation in incentive value attribution and its pivotal role on the stress response (McEwen, 2017).

(vii) the study of the BNST activation allowed approaching behavioural output signalling within the BLA-shared pathway, as well as it was relevant for its role in the integration of stress information and in food intake regulation (Herman et al., 2003; Janak and Tye, 2015).

(viii) the BLA was investigated for its pivotal contribution on motivation including reward-value encoding and processing, for its participation on the integration of incentive value dynamics, and for

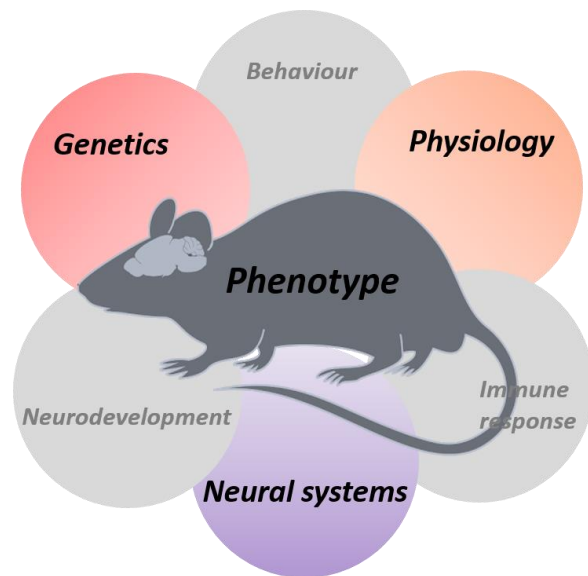


Figure 60. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Physiology, neural systems and genetics. This section aims at investigating the neurobiological underpinnings of effort valuation and processing in physiological conditions and under chronic distress. For that, the neural activation pattern of several discrete brain regions relevant for DM and stress-related symptomatology will be studied in animals chronically treated with CORT and in a health, non-treated mice population. This will help understanding the mechanisms underlying the suboptimal decisional performance of CORT-treated mice during the adapted gambling task.

its role in HPA adjustments during the stress-response (Rooszendaal et al., 2009; S.-H. Wang, 2005; Wassum and Izquierdo, 2015).

(ix) the study of the CeA allowed approaching interoceptive processing of automatic components of emotional responses, and informed about stress-related information processing and food intake regulation (Seo et al., 2016; Ulrich-Lai and Herman, 2009).

(x) the PSTN was investigated due to its significant relationship with satiation-related appetitive behaviours and food intake regulation (Barbier et al., 2020).

(xi) the VTA activation was investigated in particular for its role on reward processing and associative learning (Bouarab et al., 2019).

The experimental strategy of approaching *FosB* expression is of relevance for the present project since *FosB* plays key roles in long-term adaptive adjustments to context and upon cognitive processing, and presents persistent immunoreactivity after chronic stress (McClung et al., 2004; Perrotti, 2004) in discrete stress-sensitive areas.

A. Materials and Methods

Animals: Twenty-four hours after behavioural evaluation in the PR task (*Third Section, Chapter 3*), 12 C57BL/6JRj mice (VEH, n=6; CORT, n=6) were sacrificed for FosB immunohistochemistry.

Experimental protocol: Animals were sacrificed and brains extracted and processed as described in *Common methodology between chapters*.

Several 30 µm-thick coronal brain sections were selected in order to study the brain regions of interest: mPFC (PL and IL areas), OFC and NAc (core and shell) at [1.94 - 1.70] mm aB; rostral IC at [0.86 - 0.50] mm aB; caudal IC, PVN and dorsal BNST at [0.26 - 0.02] mm aB, amygdala (CeA and BLA) at [0.94 - 1.34] mm pB; PSTN at [2.06 - 2.30] mm pB; and VTA at [2.92 - 3.16] mm pB (see **Figure 61**; (Franklin and Paxinos, 2008).

Once mounted on gelatin-coated slides, sections were exposed to an antigen retrieval method to maximize antigenic site exposure for antibody binding (optimized protocol, data not shown). Sections were immersed for 40 min in 10 mM citrate buffer pH 6 (sodium citrate, *Sigma Aldrich*, Germany), which was previously heated at 96 °C in a water bath. The buffer was then let to cool down to room temperature before removing the sections. After washing, sections were exposed 15 min to a 0.3% hydrogen peroxide solution in order to block endogenous peroxidase activity, avoiding non-specific background staining. Next, sections were incubated 48 hours with the primary antibody at room temperature. The primary antibody (anti-FosB) was dissolved in 0.1 M PBS at pH 7.4 containing 0.3% Triton X100 (PBS-T), 1% bovine serum albumin and 10% lactoproteins. Tissues were then incubated with a secondary HRP antibody 24 hours at room temperature. After washing, the signal was amplified by using an avidin horseradish peroxidase complex (ABC Elite kit, *Vector Laboratories*) for 40 min at room temperature. A 3,3'-Diaminobenzidine (DAB) chromogen solution was used to visualize the peroxidase complex (all specific information about antibodies can be found in **Table 5**). Brain sections were dehydrated with successive alcohol baths (70°, 95°, 100° and 100°, 3 min) and cleared with xylene (*Avantor*®, 3 x 5 min). Finally, sections were coverslipped with Canada Balsam (*Roth*®, Karlsruhe, Germany). Images were acquired and analyzed as previously described in *Common*

methodology of this manuscript. Due to technical issues (e.g., deterioration of cortical regions subsequent to processing) some sections and related regions could not be included in the final analysis, and the final samples sizes were: PL n=12, IL n=12, OFC n=12, NAc n=12, rostral IC n=12, caudal IC n=11, dorsal BNST n=12, PVN n=11, amygdala n=12, PSTN n=10 and VTA n=11.

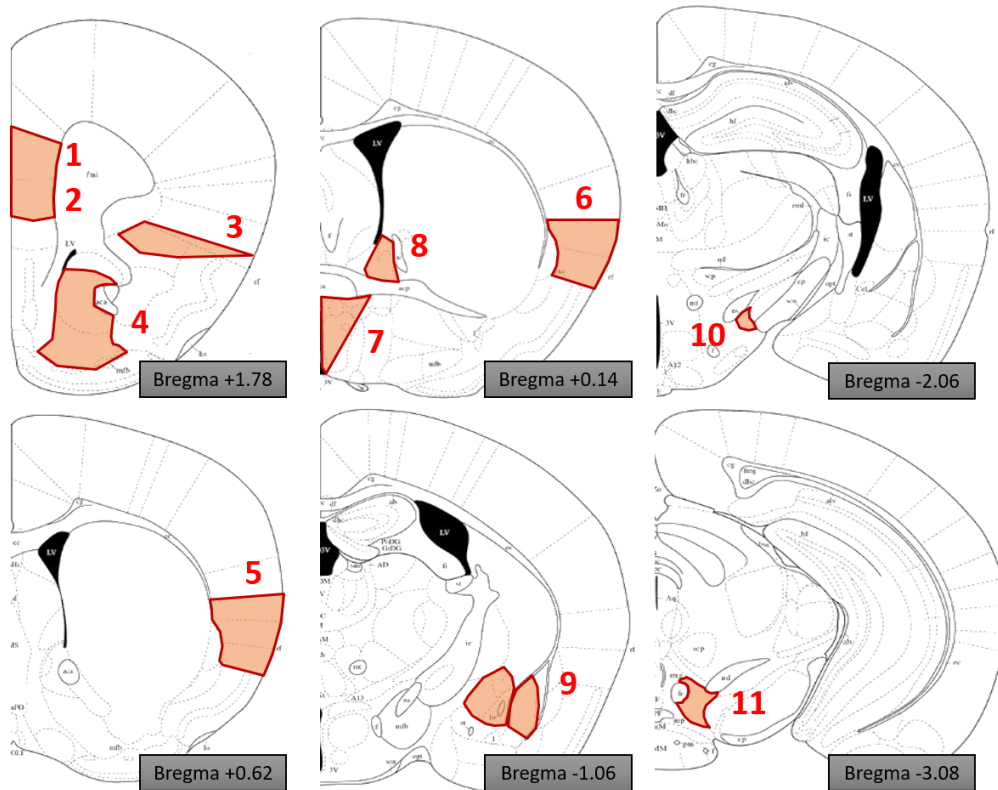


Figure 61. Coordinates of the brain sections selected for immunohistochemical analysis. Coloured areas include the brain regions of interest: (1) PL cortex, (2) IL cortex, (3) OFC, (4) NAc and NAcS, (5) rostral IC, (6) caudal IC, (7) PVN, (8) dorsal BNST, (9) BLA and CeA, (10) PSTN and (11) VTA. For the quantification of cFos and FosB expression, the regions of interest were precisely defined bilaterally (see more details in [Annex II. Image analysis, Immunohistochemistry](#)). Atlas images from Franklin and Paxinos, 2008.

B. Results

1. Decreased caudal IC and BLA FosB expression related to PR testing after chronic CORT administration

The levels of chronic neuronal activation addressed by FosB expression in the discrete brain regions of interest are presented in [Figure 62](#).

Among the targeted brain areas, the density of FosB-labelled cells was significantly decreased in the caudal IC and the BLA of CORT-treated animals (mean of FosB+ cells per mm² ± SEM, caudal IC: 265.31±6.46; BLA: 603.71±56.07) compared with control individuals (caudal IC: 369.14±38.85; BLA: 765.78±49.34) [UMW, caudal IC: Z=2.7, p<0.01; BLA: Z=2.1, p<0.05] (see [Figure 63](#)). A negative trend in FosB expression was also evidenced in the BNST (VEH: 337.41±29.97; CORT: 227.11±29.81) [Z=1.9, p=0.05] and the VTA (VEH: 342.43±26.04; CORT: 249.05±41.85) [Z=1.6, p=0.10] of CORT-treated animals ([Figure 63](#)). Otherwise, and contrary to the working hypothesis, no differences were found across conditions for neuronal activation in the other regions studied [Z<1.6, all ps>0.20] (see [Figure](#)

62 for details). Nevertheless, the large SEM values obtained for the CeA of the CORT condition suggest that this dataset should be replicated in order to circumvent inaccurate interpretation.

These results therefore indicate an impact of the pharmacological treatment on effort and reward valuation and processing during the PR testing, through the induction of aberrant functional neural network plasticity on caudal IC and BLA respectively.

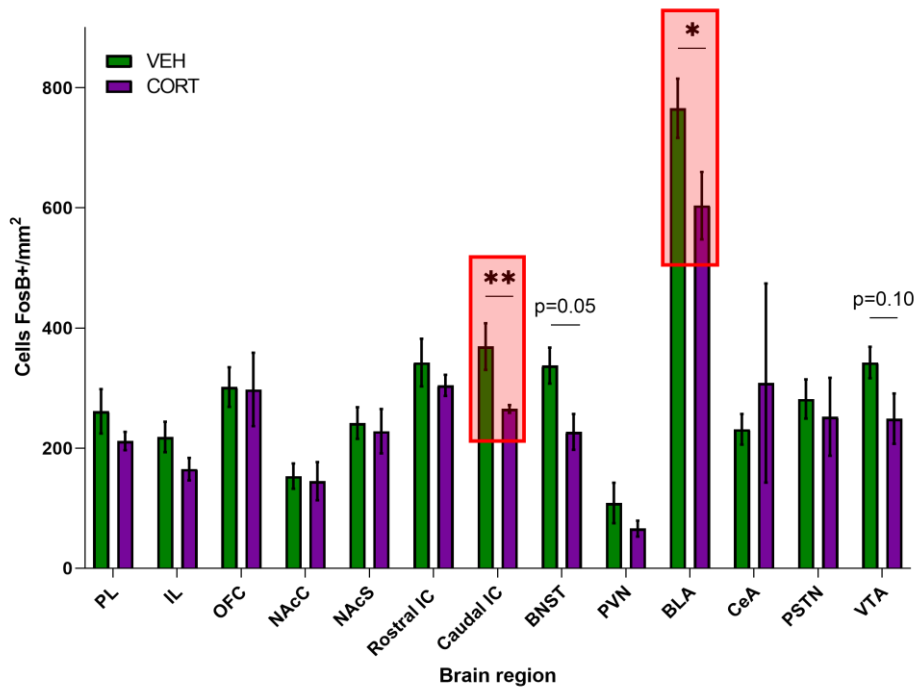


Figure 62. Relative quantification of FosB immunoreactivity in the discrete brain regions of interest following effort-allocation evaluation in the PR paradigm. FosB quantification is expressed as the mean number of immune-positive reactive cells per surface (mm²). Similar activation patterns were found in control and CORT-treated animals in the following regions: the PL (VEH: 261.31±36.82; CORT: 211.77±15.14) and IL cortices (VEH: 218.61±25.19; CORT: 165.12±18.75); the OFC (VEH: 301.67±32.81; CORT: 297.65±61.01); the NAcC (VEH: 153.46±21.15; CORT: 145.10±31.73) and NAcS (VEH: 241.737±26.34; CORT: 228.17±36.96); the rostral IC (VEH: 342.54±39.72; CORT: 304.36±17.50); the PVN (VEH: 108.68±33.85; CORT: 66.02±13.20); the CeA (VEH: 231.37±25.40; CORT: 308.48±165.63); and the PSTN (VEH: 281.70±32.64; CORT: 252.22±64.90). However, a significant effect of the pharmacological treatment was evidenced in the caudal IC (Mann Whitney U: **, $p < 0.01$) and the BLA (Mann Whitney U: *, $p < 0.05$), with reduced activation in CORT-treated animals compared with control individuals. Two negative trends of activation were also found in the BNST (Mann Whitney U: $p = 0.05$) and the VTA (Mann Whitney U: $p = 0.10$) of CORT animals.

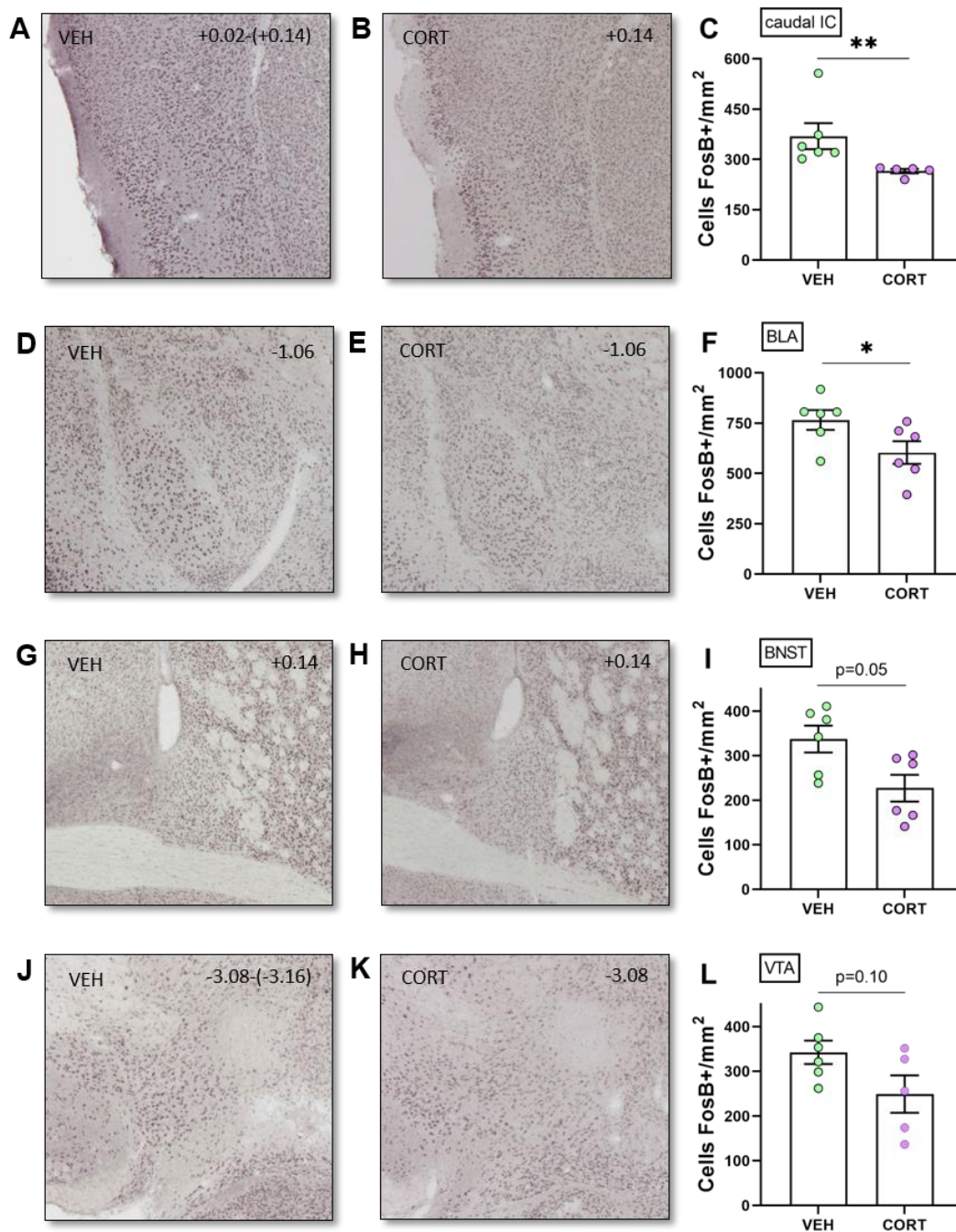


Figure 63. Illustration of the effects of chronic CORT administration on FosB expression in mice after being tested in the PR paradigm. The immunohistochemical approach here used allowed identifying an effect of the pharmacological treatment on the neural activity pattern of different brain regions. Particularly, FosB expression was significantly decreased after chronic CORT exposure (**A-C**) in the caudal IC (Mann Whitney U: **, $p < 0.01$; 0.02-0.14 mm anterior to Bregma -aB) and (**D-F**) in the BLA (Mann Whitney U: *, $p < 0.05$; 1.06 mm posterior to Bregma -pB). Two negative trends in FosB expression following exposure to CORT were also found (**G-I**) for the dorsal BNST (Mann Whitney U; 0.14 mm aB) (**J-L**) and the VTA (Mann Whitney U; 3.08-3.16 mm pB).

2. Instrumental acquisition and effort allocation for food reward in the PR paradigm do not relate to FosB expression levels

Since a detrimental effect of the pharmacological treatment was evidenced on instrumental acquisition in the FR-1 schedule of reinforcement (i.e., the number of sessions animals needed to associate correctly responding with reward presentation), FosB expression densities obtained in the different brain regions of interest were compared to FR-1 behavioural scores aiming to substantiate this finding. The working hypotheses of this project were not confirmed since no significant correlations were found between behavioural and neurobiological scores, irrespective of the brain region [r , all p 's > 0.10].

In order to directly evaluate the neuronal bases of differential animal motivation in the PR paradigm, BP scores were systematically compared to FosB-labelled cells-density for each discrete brain region of interest. Despite the fact that no significant correlations were found, including the caudal IC [$r=0.4353$, $p=0.18$] and the VTA [$r=0.5094$, $p=0.11$], two positive trends were evidenced between FosB expression and BP scores in the BLA [$r=0.5087$, $p=0.09$] and the BNST [$r=0.5621$, $p=0.06$] (see **Figure 64**). These results point towards a more intricate relationship between neural activation patterns and effort allocation and in particular for the PR paradigm, and indicate the influence of the BLA as a modulator of mice effort-based motivation in physiological and upon distress conditions.

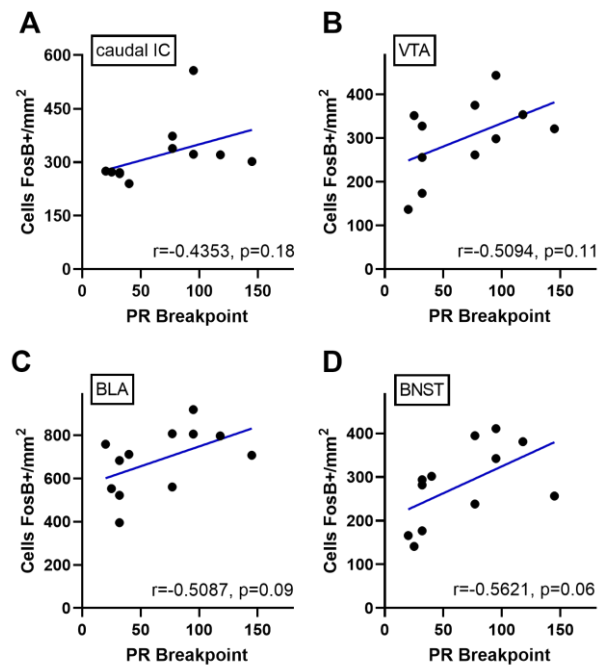


Figure 64. Study of the correlation between effort allocation in the PR paradigm and neural activation identified by FosB immunohistochemistry in different brain regions of interest. Among overall data (VEH + CORT individuals), not significant correlation was found between breakpoint (BP) scores in the PR paradigm and FosB reactivity, irrespective of the brain region of interest and (A) including the caudal IC and (B) the VTA. However, two trends between the behavioural and neurological scores were observed (C) in the BLA ($p=0.09$) and (D) in the BNST ($p=0.06$). These results suggest the existence of an intricate relationship between neural activation patterns and effort allocation valuation as major motivational component in control and stressed animals.

4.3. Neurobiological basis of CORT-induced behavioural flexibility deficits in the Reversal Learning task

Objective: The objective of this section is to identify neural networks underlying functional connectivity subsequent to behavioural flexibility during the RL task in physiological conditions and following sustained distress. Results will help to better understand the CORT-induced emergence of suboptimal DM in the ^AmGT (**Figure 65**).

Cognitive flexibility is essential for appropriate behavioural adjustment and learning in changing environments. In the preceding chapters, differential learning capacities have been evidenced in mice chronically treated with CORT, such in the case for the RL task (*Third Chapter, Section 4*). Learning in the RL task is approached from mice ability to adjust their behaviour in face of a change of the task's main rule. Animals' behavioural flexibility appears truncated after 6-8 weeks of CORT treatment, obstructing the homeostatic behavioural adjustment to the new contingencies of the task. Curtailed cognitive flexibility could therefore disrupt the acquisition of contingencies in the Δ mGT by rendering the exploration phase ineffective, which would contribute to final suboptimal DM.

In a similar manner as for the study on effort allocation, the underlying neural substrates and networks of behavioural flexibility in the CORT model were addressed by an immunohistochemical approach, aiming at disentangling the neural basis of chronic CORT-induced impaired RL. As a mean to track specific neuronal functional activity (Duman et al., 2005), the transcription factor cFos (the product of expression of the immediate-early gene *cfos*) was quantified. In parallel, *FosB* expression was addressed in order to evaluate long-term neural adjustments related to, both the behavioural paradigm and the pharmacological treatment.

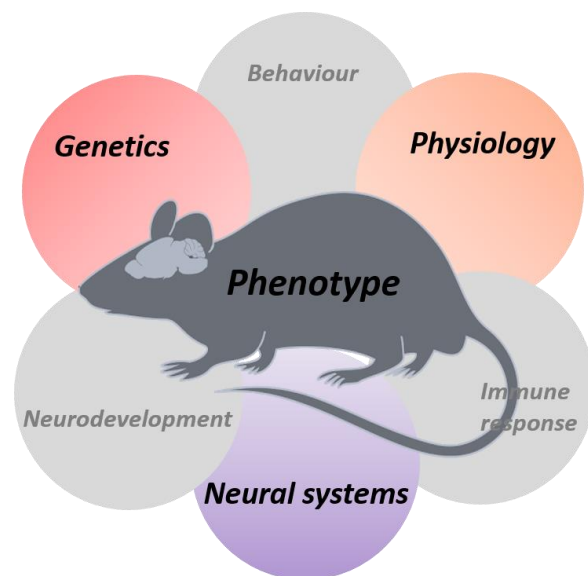


Figure 65. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Physiology, neural systems and genetics. This section aims at investigating the neurobiological underpinnings of behavioural flexibility in physiological conditions and under chronic distress. The neural activation pattern of several discrete brain regions relevant for DM and stress-related symptomatology will be studied in animals chronically treated with CORT and in a healthy, non-treated mice population. This will help understanding the mechanisms underlying the suboptimal decisional performance of CORT-treated mice during the adapted gambling task.

A. Materials and Methods

Animals: From the 30 *C57BL/6J* mice that were tested in the RL paradigm (*Third Chapter, Section 4*), 20 animals randomly chosen (VEH, n=10; CORT, n=10) were sacrificed 65 min after completion of the last RL session for cFos immunohistochemistry. The other 10 animals (VEH, n=5; CORT, n=5) were sacrificed 24 hours after the last RL session for FosB immunostaining.

Experimental protocol: Animals' sacrifice, brain collection, storing and slicing followed the same protocol described in *Fourth Chapter, Section 2*, and the same brain regions were selected. Of particular interest for this section are the PL and IL cortices, the OFC, NAc and BLA, which have been described as key players in behavioural flexibility (Bohn, 2003; Floresco, 2015; Floresco et al., 2009; Rich and Shapiro, 2009). The immunohistochemistry protocol for FosB was identical to the one described in the previous section. For the case of the cFos immunostaining protocol, sections were incubated overnight with the primary antibody (anti-cFos) without presence of lactoproteins, and 4

hours with the secondary antibody, both at room temperature (see **Table 5**). Due to technical issues (e.g., deterioration of the sections quality subsequent to processing), some samples were not included in the final analysis, so that final samples sizes were: cFos, PL n=18, IL n=17, OFC n=18, NAc n=17, rostral IC n=15, caudal IC n=15, dorsal BNST n=12, PVN n=13, amygdala n=15, PSTN n=12 and VTA n=13; FosB, PL n=9, IL n=9, OFC n=9, NAc n=9, rostral IC n=7, caudal IC n=10, dorsal BNST n=10, PVN n=10, amygdala n=9, PSTN n=9 and VTA n=9.

| Product name | Antigen | Strain | Type | Supplier | Technique | Dilution | Incubation duration | Incubation temperature |
|--------------|--------------------|--------|---------|---------------------------------|-----------|--------------------------------|-----------------------------------|------------------------|
| Sc-393232 | GR | mouse | M.clon | <i>Santa Cruz Biotechnology</i> | WB | 1:500 | overnight | 4°C |
| ab62532 | MR | rabbit | Po.clon | <i>Abcam</i> | WB | 1:1000 | overnight | 4°C |
| Sc-293187 | CRF | mouse | M.clon | <i>Santa Cruz Biotechnology</i> | WB | 1:250 | overnight | 4°C |
| Sc-47778 | β -actin | mouse | M.clon | <i>Santa Cruz Biotechnology</i> | WB | 1:1000 | 2 hours | Room temperature |
| Sc-48 | FosB | rabbit | Po.clon | <i>Santa Cruz Biotechnology</i> | IHC | 1: 500 | 48 hours | Room temperature |
| 9F6 | cFos | rabbit | M.clon | <i>Cell Signaling</i> | IHC | 1:8000 | overnight | Room temperature |
| | HRP anti-mouse Ig | goat | - | <i>BD Pharmingen™</i> | WB | 1:5000 | 2 hours | Room temperature |
| | HRP anti-rabbit Ig | goat | - | <i>BD Pharmingen™</i> | WB | 1:5000 | 2 hours | Room temperature |
| | HRP anti-rabbit Ig | goat | - | <i>Vector Laboratories</i> | IHC | 1:1000 (cFos) 1:1500 (FosB) | 4 hours (cFos) 24 hours (FosB) | Room temperature |

Table 5. Identification of the antibodies used for protein quantification and immunohistochemical staining in the study of the neurobiological correlates addressing suboptimal DM in the CORT model. Supplementary information about the antibodies and antibody-related protocol details utilized for Western Blot and immunohistochemical techniques. Relevant information for the Fourth Chapter.

B. Results

1. CORT-induced decreased VTA and dorsal BNST cFos expression related to RL final performance

Neural activation related to final RL performance and approached by cFos expression in the selected discrete brain regions of interest is presented in **Figure 66**.

The density of cFos-labelled cells in CORT-treated animals was significantly lower in the VTA (mean of cFos+ cells per mm² \pm SEM, VEH: 77.64 \pm 14.59; CORT: 41.04 \pm 20.95) [UMW, Z=2.0, p<0.05] and the BNST (VEH: 89.14 \pm 23.00; CORT: 26.18 \pm 2.08) [Z=2.0, p<0.05] compared with control animals. A negative trend in cFos-related neuronal activation was also evidenced in the caudal IC of CORT-treated animals (VEH: 101.77 \pm 12.71; CORT: 62.95 \pm 16.44) [Z=1.7, p=0.08] (see **Figure 67**). However, no differences were observed across conditions for the mPFC (PL and IL areas), OFC, NAc (core and shell), rostral IC, PVN, amygdala (BLA and CeA) and PSTN [Z<1.4, all ps>0.10]. These results are in agreement with the negligible difference observed for final RL behavioural performance between CORT and VEH-treated animals (see *Third Chapter, Section 4*). Yet, they do not directly evidence CORT-induced changes in neural activation at the level of the selected discrete brain regions and related to the task completion. They do indicate distress-induced aberrant functioning of brain networks involved in

reward-based associative learning and reward seeking, as shown respectively by the compromised VTA and BNST neuronal activity.

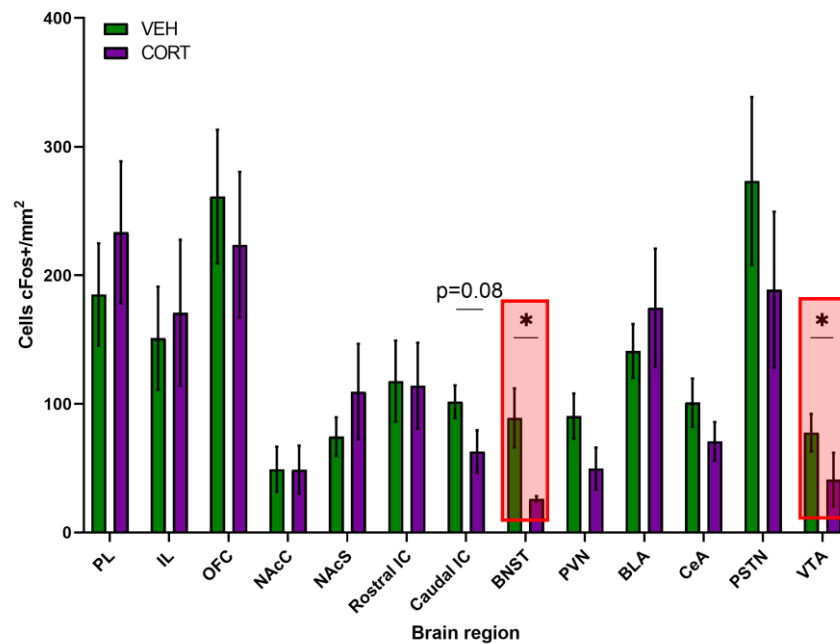


Figure 66. Relative quantification of cFos reactivity in the selected discrete brain regions of interest after completion of the RL task. cFos quantification is expressed as mean of number of positive reactive cells per surface (mm^2). Similar neural activity patterns were found in control and CORT-treated animals in the following brain regions: the PL (VEH: 185.12 ± 39.78 ; CORT: 233.45 ± 55.19) and IL cortices (VEH: 151.05 ± 40.17 ; CORT: 170.88 ± 56.73); the OFC (VEH: 261.25 ± 51.95 ; CORT: 223.72 ± 56.63); the NAcC (VEH: 49.14 ± 17.46 ; CORT: 48.84 ± 18.68) and NAcS (VEH: 74.54 ± 15.01 ; CORT: 109.52 ± 37.15); the rostral IC (VEH: 117.67 ± 31.44 ; CORT: 114.09 ± 33.40); the PVN (VEH: 90.51 ± 17.58 ; CORT: 49.70 ± 16.35); the BLA (VEH: 141.06 ± 20.95 ; CORT: 174.88 ± 45.84); the CeA (VEH: 101.00 ± 18.76 ; CORT: 70.90 ± 15.08); and the PSTN (VEH: 273.32 ± 65.31 ; CORT: 188.85 ± 60.52). However, a significant effect of the pharmacological treatment was evidenced in the BNST and the VTA (Mann Whitney U: *, $p < 0.05$), with a reduced activation in CORT-treated animals compared with control individuals. Simultaneously, a negative trend of activation was found in the caudal IC (Mann Whitney U: $p = 0.08$) of CORT animals.

2. Final RL performance negatively correlates with VTA cFos expression in CORT animals

In order to further disentangle the contribution of brain regions and networks in final RL performance, final behavioural scores (i.e., the percentage of correct answers in the last session of the RL task) were systematically compared to cFos expression. A negative correlation between parameters was highlighted in the VTA [$r = -0.7337$, $p < 0.01$]. A closer examination of this result revealed that this negative correlation was mainly accounted for by scores from CORT-treated individuals [$r = -0.7692$, $p < 0.05$] (Figure 68 A,B), whereas final RL performance does not correlate with VTA cFos-labelled cell-density in the control condition [$r = -0.5227$, $p = 0.29$]. However, no correlation was found between cFos-related neural activity and final RL performance in the BNST [$r = -0.2923$, $p = 0.36$]. Concerning the other brain regions, no difference in cFos-labelled cell-density was found across pharmacological conditions, but another negative correlation was found for the NAcC [$r = -0.5873$, $p < 0.05$] (Figure 68 C). Interestingly, final RL performance tended to negatively correlate with cFos-related neural activation in the caudal IC [$r = -0.4578$, $p = 0.09$] (Figure 68 D). No other trends or correlations were found [all $ps > 0.10$].

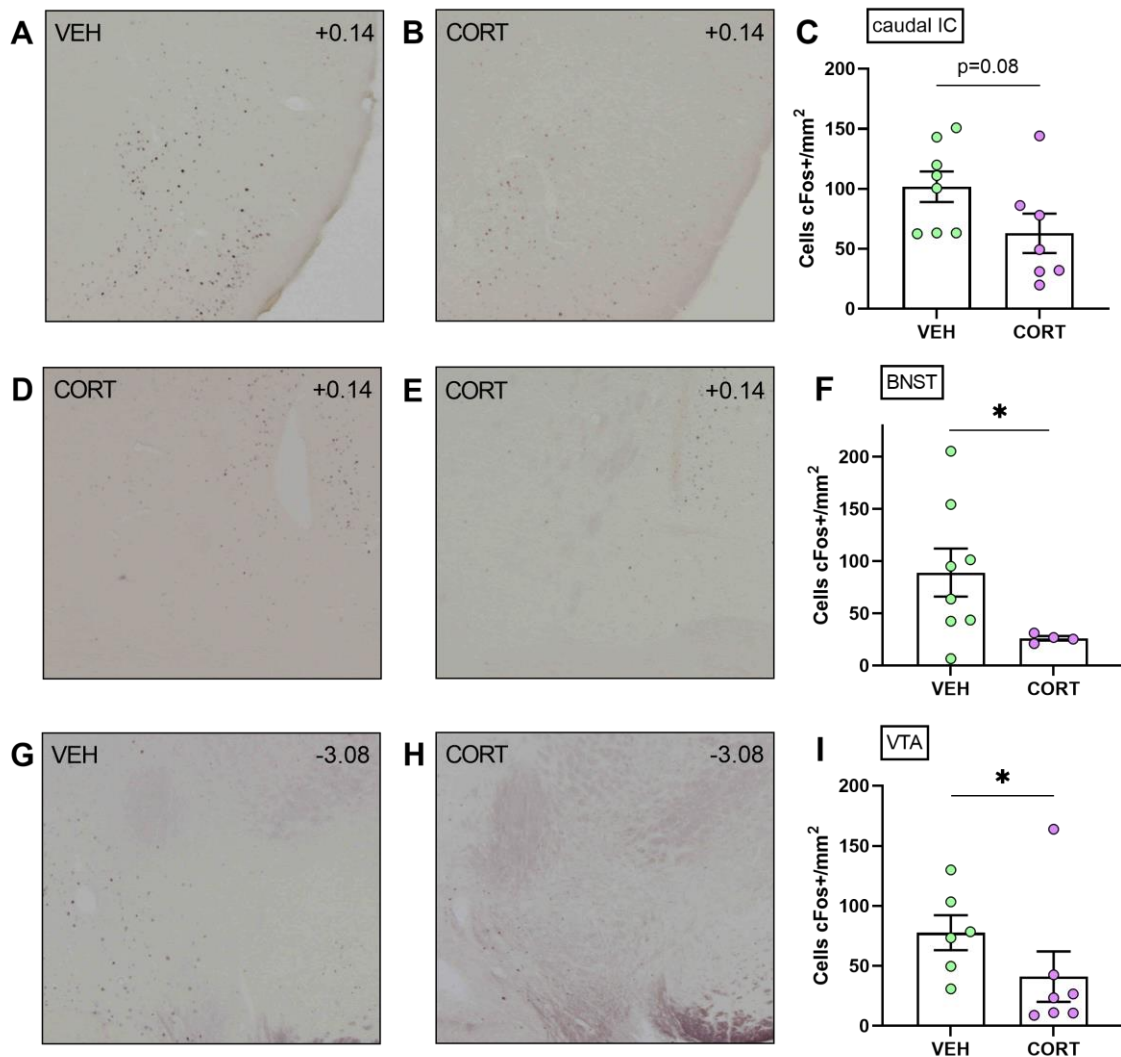


Figure 67. Illustration of the effects of chronic CORT administration on cFos expression in mice after behavioural evaluation in the RL task. Chronic CORT administration differentially impacts the neural activity pattern of different selected discrete brain regions. cFos expression was found decreased (A-C) in the caudal IC of CORT-treated animals, yet without reaching significance (Mann Whitney U: 0.14 mm anterior to Bregma -aB). However, the density of cFos-labelled nuclei was significantly decreased (D-F) in the dorsal BNST (Mann Whitney U: *, $p < 0.05$; 0.14 mm aB) and (G-I) in the VTA (Mann Whitney U: *, $p < 0.05$; 3.08 mm posterior to Bregma) after chronic CORT exposure.

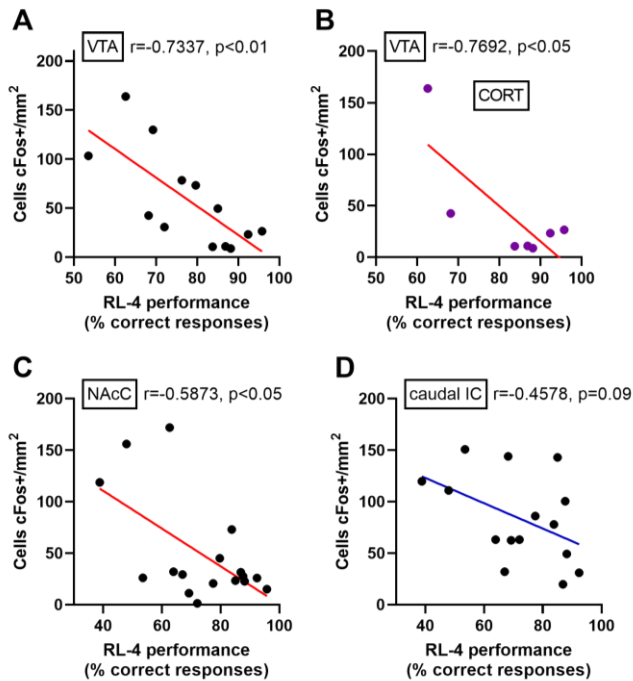


Figure 68. Study of the correlation between final behavioural performance in the RL task and neural activation identified by cFos immunohistochemistry in different selected discrete brain regions of interest. Among the overall data (VEH + CORT individuals), (A) a significant negative correlation was found between final RL behavioural scores and cFos reactivity in the VTA ($p < 0.01$), (B) which is mostly accounted for by CORT-treated individuals ($p < 0.05$). (C) Additional negative correlation between parameters was found for the NacC ($p < 0.05$), with individuals that performed better showing reduced local neural activity. (D) Finally, a negative trend between final RL performance and cFos-related neural activity was found for the caudal IC ($p = 0.09$).

3. No significant effects of chronic CORT administration on FosB expression related to RL testing

Levels of sustained neural activation revealed by FosB expression in the different selected discrete brain regions of interest are presented in **Figure 69**.

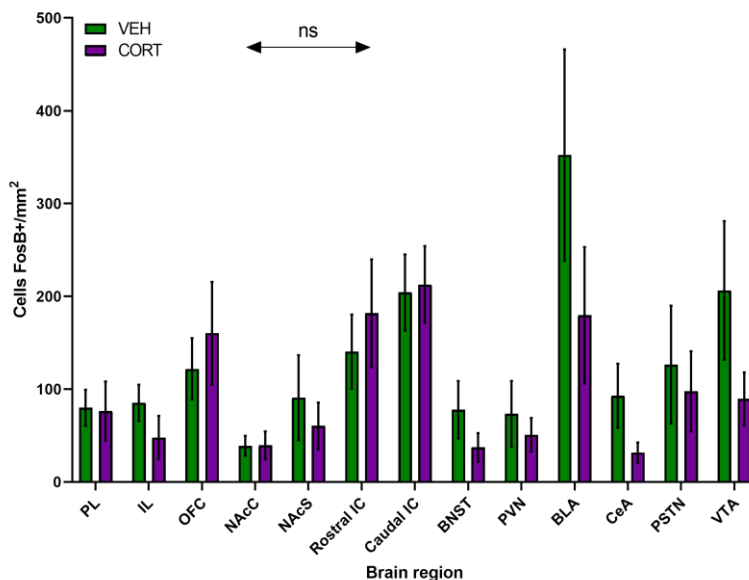


Figure 69. Relative quantification of FosB reactivity in the selected discrete brain regions of interest related to RL testing. FosB quantification is expressed as mean number of positive reactive cells per surface (mm^2). In opposition to the working hypothesis, similar activation patterns were found in control and CORT-treated animals for all the regions studied (Mann Whitney U: ns, not significant): the PL (VEH: 79.99 ± 19.40 ; CORT: 76.35 ± 31.98) and IL cortices (VEH: 85.27 ± 19.57 ; CORT: 47.80 ± 23.32); the OFC

(VEH: 121.82 ± 33.15 ; CORT: 160.26 ± 55.39); the NAcC (VEH: 38.93 ± 10.71 ; CORT: 39.51 ± 14.94) and NAcS (VEH: 90.82 ± 45.85 ; CORT: 60.46 ± 25.06); the rostral IC (VEH: 140.46 ± 39.88 ; CORT: 181.88 ± 58.04); the caudal IC (VEH: 204.19 ± 41.01 ; CORT: 212.75 ± 41.40); the BNST (VEH: 77.90 ± 31.03 ; CORT: 37.32 ± 15.28); the PVN (VEH: 73.43 ± 35.51 ; CORT: 50.85 ± 18.12); the BLA (VEH: 352.42 ± 113.76 ; CORT: 179.60 ± 73.52); the CeA (VEH: 92.96 ± 18.26 ; CORT: 31.67 ± 10.81); the PSTN (VEH: 126.32 ± 63.56 ; CORT: 97.67 ± 43.25), and the VTA (VEH: 206.34 ± 76.65 ; CORT: 89.52 ± 28.59). Wide SEM distributions indicate that data should be completed by replication of the experiment.

Against the working hypothesis, the density of FosB-labelled cells of all regions explored was similar in animals across conditions, revealing no direct effect of the pharmacological treatment on sustained neural activation related to the RL task [UMW, $Z < 1.5$, all $p > 0.10$]. However, the wide SEM linked to the small sample sizes suggest that this approach should be replicated, and interpretation on these results should be carried out with caution.

In a similar manner as for the study on cFos expression, final behavioural RL scores were systematically compared to FosB-labelled cells density for each region of interest, aiming at revealing their involvement in RL performance. Along with the precedent results, a significant negative correlation was found for the caudal IC [$r = -0.6538$, $p < 0.05$] (Figure 70). No other trends or correlations between these parameters were found in the remaining structures –mPFC (PL and IL cortices), OFC, NAc (core and shell), rostral IC, BNST, PVN, amygdala (BLA and CeA), PSTN and VTA [all $p > 0.10$].

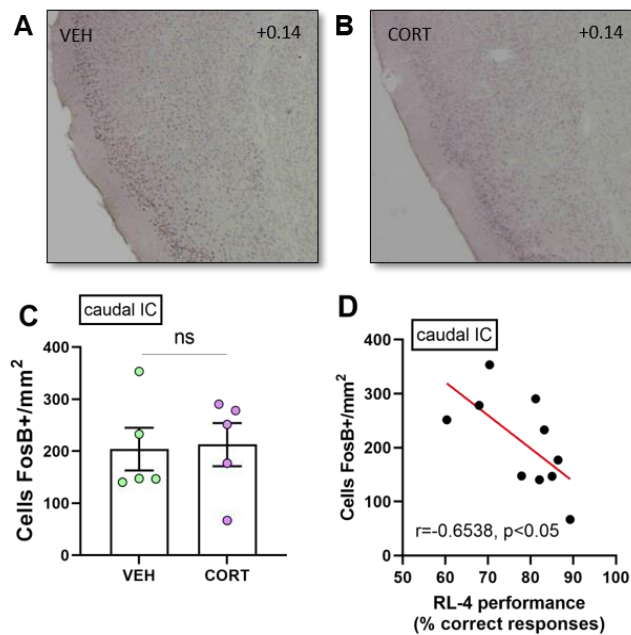


Figure 70. FosB expression in the caudal IC following RL testing. Illustration of FosB levels of expression in the caudal IC addressed by immunohistochemistry in (A) control and (B) CORT-treated animals. (C) No significant effect of the pharmacological treatment was evidenced (Mann Whitney U: ns). (D) However, a significant correlation between the density of FosB-labelled cells and final RL performance was found, suggesting a role of this brain region in the completion of the task.

4. Decreased BLA and VTA FosB expression in low-flexible individuals

As a more direct way to address the neural basis of behavioural flexibility in the RL task, cFos and FosB expression in the brain regions of interest were compared between *high-flexible* and *low-flexible* individuals. Animals forming these clusters adjusted their behaviour to the new contingency of the task at significantly different rates (referred here as “learning rate”, see *Third Chapter, Section 4*).

No differences on the cFos-labelled cells density were found across clusters, irrespective of the region studied [UMW, $Z < 1.1$, all $p > 0.20$] (see Figure 71 A). Regarding FosB expression, however, comparisons revealed a differential sustained neural activation pattern in two of the brain regions investigated: the VTA and the BLA. The density of FosB-labelled cells was significantly decreased on *low-flexible* individuals (mean of FosB+ cells per $\text{mm}^2 \pm \text{SEM}$, VTA: 79.28 ± 18.02 ; BLA: 141.56 ± 41.55) compared with *high-flexible* individuals (VTA: 304.70 ± 81.86 ; BLA: 486.11 ± 82.20) in both the VTA [$Z = 2.3$, $p < 0.05$] and the BLA [$Z = 2.3$, $p < 0.05$] (Figure 71 B).

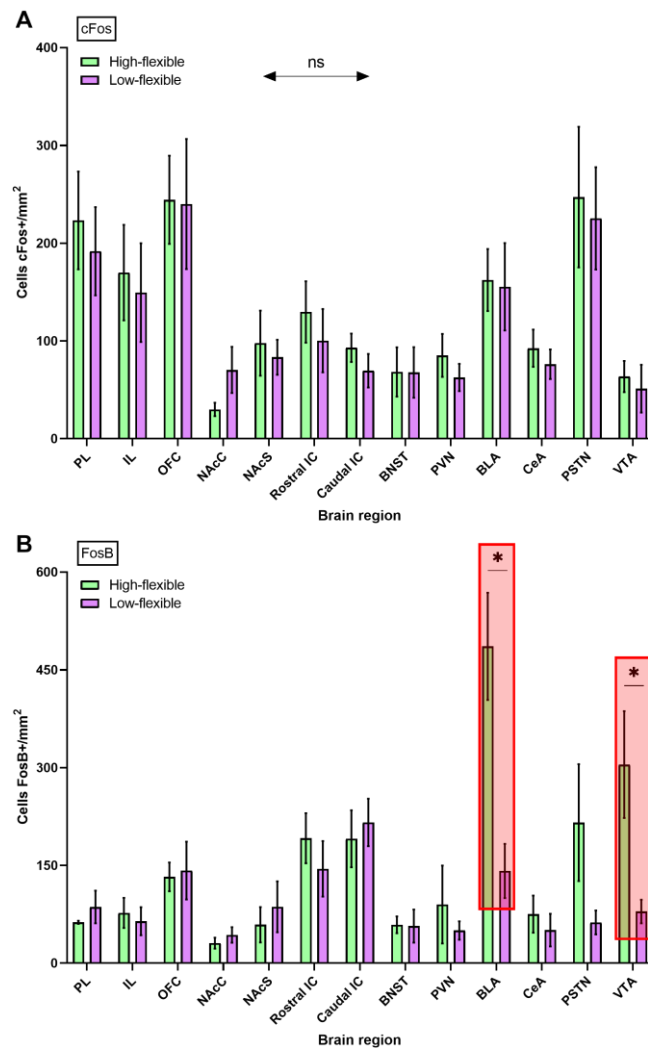


Figure 71. Comparison of neural activation patterns approached through cFos and FosB expression in selected discrete brain regions and related to RL testing between high-flexible and low-flexible individuals. Control and CORT-treated animals clustered into two subgroups of individuals that differentially adjust their behaviour to the new rule of the RL task. High-flexible mice rapidly adjusted their behaviour, significantly improving their task performance already in the second RL session, i.e., increasing the percentage of correct responses. This cluster predominantly comprises VEH-treated animals. Low-flexible animals, however, were less successful in adjusting their behaviour, thus improving significantly less their performance in the second RL session. This cluster predominantly comprises CORT-treated mice (see Third Chapter, Section 4). In the investigation of the neural activity patterns related to RL performance, **(A)** no differences were found in cFos expression between high- and low-flexible individuals (Mann Whitney U: ns, not significant) irrespective of the brain region of interest. Nevertheless, **(B)** sustained neural activation assessed by FosB expression was significantly different between subgroups in the BLA and VTA (Mann Whitney U: *, $p < 0.05$), but not in the other regions. Thus, these results support the hypothesis of the BLA and VTA-related computations implication on behavioural flexibility, and suggest that aberrant neural network activity patterns within and between these regions might result from sustained GC exposure.

These results further substantiate the modulatory role of the VTA during the RL task and confirm the implication of BLA-related networks on behavioural flexibility. They also suggest a nuanced CORT-induced aberrant neural activity underlying behavioural flexibility and contingent on BLA and VTA networks. Thus, CORT-induced slow-down in behavioural adjustment (Third Chapter, Section 4)

could ultimately result from the combination of VTA and BLA-related motivational deficits and BLA-dependent cognitive inflexibility.

4.4. Role of infralimbic cortex CRF-expressing neurons in effort-allocation and reversal learning: a chemogenetic approach

Objective: The last objective of this chapter is to causally investigate the relationship between CRF activity in the IL area of the mPFC and effort-allocation and reversal learning. Selective modulation of neural activity by the use of chemogenetic tools would help clarifying how the neuropeptide CRF is involved in animals' motivation and behavioural flexibility, as cognitive modulators of DM (**Figure 72**).

The mPFC is extensively studied to approach rodent executive dysfunction since it is involved in a wide range of cognitive dimensions such as reward evaluation (Grabenhorst and Rolls, 2011), task switching (Kehagia et al., 2010) and DM processing (Euston et al., 2012). Functional alterations in the human prefrontal region have also been reported in neuropsychiatric disorders including mood disorders like depression (Akil et al., 2018; Foland-Ross and Gotlib, 2012). Moreover, previous preclinical studies have linked motivational deficits in effort-based tasks to increased CRF activity, suggesting a CRF-based modulation on subjective valuation of effort costs (Bryce and Floresco, 2016). Here, it was found that high CRF expression levels in the mPFC could lie at the origin of suboptimal DM under uncertainty following distress, since final Δ mGT performance negatively correlates with mPFC CRF levels in CORT-treated animals (see results from *Fourth Chapter, Section 1*). This result therefore entails mPFC CRF in the regulation of executive functioning and reward processing in an intricate manner.

In order to address causal relationships, manipulations of neural networks' activity can be achieved in many ways. Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are genetically mediated drivers allowing the manipulation of cell-type-specific neuronal firing activity. The expression of GPCRs can be introduced in specific neuronal populations *in vivo* by the use of viral constructs in rodent driver lines (Roth, 2017). Most useful, DREADDs can be repetitively activated by physiologically inert synthetic molecules (designer drugs) such as clozapine-N-oxide (CNO) via intraperitoneal injection or in the

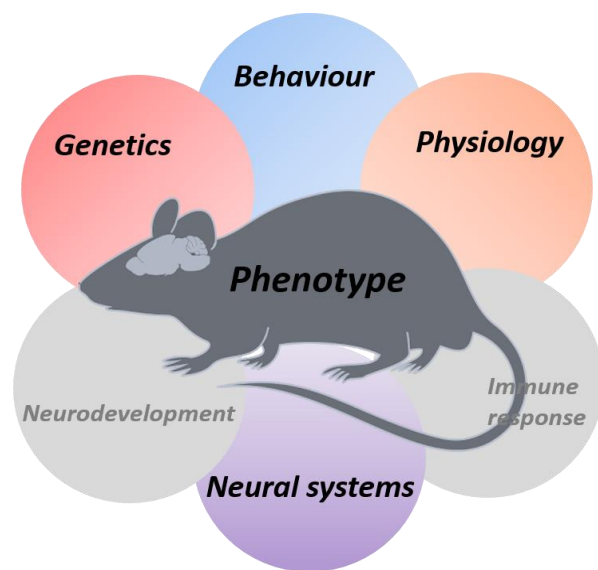


Figure 72. Illustration of a set of biological domains defining the phenotype of an animal model for the study of human biology. Behaviour, physiology, neural systems and genetics. This section aims at exploring the relationship between CRF signalling in the IL cortex and the motivational and learning deficits observed in distressed mice. Specific modulation of the IL CRF-expressing neurons' activity during the PR and RL testing will help elucidating the role of CRF signalling in modulating associative learning through reinforcement in food-rewarding behavioural tasks. It will also help understanding the relationship between high levels of CRF in the mPFC and suboptimal decisional performance in the adapted gambling task.

drinking water (Carr et al., 2018), allowing refined and reversible *in vivo* circuit-approach manipulations.

Based on the evidenced CORT-induced deficits on motivation and behavioural flexibility (see *Third Chapter, Sections 3 & 4*), and on the detrimental effects of high levels of mPFC CRF in ^AmGT performance, causally investigating the particular contribution of mPFC CRF neurons activity in the PR and RL tasks is of interest at this point. Within the mPFC, the IL region has been suggested as essential in the regulation of flexible behaviour and is required for the development of habit-based behaviours (Barker et al., 2014). Chemogenetic manipulation of CRF-expressing neurons' firing activity from the IL cortex could therefore adequately inform about the neuronal bases underlying motivation and cognitive flexibility with respect to DM modulation.

A. Materials and Methods

Animals: Twenty-three *CRF-cre* reporter mice expressing Cre recombinase under control of the human Synapsin 1 (hSyn) promoter were used for the chemogenetic approaches.

Experimental protocols:

○ *Stereotaxic viral injections for DREADD experiments*

Mice were anesthetized under a constant flow of ~1% isoflurane anesthesia (1.5L min⁻¹) and placed on a stereotaxic apparatus for viral injections. All viral injections were performed bilaterally targeting the IL cortex (coordinates from the brain surface: AP= +1.78 mm, ML= ±0.3 mm, DV= -2.25 mm) using a nanofil (*World Precision Instruments, France*) on modified microliter syringes (*Hamilton, France*) with a 35-gauge needle. After the tip of the needle was placed at the target region, microinjections were performed at a rate of 0.1 μL min⁻¹. A 300 nL total volume of AAV2-hSyn-DIO-hM4D(Gi)-mCherry or AAV2-hSyn-DIO-hM3D(Gq)-mCherry virus was injected per hemisphere for neuronal firing inhibition and enhancement respectively. The needle was left in place for 5 min after the injection was finished and thereafter, carefully removed. Animals remained in their home cages for 4 weeks before behavioural evaluation to allow axonal labelling.

○ *Chemogenetic experiments*

Animals were behaviourally tested in the PR schedule of reinforcement and the RL paradigms from the fifth week after virus injection. The behavioural protocols were slightly modified from the ones described in the *Third Chapter, Sections 3 & 4*, as follows:

PR protocol for DREADD experiments. The protocol was identical from the one previously described, but three additional sessions were included after mice individual performances stabilized. In the first and second additional sessions, mice received either CNO (*Sigma Aldrich, France*) (1 mg/kg) or vehicle (NaCl solution) via intraperitoneal injection 45 min before testing (Barbier et al., 2020). In the third additional session all mice without exception received a vehicle injection.

RL protocol for DREADD experiments. The behavioural steps of the RL protocol did not change in this chemogenetic approach, but mice received, as for the case of PR testing, an intraperitoneal injection of either CNO (1 mg/kg) or vehicle (NaCl solution) 45 min before the first RL session. Prior to the third RL session (first RL session of the second day of testing) all mice received a vehicle injection.

A subset of animals ($n=9$) was only tested in the PR paradigm and the remaining animals ($n=14$), firstly in the PR paradigm and after three days (1 resting day and 2 days of FR-5 schedule of reinforcement) in the RL task. All animals were sacrificed 24 hours after completion of the last behavioural session, and brains were removed, fixed, frozen and sectioned as described in *Fourth Chapter, Section 2*. Following verification of the injection sites by microscopic observation, three animals with injections not targeting the IL cortex were excluded from the analyses (final sample: inhibition-DREADD, $n=11$; activation-DREADD, $n=9$) (Figure 73).

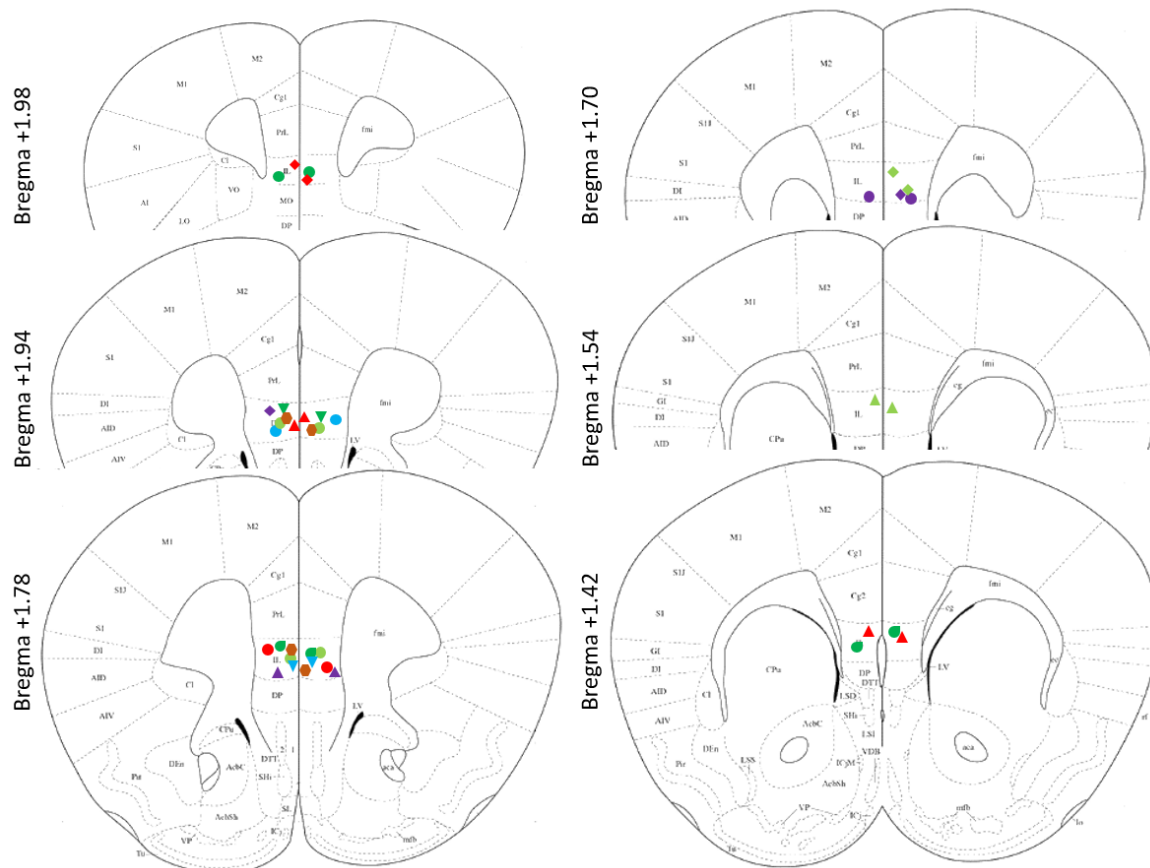


Figure 73. Illustration of the bilateral injection sites of AAV2-hSyn-DIO-hM4D(Gi)-mCherry and AAV2-hSyn-DIO-hM3D(Gq)-mCherry virus for DREADD experiments. Twenty CRF-cre reporter male mice expressing Cre recombinase under control of the human Synapsin 1 (hSyn) promoter were successfully injected for the chemogenetic experiments (inhibition DREADD, $n=11$; activation-DREADD, $n=9$). Different colours and shapes indicate different individuals. Atlas images from Franklin and Paxinos, 2008.

A. Preliminary results

The following results rely on preliminary data and cautious interpretation should therefore be applied until the investigation is completed with increased statistical power.

1. Unaltered effort-allocation during the PR task after inhibition of IL CRF-expressing neurons activity

In order to test whether inhibiting IL CRF-expressing neurons activity modifies conditioned food seeking by obstructing PR responding for reward, training behavioural performance (FR-1

schedule of reinforcement) of AAV2-hSyn-DIO-hM4D(Gi)-DREADD-injected mice ($n=11$) was firstly evaluated in absence of CNO. Statistical analyses confirmed that both groups (PR-NaCl, $n=6$; PR-CNO, $n=5$) reached the acquisition criteria after a similar number of FR-1 sessions (mean of total number of FR- sessions \pm SEM, PR-NaCl: 5.33 ± 1.36 ; PR-CNO: 4.69 ± 0.67) [UMW: $Z=1.0$, $p=0.34$]. Upon PR testing, operant responding was not different between groups that were going to be NaCl or CNO injected, neither in number of PR sessions they required to stabilize their performance (mean of total number of PR sessions \pm SEM, PR-NaCl: 8.17 ± 0.83 ; CNO: 8.23 ± 0.63) [$Z=-0.3$, $p=0.75$], nor in the effort they were willing to expend for a food reward (mean of first breakpoint –BP-1 \pm SEM, PR-NaCl: 148.17 ± 33.06 ; CNO: 119.60 ± 11.19) [$Z=0.3$, $p=0.78$]. These results confirm the absence of behavioural bias prior to DREADD testing (**Figure 74 A**).

No effect on the animals' motivation for effort allocation was observed after administration of CNO during the first and second extra PR sessions (second and third BP –BP-2 and BP-3), neither on the last testing session when all animals were NaCl injected (fourth BP, –BP-4) [PR-NaCl, $X^2F_{6,3}=1.8$, $p=0.63$; PR-CNO, $X^2F_{5,3}=0.8$, $p=0.86$] (see **Figure 74 A**). Individuals' performance remained essentially stable during the extra PR sessions, independent of the nature of the injection that they received [extra session 1: $Z=1.0$, $p=0.30$; extra session 2: $Z=0.4$, $p=0.71$; extra session 3: $Z=0.3$, $p=0.78$, post-hoc]. This suggests that basal CRF-expressing neurons' activity in the IL cortex is not directly implicated in animals' motivation for food reward in the PR paradigm (**Figure 74 A**). However, these results require further robustness through increasing the size sample, as it is not yet possible to extract reproducible conclusions within size power limits ($1-\beta > 0.8$ and $\alpha < 0.05$). Electrophysiological confirmation of CNO efficacy would be also required for corroboration, in particular since no effect of CNO injection has been evidenced. A control CNO group of animals (i.e., animals intracerebrally injected with empty virus solution, which will receive CNO intraperitoneally) will also be necessary in order to conform the results.

2. Infralimbic CRF-expressing neurons' activation might load on effort-allocation for food reward in the PR paradigm

Following the same rationale as previously, training performance (FR-1 schedule of reinforcement) of AAV2-hSyn-DIO-hM3D(Gq)-DREADD-injected mice ($n=9$) was evaluated prior to PR testing in absence of CNO. Both groups of individuals (PR-NaCl, $n=4$; PR-CNO, $n=5$) required an identical number of FR-1 sessions to learn and form the action-outcome association for reaching the acquisition criteria (mean of total number of FR- sessions \pm SEM, PR-NaCl: 7.25 ± 1.89 ; PR-CNO: 5.40 ± 0.87) [UMW: $Z=0.8$, $p=0.42$]. Through PR testing and until the first extra session, operant behaviour was similar between individuals of both groups: they stabilized their performance after a similar number of PR sessions [mean of total number of PR sessions \pm SEM, PR-NaCl: 8.25 ± 0.63 ; CNO: 7.60 ± 0.60] [$Z=1.2$, $p=0.22$] and made similar efforts to be rewarded (mean of BP-1 \pm SEM, PR-NaCl: 138.25 ± 6.75 ; CNO: 125.00 ± 12.25) [$Z=0.7$, $p=0.46$] (**Figure 74 B**).

The neural activity enhancing effect of CNO in Gq-DREADD-injected mice was revealed in their behavioural output by obstructing PR responding [PR-NaCl, $X^2F_{4,3}=3.3$, $p=0.35$; PR-CNO, $X^2F_{5,3}=8.1$, $p<0.05$] (see **Figure 74**). However, the effect of CNO on effort allocation was only significant after its first administration [extra session 1: $Z=2.5$, $p<0.05$, post-hoc], while during the second extra PR session both groups reached again similar BP-3 [$Z=1.3$, $p=0.20$, post-hoc]. As expected, similar behavioural scores (BP-4) were attained in the last session, where all animals received NaCl injections prior to testing [$Z=0.3$, $p=0.79$, post-hoc] (**Figure 74 B**). These results suggest that incremented CRF-expressing cell-firing in the IL cortex might detrimentally modify conditioned food seeking during PR paradigms, and curtail animals' motivation for expending effort in order to be food rewarded. However, further confirmation of these results is required. Firstly, it is necessary to clarify why the second CNO injection

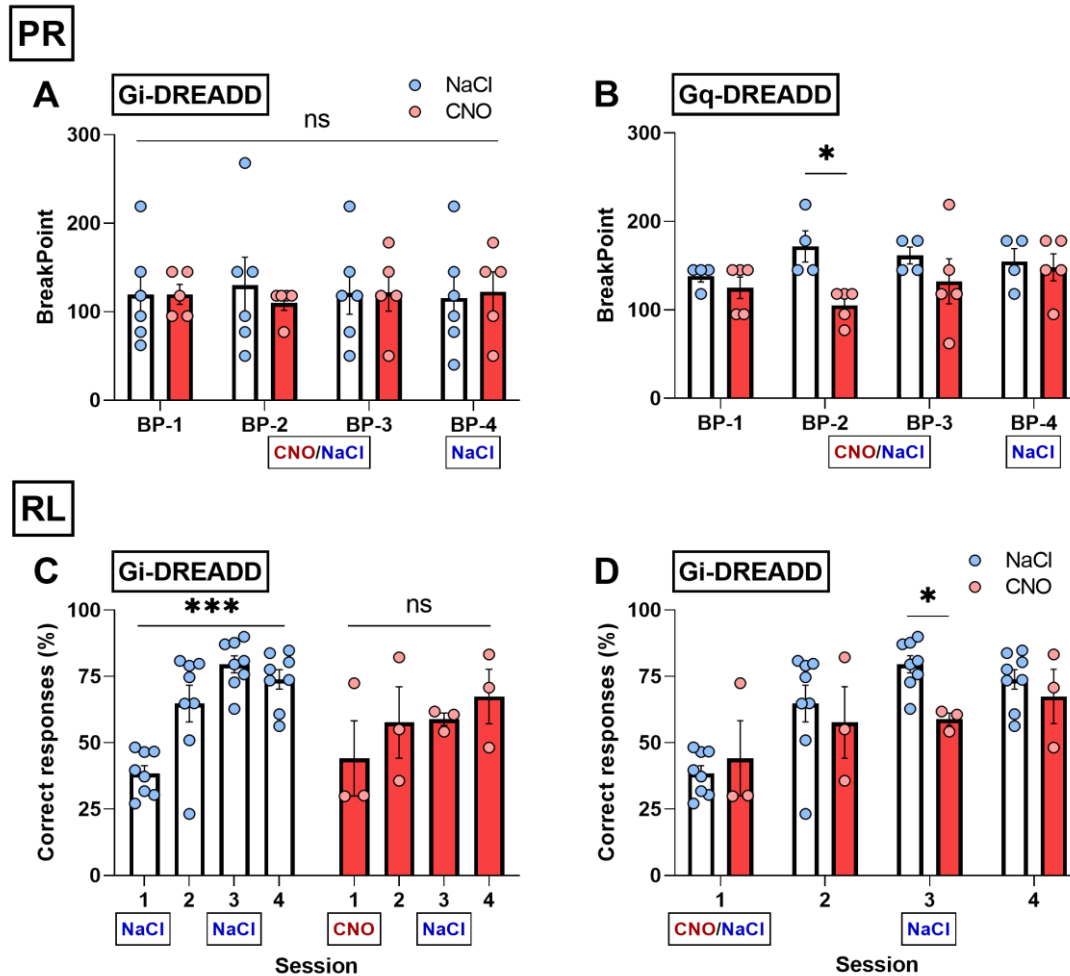


Figure 74. Infralimbic CRF-expressing neurons' activation potentially curbs effort allocation for food reward in the PR paradigm, while inhibition would reduce flexibility required for adaptive behavioural adjustment in the RL task. CRF-cre reporter mice were bilaterally infused with Cre-sensitive Gi/Gq-DREADD virus in the IL cortex (coordinates: AP= +1.78 mm, ML= ± 0.3 mm, DV= -2.25 mm) four weeks before behavioural testing in the PR schedule of reinforcement and the RL task. **(A)** During the PR testing, animals were left to stabilize their performance (i.e. to reach their breakpoint –BP-1) before being differentially CNO or NaCl intraperitoneally (IP) injected 45 min before the two following sessions. In a third extra session, all animals received NaCl. Inhibition of IL CRF-expressing neurons activity did not impact the amount of effort mice were willing to expend to be food rewarded (Mann Whitney U: ns, not significant), **(B)** while when activating IL CRF-neurons firing, CNO-treated animals reached a lower BP after the first injection than animals receiving NaCl (Mann Whitney U: *, $p < 0.05$). However, this difference disappeared for the second CNO injection, all animals allocating similar effort to be rewarded. These results need further substantiation, yet they suggest that while effort allocation does not rely on basal CRF-expressing neurons activity, incremented activity could be detrimental for animals' motivational state. **(C)** During the RL testing, animals received an IP injection of CNO or NaCl before the first session, and a NaCl injection before the beginning of the third session. Inhibition of IL CRF-expressing neurons activity impacts the ability of animals to adjust their behaviour to the new contingency of the task. Indeed, CNO-treated animals did not significantly improve their performance along the task, while NaCl-treated animals did so (Friedman: ***, $p < 0.001$). **(D)** When comparing performances for each session, CNO-treated animals performed significantly worse than animals that received NaCl in the third RL session (Mann Whitney U: *, $p < 0.05$). However, experiments should be replicated in order to increase size samples, facilitating reproducible outcomes within size power limits.

did not translate in an observable effect. Secondly, it is necessary to respect size power limits for ensuring reproducible outcomes. If the here presented results were to be confirmed, they would

substantiate the interdependence between decreased motivation, high CRF levels in the mPFC and suboptimal DM following persistent distress.

3. Infralimbic CRF-expressing neurons' inhibition might reduce flexibility required for adaptive behavioural adjustment in the RL task

With the purpose of investigating whether inhibiting IL CRF-expressing neurons' activity impacts cognitive flexibility required to adapt behaviour to new contingencies, AAV2-hSyn-DIO-hM4D(Gi)-DREADD-injected mice (RL-NaCl, n=8; RL-CNO, n=3) were tested in the RL paradigm. Interestingly, only animals that were NaCl-injected prior to the first RL session improved their performance as the task progressed [RL-NaCl, $X^2F_{8,3}=17.6$, $p<0.001$]. Animals receiving CNO, however, did not significantly increase the proportion of correct responses across the task [RL-CNO, $X^2F_{3,3}=0.2$, $p=0.61$]. Individuals receiving a NaCl injection significantly increased their performance in the second RL session compared with the first session (mean percentage of correct responses \pm SEM, session 1: 38.48 ± 2.90 ; session 2: 64.79 ± 6.95 ; session 3: 79.57 ± 3.20 ; session 4: 73.86 ± 3.67) [session 1 vs 2: W , $Z=2.2$, $p<0.05$, post-hoc], while CNO-injected animals did not (session 1: 44.15 ± 14.16 ; session 2: 57.67 ± 13.48 ; session 3: 58.89 ± 2.36 ; session 4: 67.45 ± 10.28) [$Z=0.5$, $p=0.59$, post-hoc] (see **Figure 74 C**). Behavioural scores obtained in each RL session were also compared between animal groups, revealing a significant difference only in the third session [UMW, session 1: $Z=0.4$, $p=0.68$; session 2: $Z=0.2$, $p=0.83$; session 3: $Z=2.4$, $p<0.05$; session 4: $Z=0.8$, $p=0.41$] (**Figure 74 D**). To further disentangle these differences, the learning rate (ratio between performances of the first two consecutive RL sessions) from both groups of animals were compared, which showed no significant effect of the CNO-injection [$Z=0.0$, $p=1$].

Combined, these results could seem contradictory, since a lack of learning is evidenced in animals receiving a CNO injection but performance's absolute values do not reflect this difference. This could be the consequence of the limited size sample of the CNO-injected group. Further confirmation via increasing size groups will be necessary prior to devise a hypothesis. Nevertheless, if confirmed, these results would demonstrate the implication of a basal IL CRF-expressing neurons activity in RL-related behavioural flexibility that could impact DM processing when altered. Anyhow, the effect of enhancing IL CRF-expressing neuron activation should also be explored to complete the results.

4.5. Summary and discussion of the results

In this last chapter, efforts were made to address the complex neurobiology underlying motivation and behavioural flexibility as plausible cognitive modulators of DM under uncertainty. Experiments aimed at clarifying their contribution to CORT-induced suboptimal DM.

In the first instance, protein quantification analyses have disclosed a GR/MR ratio imbalance consequent to the pharmacological treatment and accounted for by a GR down-regulation in the animals' mPFC. In fact, chronic CORT administration might be at the origin of suboptimal action-outcome encoding over cue-processing by altering GR expression in a brain region crucial for the reflective behaviour preceding optimal planning. Along with previous studies reporting negative consequences on cognition upon chronic distress and anxiety (McEwen, 2017; Park and Moghaddam, 2017; Reul and Kloet, 1985), chronic CORT administration would favour suboptimal habit formation and would yield action-outcome consolidation especially vulnerable.

In parallel, despite the fact that no obvious effect of the pharmacological treatment has been evidenced in CRF protein levels, CRF signalling in the mPFC of CORT-treated animals has been found to predict final ^AmGT performance. Synergetic effects of CRF and CORT have already been proposed in stress-induced cognitive alterations (Chen et al., 2016). Additionally, stress has been demonstrated to affect *crf* signalling, for example by compromising positive valence systems (Birnie et al., 2020) and by disrupting fronto-striatal cognition (Hupalo et al., 2019; Uribe-Mariño et al., 2016). The results here presented converge with scientific evidence of aberrant CRF signalling-related motivational deficits (Bryce and Floresco, 2016) and further support their contribution to suboptimal DM. Disrupted *crf* signalling might hinder mPFC computations supporting optimal fronto-striatal cognitive functioning and specially goal-directed behaviour. They might contribute to overreliance on negative biases to form suboptimal action-outcome associations. Therefore, the presented results point towards an integral role of CRF that further support the *Somatic marker hypothesis* (Bechara et al., 2000; Bechara and Damasio, 2005) and that allow proposing high mPFC CRF levels as neurobiological endophenotype of vulnerability to suboptimal DM under uncertainty following sustained distress.

As a second step, the FosB-based localizationist study revealed a pharmacological impact on neuronal activation of selected discrete brain regions involved in effort and reward processing. Caudal IC and BLA neural activities were decreased subsequent to long-lasting exposure to GC. More interesting here is the involvement of the IC in DM under uncertainty (Clark, 2010), already demonstrated in preclinical lesion studies. The aIC is involved in contingency acquisition on rodent gambling tasks through modulation of reward preference over punishment (Pushparaj et al., 2015). The posterior IC, however, mostly mediates emotional behaviour and transmits negative valence-related information to modulate animals' defensive reactivity during social interactions (Gehrlach, 2019). The IC is also relevant for its contribution in initiating feeding behaviour as a part of a basal ganglia-like network (Barbier et al., 2020), and depending on cognitive and emotional influences. Caudal IC coordinates used in the present project concur with the posterior region of the coordinates commonly used to address the aIC in rodents, which also is part of reward-related neural networks and participates in the representation of anticipatory cues (Kusumoto-Yoshida et al., 2015). Hence, chronic CORT administration could impact animals' motivational state by interfering aIC-related network activity, and disrupt cue-processing associated with food delivery in the PR paradigm. The CORT-induced aberrant IC neuronal activity here identified might reflect an altered anticipatory functioning that curtails reward expectation processing and consequent conditioned responding. However, the lack of correlation between caudal IC FosB expression and PR behavioural scores impedes formulating a relationship of direct causality. There is also strong evidence about the prominent role of the BLA in appetitive learning and conditioning through encoding motivational information related to instrumental association. It also participates in effort-based rewarding tasks (Beyeler et al., 2018; Ferland et al., 2018; Wassum and Izquierdo, 2015). In this regard, chronic GC exposure might impact BLA neuronal activity, obstructing animals' motivation to effort allocation for food reward likely by disrupting reward value encoding. Moreover, the positive trend found in the correlation between BLA FosB expression and BP behavioural scores aligns with this hypothesis and could also explain the trend found for the BNST. This considers the involvement of the unidirectional excitatory BLA-to-BNST projections in behavioural output signalling. Combined, these results further substantiate that CORT animals show a compromised motivational state rooted on aberrant IC and BLA-related networks' activity involved in effort and reward processing, which could hamper DM under uncertainty.

Thirdly, the cFos and FosB-related study also evidenced that GC exposure-mediated distress impacts motivational neural hubs relevant to success in tasks appealing to cognitive flexibility. Reversal learning-related cFos, but not FosB expression, was significantly decreased in CORT-treated animals in the VTA and BNST. As a key player in the reward network, dysregulated VTA activity has long been implicated in depressive symptomatology (Nestler and Carlezon, 2006 for instance) and in associative learning and reward processing deficits (Bouarab et al., 2019). In addition to its contribution on behavioural signalling, the BNST is highly responsive to stress and has been associated to distress-related behavioural dysfunction via *crf* signalling (Gray et al., 1993; Hu et al., 2020; Ulrich-Lai and Herman, 2009). Of relevance for the present project is the intimate functional connectivity between these two regions: the BNST modulate VTA DA firing primarily via GABAergic inhibitory projections even if a small population of VTA DA neurons also receive excitatory inputs from it. In addition, nearly half of VTA GABAergic neurons receive excitatory inputs from the BNST. When combined, it is justified to suggest that by disrupting HPA regulation, the BNST neural activity would be compromised, in accord with preclinical lesions studies (Gray et al., 1993). Subsequently, the VTA neural activity would be impacted. The results here obtained converge with other findings where increased *crf* activity in the VTA is suggested to result in blunted DA signalling, and to disrupt reward valuation and processing (Bryce and Floresco, 2016). They also support results on rodent optogenetic approaches showing the reversion of negative valence behaviours in mice after VTA DA stimulation (Tye et al., 2013). However, further investigation is required in order to clarify details regarding the particular neural types involved in the activity changes identified. Clustering analyses based on the neurobiological data are also in accord with the main goal of identifying neurobiological biomarkers predictive of particular behaviours.

Against the working hypotheses, the neural activation patterns of key players behind behavioural flexibility, the mPFC and OFC, were not impacted by sustained exposure to CORT. Surprisingly, neither were the BLA nor the NAc neural activity, yet a recent study has causally demonstrated their role in instrumental reward-seeking in the CORT model (Dieterich et al., 2021). The VTA has not yet been directly identified as primary player in RL tasks, but its contribution is likely essential for the modulation of reward motivated behaviours via associative learning than might contribute to the success of the task (Bouarab et al., 2019). Therefore, chronic CORT administration might reduce directly or indirectly VTA and/or BNST neuronal activity, resulting in poor associative encoding that leads to an enhanced difficulty in withholding learnt responses in favour to edit those previously learnt as useless. This explanation would endorse the finding of an aberrant neural activity pattern in the VTA (reduced FosB expression) in *low-flexible* categorized individuals. However, the results question the adequacy of the immunohistochemical-based approach here used. Neurobiological data do not directly refer to the crucial moment of behavioural adjustment (i.e., the second RL session). Ongoing investigations are to address this point of conflict to better inform about the particular contribution of the regions of interest in behavioural flexibility in physiological and distress-related conditions.

Finally, in consideration to the dissociable roles attributed to the ventral and dorsal mPFC in DM (van Holstein and Floresco, 2020), the role of IL CRF signalling was approached in a first reversible *in vivo* circuit-approach. Despite the fact that the preliminary DREADD results require confirmation, they suggest that IL CRF signalling contributes to cognitive computations necessary for goal-directed behaviours, with motivation and flexibility as potential major modulators.

GENERAL DISCUSSION

The results obtained during this investigation have demonstrated that human decisional deficits observed in individuals that have been exposed to sustained distress can be modelled in mice chronically treated with CORT. This paradigm allows mimicking human hypercortisolaemia and dysfunctional HPA axis activity, which are reported symptoms in patients suffering from various distress-induced disorders. Most interestingly, the decisional deficits observed in the CORT model likely result from disrupted learning and motivational processes relying on the activity of the mPFC and the reward-system. In the investigations regarding potential biomarkers of distress-induced decisional impairments and based on the here obtained results, disrupted mPFC *cfr* signalling has been proposed in this thesis as endophenotype of vulnerability.

The results of this doctoral investigation are discussed below and summarized in **Table 6**. An illustration of the processes involved in DM and identified as sensitive to chronic CORT exposure can be found in **Figure 75**.

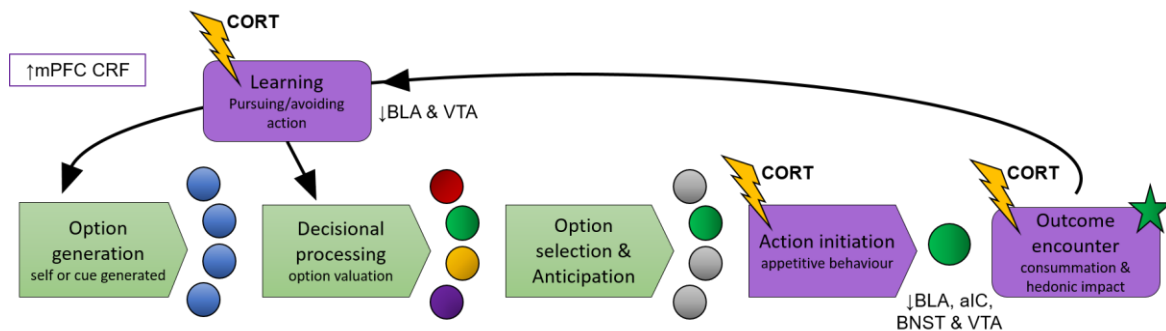


Figure 75. Chronic CORT administration induces suboptimal DM likely, through impingement of appetitive and consummatory motivational dimensions of behaviour and through obstruction of cue-based learning in dynamic environments. Disrupted motivational and learning processes in the CORT model rely on the activity of the mPFC and the reward-system. Particularly, a decreased neural activity in the BLA, the aIC, the BNST and the VTA might be at the origin of alterations observed involving action initiation and consummatory behaviour. A disrupted mPFC *cfr* signalling is proposed here as endophenotype of vulnerability to suboptimal DM under chronic distress due to its predictive value in the ^AmGT. Adapted from Husain and Roiser, 2018.

Modelling DM under uncertainty

Robust and valid animal models are essential to investigate the behavioural and neurobiological bases of human psychopathology. A good correspondence between human and animal data is therefore essential to arrive at conclusions and to predict risks and vulnerabilities to these pathologies, as well as to understand physiological processes.

The first objective of this doctoral project was to adapt a murine gambling protocol in order to limit satiation bias during the evaluation of DM under uncertainty. The successful modifications implemented to the original protocol enabled facilitating appetitive behaviours in mice and ensured significant reward consumption across conditions. This was indeed pivotal for the success of the doctoral project, which relies on the animal model's suitability in order to address DM under uncertainty during distress.

The translational validity of the model was then evaluated. A direct comparative study between behavioural human and mice data demonstrated that the ^AmGT has a remarkable face validity when comparing healthy populations. Gambling performances were demonstrated to be similar across species despite protocol variations. Direct comparisons of human and animal data using common methods of statistical analysis has not been reported before, and the results presented here clearly demonstrate that such an approach helps bridging findings between clinical and preclinical investigations. This doctoral project stands on the conviction that comparability between human and animal data are possible when protocols are optimized to fit species needs. Human IGT studies most commonly use single-session automatized versions of the task with money gains and losses as potential outcomes. Rodent versions, however, preferentially rely on multi-sessions tasks with food-related gains and losses. Modelling money gain and losses is challenging task in rodent versions. Yet it is accepted that money motivates humans to act and make decisions on a level comparable to the primary reinforcer which is food. Thus, food is a self-evident driver for rodents to motivate behaviour, particularly in situations of alimentary deprivation. Such differences in protocol depend on a species' nature, and unfortunately are difficult to minimize, if not impossible. This project therefore favoured the strategy of direct comparison between species by using common statistical methods.

Interestingly, the study revealed a difference in the onset of reward responsiveness between species. Mice learnt the contingencies of the task faster than humans. This interesting finding has here been interpreted in terms of the different nature of rewards and penalties used in the human and mouse gambling tasks. This is the most plausible explanation for this result, as the outcomes' nature has been extensively identified as a major limitation of these decisional tasks. However, other potential explanation arises under the premise that humans and mice were equally motivated. In that case, the earlier emergence of reward responsiveness in mice compared to humans might reflect safeness-related behaviour in mice rather than faster learning rate. Humans consented their participation in the task and were aware of the single-session nature of the task. They gained these *a priori* "not so valuable" information by personally interacting with the researchers. Mice, however, were individually placed in a maze and left free to behave. Even if they were habituated to the device and to the researcher's manipulation, food-deprived mice were removed from their home cages and isolated. Hence, it is also justified to hypothesize that the differences in the onset of reward responsiveness might reflect a "safer" mice behavioural strategy as a way for them to escape internal stressful cues derived from the uncertain intrinsic nature of the task. Another possible explanation relies in the different task-schedules. Multi-session tasks might offer animals the opportunity to consolidate the acquired knowledge before each new session, and to "re-calibrate" their internal/emotional state. Humans, who chose consecutively to complete the task in a single session, might be at disadvantage compared to mice.

The translational value of the murine model was further substantiated with study of the interindividual variability across species. Humans and mice distributed in a similar manner across the three identified decisional profiles. A k-mean clustering approach was chosen to identify the different behavioural strategies, but other clustering methods could have also served this purpose. This method was selected for its novelty as it was not yet applied to interindividual characterization of human IGT studies. However, this method entails other limitations such as the impossibility of *a priori* defining what is a good or a poor gambling performance. Nevertheless, humans and mice share similar decisional strategies when their performances are analysed in a k-mean-based approach, which supports the suitability of the animal model to reproduce human behaviour.

The study of behavioural domains within the gambling tasks has been very informative for strategy characterization in humans and mice. Although similar, the decisional clusters across species did not behave identically, and the differences might again be a consequence of protocol limitations or stress-related behavioural adjustments, apart from species specifics. Unfortunately, the behavioural dimensions of rigidity/stickiness, flexibility, loss-shift and wins-stay are here insufficient to evaluate the relationship between gain maximization and loss aversion. In this frame, computational models of DM may prove very useful to obtain better insight into “discrete” behavioural tendencies modulating gambling performance in humans and mice. Notably, an ongoing project in computational modelling of these human and mice gambling data aims at elucidating behavioural factors that could not be identified with the classical statistical approaches, especially during early phases of the tasks.

Since their origin, IGT studies have referred to disadvantageous performances as risky behaviours. In the original protocol from Bechara and colleagues, the disadvantageous decks are called “risky” in comparison to the “safe” advantageous ones (Bechara et al., 1994). By inertia, this interpretation has been adopted also in preclinical studies. However, the rationale behind this interpretation is controversial and not always shared across the scientific community. In the ^AmGT, for example, individuals of the intermediate decisional profile developed a preference for the advantageous options, but they did not succeed in maximizing their gains. Intermediate DMs’ performance is interpreted as worse than the one from good DMs, but that might result from the limited number of choices the animals could make. Are intermediate DMs worse than good DMs or just slower learners? It would be interesting to know if they would reach good DMs endpoint performance if they had the possibility to continue choosing. An extended exploration phase in these intermediate individuals might result in a better recognition of options and subsequent better management of available cues during the exploitation phase. Interpretations about risk in the rodent adaptations should therefore be made with caution and be corroborated with supplementary data from tasks specifically evaluating risk.

Modelling distress-induced DM alterations

Organisms face stressful situations through behavioural and physiological adjustments enabling them to recover homeostasis. However, when these adjustments are not sufficient for organisms to recover a homeostatic state, the allostatic load impacts their adaptability to the environment and may result in states of exhaustion. This is common in situations of sustained distress, which have in turn been associated to increased levels of GC circulating in the plasma. Thus, the present doctoral project has used a protocol of chronic CORT administration to model distress-related symptomatology in mice.

The investigation on the effects of long-lasting exposure to GC on DM under uncertainty was successful. Chronic CORT administration induced suboptimal DM in mice during the ^AmGT. Moreover, CORT-induced delay on reward responsiveness reflects an alteration in the exploration-exploitation trade-off that might result from a learning slow-down. The study on the behavioural interindividual variability of the mice population revealed a different distribution of individuals into the three decisional profiles identified. Particularly, chronic CORT exposure induced suboptimal DM by favouring the intermediate decisional strategy. At this point it is important to highlight that the clustering method used in this approach is different from the one used in the evaluation of the translational value of the ^AmGT. In fact, the limits for good and poor performances were defined since the beginning in

order to facilitate further comparisons with other preclinical studies. The frequency distribution into the three clusters in physiological conditions consequently differed across studies.

The success on modelling DM-related alterations strengthens the validity of the pharmacological treatment as a mean to reproduce distress-related human symptomatology. The results therefore substantiate the suitability of the CORT model as a useful tool in preclinical investigations on human pathology resulting from maladaptation to sustained stress. However, future studies should include females in order to evaluate gender-specific effects of CORT on Δ mGT performance. Scientific evidence has demonstrated that female mice are resilient to chronic CORT administration concerning the emergence of negative valence behaviours (Mekiri et al., 2017). It should be demonstrated that that would be also the case for DM under uncertainty.

Operant approaches of the Δ mGT need to be beneficial in refining protocol limitations. During these investigations, DM under uncertainty was evaluated in a maze-based paradigm, and its translational validity was demonstrated. However, operant approaches with automatized recording of parameters certainly offer several advantages over maze versions. One major limitation is the active participation of the researcher, which individually relocates the animals inside the maze or the home cages. Contrary to operant approaches, researchers' active implication in maze-based tasks significantly increases the time necessary for the completion of the experiment and does not allow parallel performance. Ongoing operant investigations will offer the possibility to record complementary parameters within the Δ mGT that might help understanding the CORT-induced behavioural alterations. Nevertheless, mazes are useful for approaching motor aspects, and potentially might better reconcile the exploratory nature of mice. At any case, the maze-based Δ mGT was appropriate for the experimental design since the device was used for the evaluation of different behavioural tasks, which diminished stress-related behaviour due to animals' device exposure.

Distress-induced suboptimal DM grounds on the alteration of HPA axis components in the mPFC

Chronic CORT administration is a tractable mean to model dysregulated HPA axis activity subsequent to sustained distress. As a way to counterbalance the allostatic load, several physiological mechanisms within the HPA axis act in a coordinated manner to recover the pre-stress state. However, these regulatory mechanisms might be inefficient in the CORT model since the hormone is administered uninterruptedly. This doctoral investigation focused on the three key players involved in these regulatory mechanisms: the GC receptors GR and MR, and the major HPA axis activator, which is the CRF neuropeptide. Their contribution was studied in three telencephalic areas involved in associative learning and emotional processing: the mPFC, DLS and VH.

This investigation highlighted a CORT-induced decrease of the GR/MR ratio of expression in the mPFC of mice, which resulted from a downregulation of GR expression. This alteration probably arises from local sensitization subsequent to hormonal overstimulation, yet literature also relates to MR downregulation in distress-related pathology (see for instance de Kloet et al., 2018). The mPFC is crucial for reflective behaviour preceding adaptive planning, and therefore, CORT-induced altered GR expression might be at the origin of suboptimal action-outcome encoding over cue-processing. Unfortunately, differential GR expression does not directly predict Δ mGT performance, neither in pathological nor in physiological conditions. Unexpectedly, this study did not reveal any alteration in the GR/MR ratio of expression in the other two regions of interest. Differential DLS GR/MR ratios of

expression were expected since the reinforcement of actions for the exploration-exploitation transition (i.e., the emergence of habitual behaviour) depend to a large extent on striatal activity. In a similar manner, VH GR/MR ratios of expression do not evidence neither HPA dysfunctionality, nor altered emotional processing. In any case, before rejecting these possibilities altogether, future studies will compare GR/MR ratios of samples following a more precise timing. Indeed, protein extracts in this study correspond to 13 weeks of cumulative CORT treatment, while Δ mGT performance relates to 7-8 weeks. A variation in the expression of these HPA axis elements might have occurred that could bias the conclusions of this investigation.

In the study of the contribution of CRF activity to suboptimal DM, this investigation showed that CRF expression in the mPFC predicts Δ mGT performance under distress. The negative correlation found between these parameters has motivated the proposal of high mPFC CRF levels as neurobiological endophenotype of vulnerability to suboptimal DM under uncertainty after sustained distress. Medial PFC computations behind suboptimal frontostriatal cognitive functioning and especially goal-directed behaviour, might therefore result from locally disrupted *crf* signalling. However, this investigation failed to reveal any differences in the DLS and VH CRF expressions and, consequently, future studies shall consider sampling at more accurate timelines of correspondence in order to further substantiate these findings. Another possibility is to address the contribution of these HPA axis elements in other brain areas relevant for DM such as the IC.

During this investigation, mPFC samples were integrally processed without discerning dorsal from ventral parts. However, the PL and IL cortices are known to exert complementary but dissociable roles in value-based DM (van Holstein and Floresco, 2020). In the particular case of the CRF, for example, the negligible difference between pharmacological conditions might result from a veil-effect from the sum of the PL and IL levels of expression. To obtain better insight into the contribution of these subregions to mPFC and CRF signalling during goal-directed behaviour, this investigation has initiated a series of causal experiments. From a chemogenetic approach, IL CRF signalling was modulated during two different operant tasks, specifically addressing effort allocation and behavioural flexibility. Preliminary data showed that IL CRF-related neural activity activation might reduce effort allocation in reward-based contexts. Besides, basal IL CRF-related neural activity is apparently not involved in effort allocation, yet it might be required for behavioural adjustment and learning in operant tasks. If these results were to be confirmed, high IL CRF levels could be considered at the origin of the obstruction of associative learning and potentially impact DM. Despite their preliminary nature, these results converge with the precedent findings in the CORT model and further support the hypothesis of high mPFC CRF levels as neurobiological endophenotype of vulnerability to suboptimal DM under uncertainty during distress. Ongoing experiments aim at completing these findings and to encompass CRF activity in the PL cortex.

Distress-induced suboptimal DM relay on appetitive-related motivational deficits

Motivational dimensions have been demonstrated to be major modulators of DM under uncertainty. In the process of making decisions, the generation of options and their valuation depend on their encounter by the individual. For that, individuals must initiate a course of action that can be maintained and invigorated or adjusted. Thus, appetitive behaviours comprise a key motivational dimension affecting DM and especially in dynamic environments and during explorative phases.

The investigation on the effects of sustained GC exposure on appetitive behaviours required for optimal DM under uncertainty showed unequivocal deficits in CORT-treated individuals. Importantly, distressed mice showed self-oriented behavioural deficits, as reported by the FCS evaluation, and special difficulty in the initiation of actions, as shown by increased feed latencies in the NSF test and FFT evaluations. Moreover, this state of vacillation or difficulty in initiating a course of action was accentuated in situations including unfamiliar (and potentially stressful) cues, as demonstrated in the FFT-related neophobia evaluation. Yet, the pharmacological treatment did not impact animals' choice between rewards when they were familiar, in contrast to when they were unfamiliar. These last results further substantiate that unfamiliar, uncertain situations impact the ability of CORT-treated animals to settle on choices. Additionally, the evaluation of effort allocation demonstrated that chronic CORT administration leads to deficits in operant-related maintenance and invigoration of rewarded actions. This result is also supported by an altered operant conditioning in the RL evaluation, where CORT-treated mice were significantly less rewarded than control individuals, despite their similar proportion of correct responses compared with the control group. These deficits reflect a CORT-induced disruption of the maintenance and pursue of course of actions that in combination with the difficulties on behavioural initiation might explain the delayed reward responsiveness during the ^AmGT. These deficits were also predictive of alterations on reward and effort processing that could disrupt subjective valuation of options and learning before option generation.

Appetitive behaviours refer to the motivational dimension of the so-called “wanting” the outcomes. Among the evidenced deficits on appetitive behaviour, the increased NSF and FFT latencies might illustrate a CORT-induced cognitive interlock-like phenotype in mice similar to the one described for humans with depressed moods (Belzung et al., 2015). In fact, it is plausible that a negative valenced emotion similar to human frustration would emerge in parallel to the increasing time required to start a course of action. This would therefore contribute to the allostatic load, obstructing the individuals' capability to make new decisions. These states reminiscent to human avolition and abulia might block animals' process of make new decisions. This interpretation is again in accord with clinical reports including patients of depression (Husain and Roiser, 2018).

Decreased effort allocation in the PR task after sustained exposure to CORT might also reflect energetic deficits, since GC are directly involved in the glucose metabolism and participate in different processes serving the modulation of glucose concentration in blood. For example, they stimulate gluconeogenesis in the liver and provide its substrates through mobilization of amino-acids from extrahepatic tissues, they increase sodium retention and potassium excretion, etc. (M. Wang, 2005). Energetic metabolism-related measurements are not included in the here presented study, and future work shall better discern between motivational and energetic CORT-induced effects in the PR paradigm. In any case, both avolition/abulia and anergia/fatigue-related symptoms find their correspondence in human pathology and further substantiate the suitability of the CORT model in translational investigation.

Finally, different behavioural evaluation scores were compared to ^AmGT performances to clarify how CORT-induced appetitive deficits impact decisional processing. Unfortunately, FCS and FFT scores did not offer a causal explanation for suboptimal DM in the ^AmGT, and NSF and operant scores are related to different animal cohorts. Yet, the present study offers substantial evidence regarding the role of appetitive behavioural deficits in the CORT model, as well as for their potential contribution to suboptimal DM processing under dynamic conditions.

Distress-induced suboptimal DM relay on consummatory-related motivational deficits

Another motivational dimension influencing the process of making decisions is the hedonic impact of the rewards and penalties. In the case of rodents tasks relying on food rewards and penalties, encountering but also actively consuming the outcome is required for the hedonic impact to occur. Thus, the study of consummatory behaviours is relevant to address animals' appreciation of rewards and penalties, i.e., an interpretation of how much they "like" them, and for the investigation of the dynamics of incentive valuation.

The study on the effects of long-lasting exposure to high levels of circulating GC evidenced alterations in the consummatory behaviour of CORT-treated animals. During the Δ mGT, stagnated dynamics of the loss-shift behavioural dimension is interpreted as overappreciation of the penalty, classically referred in the literature as hypersensitivity to penalty. Reward appreciation, addressed through evaluation of the win-stay behavioural dimension however, was not influenced by chronic CORT exposure. Yet, again, the results obtained for the decisional profiles do not allow the identification of direct causality between hedonic impact and choice strategy.

Reward appreciation was found unaltered during the SPT, while CORT-treated animals' consumption was significantly lower in the FFT. However, intake scores of both tests significantly correlate, suggesting that the negligible difference in sucrose consumption between pharmacological conditions might be attributable to protocol variations. Scientific literature frequently reports decreased sucrose solution consumption in stress-related animal models as well as interindividual variability in physiological conditions (see for instance Pittaras et al., 2016). Unfortunately, different sucrose solution concentrations and experimental designs are reported, which prevents objective comparisons. Divergent consummatory behaviours observed in the SPT and FFT evaluations might also reflect putative effects of the environment. During the SPT, for instance, animals were isolated during the dark cycle and for a prolonged period of time, whereas the FFT lasted only five minutes and was performed during the light cycle and in presence of the researcher. The optimization of protocols is therefore essential for disentangling these discrepancies and would help addressing other motivational aspects of animals' behaviour.

The penalty overappreciation and reward underappreciation observed in mice chronically treated with CORT are consistent with the coping deficits demonstrated through the FST evaluation, and with the decreased food intake after the NSF test, respectively. Coping deficits might evidence an enhanced impact of negative emotions on animals' behaviour, as well as the influence of a high risk situation for animals' safety. Altered hedonic appreciation might influence reward processing and revaluation required for adaptive reinforcement learning, and might therefore influence the onset of reward responsiveness and the exploration-exploitation trade-off during the Δ mGT. Hence, this investigation endorses that sustained distress in mice alters the impact that outcomes may have in DM under uncertainty. This happen through modifying the incentive value of gains and losses in a similar way as human anhedonia and apathy disrupt decisional quality in humans.

Distress-induced motivational deficits involve brain networks within the reward system, and primarily concern the BLA, aIC, dorsal BNST and VTA

Motivational deficits including abulia, anhedonia and apathy, are inscribed in the symptomatology of various pathologies resulting from exposure to chronic distress, such as depression and schizophrenia. Hence, these motivational deficits characterize transdiagnostic dimensions

expressed across discrete diagnostic entities. The neural bases of these symptoms are connected to the reward system, and consequently they are primarily localized within the cortico-basal ganglia-thalamo-cortical loop.

This investigation demonstrated that appetitive and consummatory behavioural deficits resulting from sustained distress in mice are connected to aberrant activity patterns of several structures within the reward system. Particularly, appetitive deficits related to decreased effort allocation in CORT-mice are concomitant with decreased neural activity in the BLA and the aIC (caudal IC in the present study). Though not reaching significance, dorsal BNST and VTA discrete activation patterns were also decreased in mice after chronic CORT exposure. Furthermore, final performance in the RL task, which involved a decreased conditioned responding in CORT animals, relates to decreased VTA, dorsal BNST and aIC neural activity, the latter without reaching significance. Thus, aberrant neural activity subsequent to chronic CORT exposure affects substrates within neural networks participating in associative learning, in reward and effort processing. Particularly, aberrant BLA and aIC neural activity might impact cue-encoding primarily through obstruction of reward valuation, of interoceptive and effort-related processing. In parallel, VTA and BNST aberrant neural activity might explain why CORT-treated animals committed to the completion of the task in a lesser extent. The VTA activity is crucial for motivating behaviour in goal-directed contexts, while the BNST plays a role in the translation of emotional information to subsequent behavioural adjustment.

Despite the fact that the OFC is relevant for effort-based DM, this investigation did not succeed in demonstrating its direct involvement in CORT-induced alterations in operant behaviour. Likewise, it was unexpected not to find a stronger effect of distress on VTA activity related to effort allocation, since PR tasks are commonly used to approach motivational deficits. Considering that decreased VTA neural activation was found to relate to motivational aspects of the RL task, it will be interesting to address PR final performance in a similar manner. In other words, to better understand the involvement of the VTA activity in the behavioural deficits revealed through PR evaluation, local cFos expression should be related to final performance (breakpoints). This could help in discerning the involvement of the VTA activity on state vs trait motivation-related deficits.

It was also unexpected not to encounter an effect of chronic CORT exposure on NAc neural activity. The intimate connectivity between the NAc and the BLA is accepted to be essential for mediating appetitive conditioning and reward-related behaviours. Indeed, BLA-to-NAc glutamatergic projections potentiate instrumental reinforcement (Amir et al., 2015) and may increase reward-seeking in response to reward predictive cues (Namburi et al., 2016). Moreover, recent investigations have causally demonstrated the involvement of NAc-BLA connections in DM under risk (Bercovici et al., 2018) and, of particular interest for the presented investigation, in CORT-induced appetitive deficits (Dieterich et al., 2021). This investigation showed that cFos, but not FosB expression in the NAcC predicts overall final RL performance. However, similar patterns of NAcC cFos expression were found across pharmacological conditions. Considering the relevance of the NAc within the reward system and since motivational deficits are unequivocal in the CORT model, future studies shall investigate causal relationships between BLA-NAc connectivity and suboptimal DM under uncertainty.

The study on the neural underpinning of motivational deficits in the CORT model expected also to evidence an aberrant pattern of neural activity within the IC-CeA-PSTN network. The connectivity of this network participates in the control of feeding behaviour and might regulate appetitive behaviours in mice. Concerning the particular contribution of the PSTN within the network, Barbier

and collaborators explained its role like follows: “*I don’t want to consume this food, recognized as good, because I am not in a conducive emotional or physiological state*” (Barbier et al., 2020). Thus, the PSTN modulates appetitive behaviour in reward-related contexts and subsequently influence the hedonic impact of the rewards. Unfortunately, this study did not reveal altered patterns of neural activation, neither is this region, nor in the CeA. However, whether due to the small sample size or other methodological limitations, SEM values related to FosB expression in the CeA are too wide to be considered accurate. Consequently, before rejecting the possibility of the IC-CeA-PSTN network involvement in CORT-induced alteration of appetitive behaviours, immunohistological approaches should be replicated.

Distress-induced suboptimal DM relay on learning deficits

Learning from experience is necessary for individuals to successfully adapt to new situations. Different cognitive functions participate in the process of making decisions, ranging from memory processes allowing the recall of pertinent information to guide future behaviour, to flexible thought allowing disengaging from rules when overtaken.

This study is conclusively demonstrating detrimental effects of sustained CORT exposure on mice learning capabilities. In relation to the delayed reward responsiveness in the [^]mGT, the study on choice strategy showed that learning became more sensitive towards penalty than towards reward encounter after chronic CORT exposure. Besides, the pharmacological treatment impacted learning relying on spatial mnesic capacities during the dWST. Although memory scores do not directly predict differential [^]mGT performance, mnesic deficits might had had compromised several processes involved in DM. Continuous reevaluation of options requires from memory recall in order to juxtapose output information before the generation of new options. Thus, the transition from goal-directed to habit-based behaviour in the [^]mGT, i.e., from exploration to exploitation, might be compromised in CORT animals.

This investigation again demonstrated a disruption in the learning capacities of CORT-treated animals during operant tasks. Indeed, instrumental acquisition was impaired after sustained hormonal exposure, as shown by the delay in reaching acquisition criteria. Thus, the learning processes leading to the formation of action-outcome associations was disrupted. Moreover, chronic CORT administration prompted behavioural inflexibility in animals through the delay of their behavioural adjustment in early phases of the RL task. These learning deficits are likely to affect the ability of animals to reevaluate options in face of their experience and will therefore impact the generation of new options. Following a similar rationale compared to the one used for motivational aspects, learning deficits in mice might provoke the emergence of additive negative emotions potentially contributing to an overall frustration-like state. This complex emotional state might lead animals to behave in non-adaptive ways and affect the initiation of actions, or even facilitate perseverative or impulsive actions. The learning deficit itself could therefore contribute to the emergence of states reminiscent to human rumination or behavioural misbalance. Unfortunately, relationships of direct causality cannot be claimed here since different animal cohorts were used during this investigation. Ongoing projects would help elucidating the particular contribution of these particular learning deficits in CORT-induced suboptimal DM. In particular, more complex experimental designs of RL, as well as strategy shifting tasks, will complete the findings of the presented investigation on behavioural flexibility. Operant approaches of gambling performance will offer further information about behavioural dimensions affecting DM under uncertainty, such as animals’ tendency to choose in an impulsive manner.

Interestingly, learning deficits in the CORT model did not encompass psychomotor components and motor scores do not directly predict mice Δ mGT performance. The behavioural adjustment in a rotarod-based task was delayed after chronic CORT administration, but mostly appeals to motor components. Future studies should include additional examinations in order to discern cognitive from metabolic effects of CORT administration on the MLT performance. These results also invite to further substantiate the effects of chronic CORT administration on skeletal muscle metabolism potentially yielding to muscle atrophy, in accord with previous studies (Harlé et al., 2017; Kang et al., 2020).

Conclusively, delays in the encoding of contingencies and subsequent slower learning rates in the DM context are likely to reduce gambling success by preventing reward responsiveness at early learning stages. This obstruction of associative learning over cue-processing will consequently frustrate gain maximization.

Distress-induced learning deficits do not ascribe to cortico-striatal brain networks primarily involving the mPFC, OFC and DLS.

Learning impairments are usually reported in individuals following sustained distressful events. Patients suffering from depression, for instance, show difficulties in forming new instrumental associations and their ability to adaptively adjust their behaviour in dynamic environments is compromised. Thus, PFC regions might play a key role in these learning impairments. Moreover, patients suffering from depression and other neuropsychiatric disorders often found themselves overwhelmed by the effect of negative stimuli and end in a cognitive interlock that can bias their cognitive capabilities (Belzung et al., 2015). Cortical areas such as the mPFC and OFC, as well as interoceptive regions like the insula, have been proposed to participate in this negative state of mind.

This doctoral investigation demonstrated that high mPFC CRF levels predict suboptimal Δ mGT performance in distressed mice, as previously reported. However, mPFC CRF expression was not predictive of neither spatial WM, nor of psychomotor learning scores. Similar results were obtained for GR/MR ratios of expression in the other telencephalic regions of interest. Unexpectedly, striatal activity after chronic distress was not found altered during this investigation. Likewise, striatal-related neurobiological data was not predictive of learning deficits. Considering these findings and scientific evidence from the literature, future studies should interrogate the role of these HPA axis elements in other cortical regions, such as the OFC and the ACC, in CORT-induced learning deficits and particularly, in suboptimal Δ mGT performance.

Differential patterns of neural activation in discrete cortical areas related to operant learning deficits were also expected. Particularly, this investigation sought for PL and IL variations explaining CORT-induced delay in instrumental acquisition, since these subregions act complementary during the process of formation of action-outcome associations. However, PL and IL patterns of neural activation were always similar in mice across pharmacological conditions. The investigation did not demonstrate a direct involvement of the OFC in operant learning deficits, despite the fact that the BLA activation pattern was found aberrant, and that the BLA-OFC connectivity is thought to be pivotal in instrumental acquisition (Zeeb and Winstanley, 2013). CORT-induced learning deficits were well established after studying how animals individually adapt their behaviour in early phases of the RL task. Interestingly, animals that took longer to learn the new rule of the task, so-called *low-flexible* individuals, showed aberrant patterns of neural activity in the BLA and VTA compared with *high-flexible* individuals. These

results are consistent with scientific evidence supporting the role of the BLA in mediating behavioural adjustment following outcome conflicts (Wassum and Izquierdo, 2015). Besides, the decreased VTA activity might reflect motivational deficits in *low-flexible* individuals. It is therefore plausible that the ability to learn a task's new rule is compromised in *low-flexible* individuals, firstly because a BLA aberrant activity might obstruct the disengagement from the former rule to form a new action-outcome association, and secondly because they were intrinsically less motivated, as is indicated by the decreased VTA activity. However, a lack of motivation in these *low-flexible* individuals (that are predominantly distressed individuals) could also derivate from their difficulty to learn the new rule.

| Decisional process | Behavioural paradigm | CORT-induced/Parameters | Underlying neurobiology |
|--|--------------------------------|--|---|
| Overall DM | ^A mGT | Delayed reward responsiveness & suboptimal final performance | mPFC GR downregulation & mPFC CRF predictive value |
| Appetitive behaviour/behavioural initiation | FCS | FCS degraded | - |
| | NSF test | ↑ Latency to feed | - |
| | FFT | ↑ Latency to feed | - |
| | PR schedule of reinforcement | ↓ Breakpoint (effort allocation) | ↓ BLA & aIC activity & negative effect of IL CRF activation |
| | RL task* | ↓ Obtained rewards | ↓ VTA & BNST activity |
| Consummatory behaviour/hedonic appreciation/reward valuation | ^A mGT | ↓ Total intake | - |
| | | ↑ Lose-shift | |
| | | = Win-stay | |
| | FST | = Immobility latency & ↑ immobility duration | |
| | SPT | = Sucrose solution consumption | |
| | FFT | = Choice & ↓ total intake | |
| Reinforcement learning/psychomotor learning | ^A mGT | = Rigidity score = Flexibility score | - |
| | dWST | ↑ Total errors & ↑ test latency | |
| | FR-1 schedule of reinforcement | Delayed instrumental acquisition | - |
| | RL task* | = Correct responses & ↓ learning rates – behavioural flexibility | ↓ VTA & BNST activity & ↓ BLA & VTA in low-flexible |
| | MLT | ↓ Time on the rotor | - |

Table 6. Summary of the results obtained during the behavioural evaluation and the study of the neural underpinnings of the direct or indirect processes subserving DM under uncertainty.

Unfortunately, the experimental design used during this investigation does not allow discerning these two possibilities. Ongoing projects will help answer these questions by investigating patterns of neural networks' activation in *high-* and *low-flexible* individuals directly related to learning rates in early stages of the RL task.

In the search of biological markers of suboptimal DM vulnerability, this investigation has addressed neural activation in physiological and pathological conditions from a localizationist strategy.

However, data obtained during this research is being analysed from a functional neural network's connectivity perspective that likely adjusts better to actual brain functionality. This approach will also allow hierarchical clustering of functional modules, which will help understanding the underpinnings of distress-induced suboptimal DM under uncertainty. Combined, this investigation demonstrated that chronic CORT administration is an effective paradigm to reproduce human decisional impairments in dynamic environments subsequent to sustained distress, and that these impairments are primarily mediated by learning and motivational deficits.

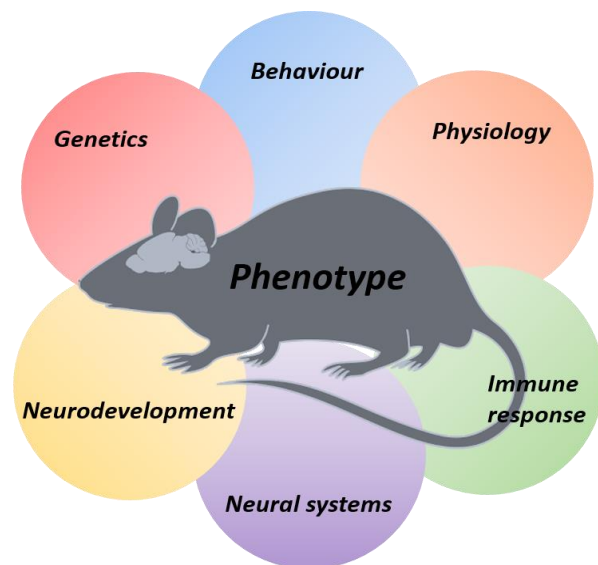
Various neural correlates have been proposed to be at the origin of these deficits. Firstly, aberrant *crf* signalling in the mPFC might obstruct optimal fronto-striatal cognitive functioning, and consequently impact goal-directed behaviour and subsequent learning necessary for optimal DM processing. And secondly, motivational deficits might primarily arise from aberrant BLA, aIC and VTA activity that would impact DM through disruption of reward and effort valuation and processing.

CONCLUSIONS AND OUTLOOK

The understanding of the neurobiological underpinnings of DM has significantly progressed in the past decades, however, much remains to be done. Decision-making is a highly complex process, occurring almost constantly during an individual's life. Investigation on DM rely on a large number of tasks that interrogate specific components of this function that appeal to particular underlying neural circuits. Although this large set of tasks has significantly contributed to the general understanding of the neurobiology behind DM, findings are often controversial and interpretation based on their comparison remains cautious. Investigation on DM benefits from translational research, which includes numerous non-human species and behavioural paradigms. Indeed, the development and refinement of animal paradigms is constantly providing additional tools for dissecting the neural substrates and networks that subserve phenotypes in a physiological-to-pathological continuum. Thus, systematic application of translational valuable paradigms might further contribute to identify particular biological elements affecting DM across clinical conditions. In parallel, clinical studies could nourish translational research on DM through incorporation of interventions based on phenotype specifics, since clinical definitions might not be sufficient to encompass interindividual variability neither in health, nor in disease.

In this framework, the CORT model offers opportunities to investigate whether the major alterations identified in the mPFC, BLA, aIC and VTA underlie the cognitive and motivational alterations observed across neuropsychiatric and other clinical conditions subsequent to sustained distress. The CORT model, for example, successfully reproduces an abulia-like phenotype, that is often central in amotivation-related symptomatology across different clinical conditions. However, a detailed understanding of the biology behind GC and their receptors is essential for the interpretation of findings, as otherwise this could lead to unfortunate conclusions. The

CORT model also allows the possibility to address the effects of sustained distress during early-life periods and its consequences on adult DM quality. Of great interest are the epigenetic processes induced by distressful events that can impact GR and MR expression, which would impact in turn the stress response mechanisms. Glucocorticoids receptors are essential mediators during the stress response and affect synapse plasticity and cellular energetic activity via mitochondrial mechanisms. Thus, neural networks reacting to distress rely on GC receptors temporal availability and activity at least to a certain extent (Hollis et al., 2015). Understanding how GC molecular signalling and pathways mediate neural and behavioural adjustments in a dynamic environment is therefore indispensable to address the physiopathology of distress-related disorders.



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ANNEXES

ANNEX I: Supplementary results

Animals' weight monitoring during food deprivation

In order to maintain animals' motivation for food reward in the behavioural approaches, animals were subjected to a continuous food deprivation protocol to 80-90% of their free-feeding weight, starting a week before the start of behavioural testing. Mice were weighted daily at the beginning of the light cycle and the amount of food presented in the home cage was progressively decreased until all mice met the weight limit criteria.

Mice needed approximately a week of alimentary deprivation to reach the weight limit criteria. From that point, the amount of food in the home cage was maintained, and recalculated to better adjust the weight of the animals whenever necessary. **Figure 76** illustrates the mean body weight's evolution of 122 C57BL/6JRj mice during their behavioural evaluation. Animals conforming both pharmacological conditions did not significantly differ in their body weight (VEH, n=61; CORT, n=61) [$F_{2,120}=0.001$, $p=0.97$].

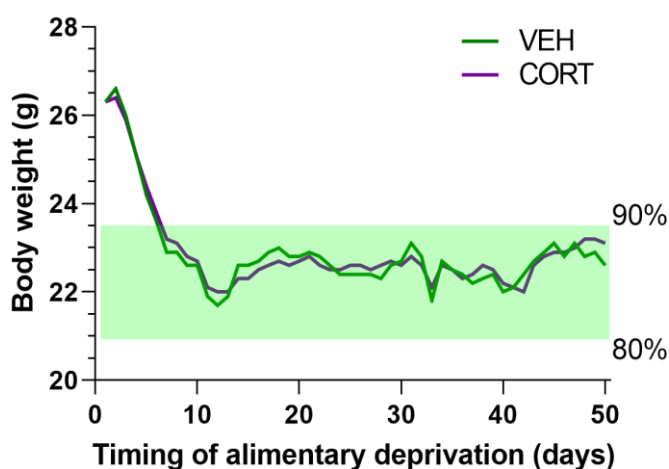


Figure 76. Mice weight monitoring during food deprivation.

Distribution of human and mice gambling data

In the study of the translational value of the Δ mGT, intraspecies variability was investigated using a k-mean clustering stratification according to the endpoint performance (% of advantageous choices in the last 30% of the task) discriminating three decisional profiles: good, intermediate and poor DMs. **Figure 77** illustrates individual data (endpoint performance) showing larger dispersion in humans than in mice.

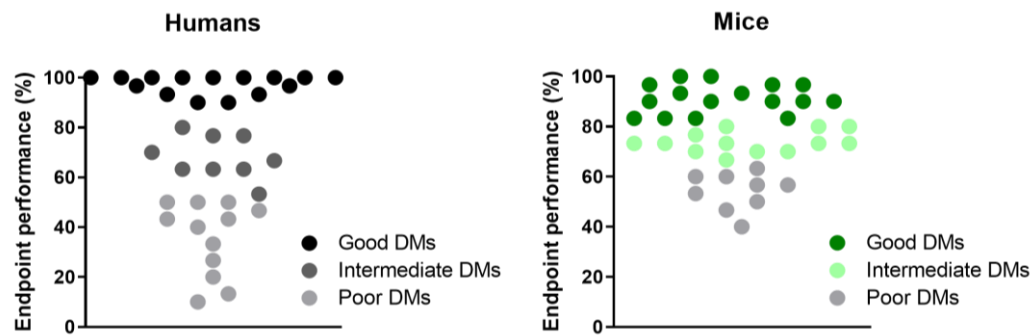


Figure 77. Distribution of endpoint gambling performance (% advantageous choices in the last 30% of the task) of the three decisional profiles (good, intermediate and poor DMs) in humans (left) and mice (right).

Gambling preferences in human and mice DM subgroups

Statistical details from the translational investigation on choice strategy of the three decisional profiles identified in the IGT and ^AmGT.

| Mice | | | | Humans | | | |
|--|------------|------------------|------------------|--|------------|--------------------|------------------|
| Bonferroni's multiple comparisons test | Mean Diff, | 95% CI of diff, | Adjusted P Value | Bonferroni's multiple comparisons test | Mean Diff, | 95% CI of diff, | Adjusted P Value |
| Good DMs | | | | Good DMs | | | |
| C' vs. D' | -5,417 | -23,54 to 12,70 | > 0,9999 | C vs. D | 26,37 | 8,077 to 44,67 | 0,001 |
| C' vs. B' | 38,33 | 20,21 to 56,45 | < 0,0001 | C vs. B | 60,29 | 42,00 to 78,59 | < 0,0001 |
| C' vs. A' | 38,75 | 20,63 to 56,87 | < 0,0001 | C vs. A | 61,08 | 42,78 to 79,37 | < 0,0001 |
| D' vs. B' | 43,75 | 25,63 to 61,87 | < 0,0001 | D vs. B | 33,92 | 15,63 to 52,22 | < 0,0001 |
| D' vs. A' | 44,17 | 26,05 to 62,29 | < 0,0001 | D vs. A | 34,71 | 16,41 to 53,00 | < 0,0001 |
| B' vs. A' | 0,4167 | -17,70 to 18,54 | > 0,9999 | B vs. A | 0,7843 | -17,51 to 19,08 | > 0,9999 |
| Intermediate DMs | | | | Intermediate DMs | | | |
| C' vs. D' | 11,11 | -7,603 to 29,83 | 0,6869 | C vs. D | -2,949 | -23,87 to 17,97 | > 0,9999 |
| C' vs. B' | 31,56 | 12,84 to 50,27 | < 0,0001 | C vs. B | 20 | -0,9219 to 40,92 | 0,0695 |
| C' vs. A' | 27,78 | 9,064 to 46,49 | 0,0007 | C vs. A | 6,282 | -14,64 to 27,20 | > 0,9999 |
| D' vs. B' | 20,44 | 1,730 to 39,16 | 0,0242 | D vs. B | 22,95 | 2,027 to 43,87 | 0,0233 |
| D' vs. A' | 16,67 | -2,047 to 35,38 | 0,1111 | D vs. A | 9,231 | -11,69 to 30,15 | > 0,9999 |
| B' vs. A' | -3,778 | -22,49 to 14,94 | > 0,9999 | B vs. A | -13,72 | -34,64 to 7,204 | 0,4896 |
| Poor DMs | | | | Poor DMs | | | |
| C' vs. D' | 8,148 | -16,01 to 32,31 | > 0,9999 | C vs. D | -1,333 | -25,19 to 22,52 | > 0,9999 |
| C' vs. B' | 8,889 | -15,27 to 33,05 | > 0,9999 | C vs. B | -4,333 | -28,19 to 19,52 | > 0,9999 |
| C' vs. A' | 7,778 | -16,38 to 31,94 | > 0,9999 | C vs. A | -28,33 | -52,19 to -4,479 | 0,0109 |
| D' vs. B' | 0,7407 | -23,42 to 24,90 | > 0,9999 | D vs. B | -3 | -26,85 to 20,85 | > 0,9999 |
| D' vs. A' | -0,37 | -24,53 to 23,79 | > 0,9999 | D vs. A | -27 | -50,85 to -3,145 | 0,0175 |
| B' vs. A' | -1,111 | -25,27 to 23,05 | > 0,9999 | B vs. A | -24 | -47,85 to -0,1454 | 0,0477 |
| Bonferroni's multiple comparisons test | Mean Diff, | 95% CI of diff, | Adjusted P Value | Bonferroni's multiple comparisons test | Mean Diff, | 95% CI of diff, | Adjusted P Value |
| C' | | | | C | | | |
| Good DM vs. Intermediate DM | 0,4722 | -16,21 to 17,15 | > 0,9999 | Good DM vs. Intermediate DM | 31,09 | 13,30 to 48,89 | 0,0001 |
| Good DM vs. Poor DM | 11,81 | -7,531 to 31,14 | 0,4243 | Good DM vs. Poor DM | 45,36 | 26,11 to 64,61 | < 0,0001 |
| Intermediate DM vs. Poor DM | 11,33 | -8,234 to 30,90 | 0,4886 | Intermediate DM vs. Poor DM | 14,27 | -6,047 to 34,59 | 0,2733 |
| D' | | | | D | | | |
| Good DM vs. Intermediate DM | 17 | 0,3211 to 33,68 | 0,0442 | Good DM vs. Intermediate DM | 1,772 | -16,02 to 19,57 | > 0,9999 |
| Good DM vs. Poor DM | 25,37 | 6,034 to 44,71 | 0,0054 | Good DM vs. Poor DM | 17,66 | -1,592 to 36,91 | 0,0836 |
| Intermediate DM vs. Poor DM | 8,37 | -11,20 to 27,94 | 0,9059 | Intermediate DM vs. Poor DM | 15,88 | -4,432 to 36,20 | 0,1808 |
| B' | | | | B | | | |
| Good DM vs. Intermediate DM | -6,306 | -22,98 to 10,37 | > 0,9999 | Good DM vs. Intermediate DM | -9,201 | -27,00 to 8,595 | 0,6377 |
| Good DM vs. Poor DM | -17,64 | -36,98 to 1,698 | 0,0862 | Good DM vs. Poor DM | -19,26 | -38,51 to -0,01573 | 0,0497 |
| Intermediate DM vs. Poor DM | -11,33 | -30,90 to 8,234 | 0,4886 | Intermediate DM vs. Poor DM | -10,06 | -30,38 to 10,25 | 0,6967 |
| A' | | | | A | | | |
| Good DM vs. Intermediate DM | -10,5 | -27,18 to 6,179 | 0,3886 | Good DM vs. Intermediate DM | -23,7 | -41,50 to -5,907 | 0,0046 |
| Good DM vs. Poor DM | -19,17 | -38,50 to 0,1700 | 0,0529 | Good DM vs. Poor DM | -44,05 | -63,30 to -24,80 | < 0,0001 |
| Intermediate DM vs. Poor DM | -8,667 | -28,23 to 10,90 | 0,8557 | Intermediate DM vs. Poor DM | -20,35 | -40,66 to -0,02996 | 0,0495 |

ANNEX II:

Image analysis, *Western Blot*

This protocol was used to relatively quantify GR, MR and CRF protein bands obtained in the Western-blot experiment (*Forth Chapter, Section 1*). *ImageJ* software was chosen as working platform. The quantification reflects the relative amount of targets as a ratio of each protein band relative to the lane's loading control (β -actin).

1. Images preparation

- JPEG file format was used to save scanned-band raw images.
- Images' picture mode was then changed to *Greyscale*.

2. Regions of Interest definition

- For each row of protein band across lanes, a single region of interest was defined. This step was repeated for each protein band lane and lanes were treated separately.
- With the *rectangle* tool from *ImageJ*, a frame was drawn around the largest band of the row. This frame covered the minimum area to contain the whole of the band. This frame was saved as the region of interest for this specific row. The same procedure was applied for each target and the loading control.

3. Measurements

- Taking protein measures required to use the same frame for all the protein bands across the other lanes. Frames fit all the bands in a row since the region of interest was sized according to the largest one.
- Starting from the first band of a lane, each band was consecutively centred inside the frame and with the tool *measure* from the *Analyse* menu, data about the pixel density was obtained.
- With the same frame as the one used for protein bands (row), measurements about the background were taken. For that, the frame was placed just above or below every band in the row, where there were no other bands and avoiding eventual artefacts.

4. Calculations for final relative quantification

- Once all data for the measurement of the protein bands and their backgrounds, as well as for the loading control bands and their backgrounds were saved, different formulae were applied:
 - Pixel density values were inverted for all data (bands, controls and backgrounds), using the formula $255-X$. Since *ImageJ* recorded values from 8-bits images, pixel densities values were expressed in a range between 0 and 255, corresponding to X on the formula.
 - To quantify the net value of each protein or loading control band, the value of the inverted background was deducted from the inverted band value.
 - To finally quantify the relative value of the targets, the ratio of the net protein band to the loading control was calculated.

Image analysis, *Immunohistochemistry*

This protocol was used to quantify cFos and FosB labelling in brain section acquired in the immunohistochemistry experiments (*Forth Chapter, Sections 2 & 3*). *ImageJ* software was chosen as working platform. The quantification reflects the density of labelled cellular nuclei per unity of density (cFos+/mm² and FosB+/mm²).

1. *Images preparation*

- JPEG file format was used to save acquired images of brain-sections.
- Images were calibrated using the *set scale* tool.

2. *Regions of Interest definition*

- For each brain structure and substructure of each brain section, a single region of interest was defined.
- With the *freehand* tool from *ImageJ*, a frame was drawn delimiting the structure or substructure to be analysed. Each frame was saved as the region of interest for each specific brain section.
- The area of each region of interest was measured using the tool *measure* from the *Analyse* menu.

3. *Image processing for contrast enhancement*

- Images' picture mode was changed to *Greyscale*.
- For foreground extraction, images were processed by using the *subtract background* tool from the *Process* menu.
- They were then transformed to 8-bits images.

4. *Detection and quantification of labelled nuclei*

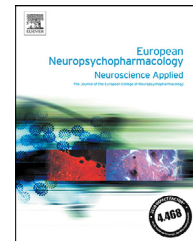
- Following background subtraction and 8-bits images transformation, the threshold for particles' detection was manually determined for each region of interest of each brain section. For that, the tool *Adjust-threshold* from the *Image* menu was used.
- Before labelled nuclei quantification using the tool *measure* from the *Analyse* menu, the minimum size of particles was set up at 10µm, and images were processed for over-segmentation in order to separate "touching" objects.

ANNEX III: Cabeza *et al.*, 2020



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Modelling decision-making under uncertainty: A direct comparison study between human and mouse gambling data



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Abstract

Decision-making is a conserved evolutionary process enabling us to choose one option among several alternatives, and relies on reward and cognitive control systems. The Iowa Gambling Task allows the assessment of human decision-making under uncertainty by presenting four card decks with various cost-benefit probabilities. Participants seek to maximise their monetary gain by developing long-term optimal-choice strategies. Animal versions have been adapted with nutritional rewards, but interspecies data comparisons are scarce. Our study directly compares the non-pathological decision-making performance between humans and wild-type C57BL/6 mice. Human participants completed an electronic Iowa Gambling Task version, while mice a maze-based adaptation with four arms baited in a probabilistic way. Our data shows closely matching performance between both species with similar patterns of choice behaviours. However, mice showed a faster learning rate than humans. Moreover, both populations were clustered into good, intermediate and poor decision-making categories with similar proportions. Remarkably, mice characterised as good decision-makers behaved the same as humans of the same category, but slight differences among species are evident for the other two

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subpopulations. Overall, our direct comparative study confirms the good face validity of the rodent gambling task. Extended behavioural characterisation and pathological animal models should help strengthen its construct validity and disentangle the determinants in animals and humans decision-making.

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1. Introduction

Do animals gamble for food just like humans gamble for money? Most species, including human beings, have to make accurate assessments of the cost-benefit trade-off in reward-driven contexts. For example, the longer an animal is searching for food, the more likely it is to accumulate more and better rewards, however, the more likely it is to encounter a predator. Thus, decision-making (DM) is an essential mechanism for survival in partly predictable situations (de Froment et al., 2014). The underlying principles of how living beings achieve efficient DM are still not fully understood, and assessing human DM using animal models requires further validation. Laboratory tests simulating real-life DM, such as gambling tasks, have been developed. Particularly, the Iowa Gambling Task (IGT) is widely used to assess human DM under uncertainty. Participants are required to maximise the monetary gains by selecting cards from four decks with various cost-benefit probabilities (Bechara et al., 1994). Players face a choice-conflict between playing from long-term disadvantageous decks yielding higher gains but frequent losses, or from advantageous ones associated with smaller immediate rewards and less frequent penalties. Thus, optimal decisions involve the animal refraining from the preferred large immediate rewards whilst opting for lower but more frequent gains, leading to long-term benefits.

The IGT was originally created to study DM impairments in patients with ventromedial prefrontal cortex damage. Compared to healthy participants who progressively learn to select the advantageous decks, the impaired patients decisively underperform by being unable to set the optimal strategy from repeated negative outcomes. Besides this, many IGT studies with healthy populations have shown a high interindividual performance variability (Bechara and Damasio, 2002; Bechara et al., 2002; Steingroever et al., 2013). Indeed, several clinical reports indicated that, while a majority of healthy participants develop the optimal strategy (good DM individuals), others do not acquire a preference for one deck over the others, which is indicative of a lack of learning (poor DM individuals) (Bechara et al., 2001, 2002; Glicksohn et al., 2007a). The literature has shown that many factors may account for this variability in gambling performance. For example, Bechara et al. explain the poor IGT performance in terms of atypical sensitivity to the reward or punishment. Mechanisms underlying this atypical sensitivity are numerous and still not fully elucidated. Among the potential contributors to IGT failure, the presence of personality characteristics associated with risky behaviour (Buelow and Suhr, 2009), of weaknesses in executive functioning (Brand et al., 2007), a low motivation (Giustiniani et al., 2015), a negative affect at the time of task (Suhr and Tsanadis, 2007) as well as low educational

(Davis et al., 2008) and low intellectual levels (Barry and Petry, 2008) have been reported. These data illustrate a behavioural continuum with an overlap in choice strategies between human non-pathological and pathological conditions.

Interestingly, animal versions of the IGT with species-appropriate adjustments to assess DM in a design comparable to humans (van den Bos et al., 2006; van Enkhuizen et al., 2013; Heilbronner, 2017; Pittaras et al., 2016; Rivalan et al., 2009, 2013; de Visser et al., 2011; Young et al., 2011; Zeeb et al., 2009) have been developed. For example, in rodent gambling tasks the decks of cards have been replaced by mazes or operant chambers equipped with different options of varying outcomes; monetary gains are replaced with nutritional rewards since money and food are thought to drive similar behaviours (Lehner et al., 2017).

Similar to human studies, rodents with lesions perform sub-optimally in adapted IGT versions and show a delay in adopting a consistent strategy compared to healthy individuals (Rivalan et al., 2011; Winstanley and Clark, 2015). For the majority of healthy rodents, as the task progresses a raise in advantageous choices is observed, which is commonly accompanied by individuals clustering in three subpopulations who reflect the variable choice strategies (van Enkhuizen et al., 2014; Pittaras et al., 2016; Rivalan et al., 2009). Good DM individuals quickly develop a strong preference for the advantageous options, while poor DM individuals display the worst performances, either not showing any preference for neither advantageous nor disadvantageous options or displaying a long-term preference for the disadvantageous ones. Intermediate DM individuals perform between the other two subgroups. Whether these interindividual variabilities observed in both healthy human and animal populations are similar has never been directly assessed to substantiate the face validity of the models.

Animal and human versions of gambling tasks usually evaluate performance with different analytical methods (Bechara et al., 2001; Zeeb and Winstanley, 2013) and are hence not compared in a direct manner even if animal gambling tasks have been specially created to simulate human DM processes. However, direct comparative research will be a valuable approach allowing clinicians and researchers to bridge their findings, rendering translational studies as a direct tool genuinely meaningful (van Enkhuizen et al., 2014).

Here, we bridge the clinical and preclinical findings on DM processes featuring uncertainty by directly comparing the performance of healthy humans and mice for the first time in the same study using IGT-adapted tasks and identical analytical methods.

Based on the recent literature addressing the validity of mouse gambling tasks (mGT) (van den Bos et al., 2013; van Enkhuizen et al., 2014; Heilbronner, 2017; Pittaras et al., 2016; Winstanley and Clark, 2015), we

forecasted a good face validity of our animal model with a similar overall performance regarding the human population (Giustiniani et al., 2015). To check for the existence of a similar variability in the choice strategies among the species, we then compared the stratification of both populations according to the endpoint performances. To evaluate the construct validity, we further studied the behavioural indexes of the cognitive processes sub-serving the optimal choices. Finally, we sought to find the correlations with an endpoint performance, focusing on the parameters of rigidity (Pittaras, 2013), flexibility (van Enkhuizen et al., 2014) and win-stay and lose-shift choices (de Visser et al., 2011). We also analysed the psychometric scores from human participants and reward sensitivity data in mice, to relate the performance or choice strategies to additional behavioural traits.

2. Experimental procedures

2.1. Participants

To reduce conceptual and methodological differences between the tasks, we controlled factors known to interfere with the results such as sex differences and the presence of instructions, especially in humans. In that respect, we recruited only male subjects because they have been described as less risky than females, choosing the advantageous options more frequently in the IGT (Singh, 2016) and its rodent adaptations (van den Bos et al., 2006). Further characterisations will include both genders, allowing us to take into account the variability we accepted to overlook in the present study (Prendergast et al., 2014).

2.2. Humans

Forty healthy right-handed participants (mean age \pm SEM = 24.7 \pm 5.1; range 19-38) were involved in the study. None reported a previous medical history of psychiatric disorders, substance or alcohol abuse, neurological diseases, traumatic brain injury or stroke, and none reported taking any medication.

Participants received information regarding the aim of the task and gave their written informed consent to take part in the study. Given the influence of real money playing a significant role on motivation, subjects were informed that the monetary payment would be proportional to the global gain obtained in the task (Meyer, 2004; Meyer et al., 2000; Miedt et al., 2010). Due to the ethical considerations and independent of individual performance, all participants received the maximum amount of €85 at the end of the experiment. The protocol was approved by the Committee of Protection of Persons (CPP-Est-11; authorisation given by the General Health Administration (ANSM 2016-A00870-51 and NCT 02862821)).

2.3. Mice

Forty C57BL/6J mice (Ets Janvier Labs, Saint-Berthevin, France) were used for this study. Inbred animals such as C57BL/6J are known to behave uniformly and display low interindividual variability (Festing, 2014) (but see recent work by Tuttle et al., 2018). All animals were 3-5 months old at the time of testing, group-housed and maintained under a 12 h-circadian cycle with a constant temperature (22 \pm 2 °C). Water was available ad libitum and all mice were food-restricted at 80-90% of their free-feeding weight (mean weight (g) \pm SEM = 22.2 \pm 0.2) in order to motivate

exploration and completion of the nutritionally rewarding task (Rivalan et al., 2009). Experiments were performed in behavioural rooms with tight luminous intensity. All procedures met the NIH guidelines for the care and use of laboratory animals and were approved by the University of Franche-Comte Animal Care and Use Committee (CEBEA-58). All efforts were taken to minimise animal suffering during the testing according to the Directive from the European Council on the 22nd of September 2010 (2010/63/EU).

2.4. Human gambling task

The task was an adapted electronic version of the IGT (Giustiniani et al., 2015), whose aim was to maximise monetary gain through successive selections between four decks. The decks' composition, values and scheduled reward-penalty were predetermined identically to match the original IGT (Bechara, 1997; Bechara and Damasio, 2005; Bechara et al., 1994). The decks looked identical, but differed in composition. Decks A and B were disadvantageous, yielding immediate rewards but involved major economic losses on the long run. Decks C and D were advantageous, yielding frequent small wins and smaller long-term penalties, resulting in long-term gain. The decks also varied in their schedule of losses, with decks B and D featuring infrequent, decks A and C frequent losses, respectively. All decks contained an infinite number of cards in this computerised version of the IGT. The monetary reward was converted from US Dollars to Euros in order to match the currency of the test subjects. At the beginning of the task, participants had a loan of €2000.

Contrary to most IGT experiments, participants were not provided with information regarding the presence of advantageous or disadvantageous decks and the number of trials. This ensured that a somewhat partial advantage compared to the animals was avoided (Macphail, 1996) (but see Rivalan et al., 2011 work). In the absence of such information the final performance usually worsens; therefore, the exploration phase typically lengthens and the optimal strategy is hardly found in 100 trials (Balodis et al., 2006; Fernie and Tunney, 2006; Glicksohn et al., 2007). However, when more trials are allowed, many individuals performing poorly in the first 100 trials are able to achieve good final performance (Balodis et al., 2006; Buelow et al., 2013; Bull et al., 2015). To that purpose, the number of trials was increased from 100 to 200. A full description of the electronic version of the IGT is given in Giustiniani et al. (2015).

2.5. Mouse gambling task

DM was evaluated using an mGT adapted from published protocols (Pittaras et al., 2016; de Visser et al., 2011). The experiment took place in a completely opaque four-arm radial maze (identical and equidistant arms, 37 cm long and 5.7 cm wide), with the common central zone used as a start-point. Mice were rewarded with grain-based pellets (20 mg Dustless Precision Pellets® Grain-Based Diet, PHYMEP s.a.r.l., Paris, France) or punished with grain-based pellets previously treated with quinine (180 mM quinine hydrochloride, Sigma-Aldrich, Schnellendorf, Germany). Quinine pellets were poorly palatable but edible.

Prior to every experimental session, the animals were acclimated to the behavioural room for 30 min. The experimental design was composed of 5 blocks of 20 trials over five days (a total of 100 trials per animal). The first ten trials of each block took place during the morning, the second ten trials during the afternoon. Before the first trial of the first block, mice had 3 min to explore and eat inside the maze (*first habituation period*). From the second block, mice had 2 min to explore the maze before the first trial, but no food was available (*general habituation period*). These habituation periods aimed to reduce the stressing effect of the

animals' first exposure to the dispositive. After this habituation, mice were placed at the start-point inside an opaque cylindrical structure to avoid early orientation. The cylinder was removed after 5 s and animals were allowed to choose an arm. Mice had one minute to choose an arm, explore it and eat the reward. An extra-minute was given if the choice was not made in time.

Our mGT was adapted in order to minimise the effect of satiety during the task. For that, two arms gave access to a small reward (1 pellet) in the first trial of each half block (trials 1, 11, 21, 31, 41, 51, 61, 71, 81 and 91) and bigger rewards (3-4 pellets) in the other 18 choices of each block with a small probability of presenting a punishment (3-4 quinine pellets, twice in 18 possible choices) (*advantageous arms C' and D'*). Arm C' and D' provided a maximal amount of 50 and 66 rewards per block, respectively.

The other two arms (*disadvantageous arms A' and B'*) gave access to a bigger reward (2 pellets) during the first trials of each half block, but bigger punishments (4-5 quinine pellets) and a smaller reward-possibility (4-5 pellets, once in 18 possible choices) in the block's remaining 18 choices. Arm A' gave access to a maximal amount of 10 rewards per block, arm B' until 14. Between consecutive trials, the animals were placed in their home cages for 90 s. The localisation of advantageous and disadvantageous arms was randomised with different award and punishment sequences for each animal.

2.6. Determination of interindividual differences

A k-mean clustering method, already used in mouse gambling tasks (Pittaras, 2016; Pittaras et al., 2016), was applied to identify interindividual differences in both, human and mice populations. This enabled the automatic identification of the objects' optimum partition into a specific number of clusters, minimising and maximising the intra and inter-cluster variance, respectively (Timmerman et al., 2013). In accordance with the literature (Pittaras et al., 2016), the mean percentage of advantageous choices was calculated for the tasks' final 30% when performance was highly stable ($W: p < 0.01$ in mice and humans). The individual performances were then divided into three groups: *good*, *intermediate* and *poor decision-makers (DMs)*.

2.7. Choice behaviours: rigidity, flexibility, lose-shift and win-stay scores

Maximisation of benefits and the reduction of costs, which characterise optimal performances, require flexibly adapting contingencies in order to favourably orient future choices. To compare different DM strategies, behavioural measures of cognitive processes at the beginning and the end of the task have been calculated. We measured our populations' rigidity score by calculating the highest percentage of choice of a deck or arm. The flexibility score is then the proportion of switches from one deck or arm to another. The lose-shift score, as a measure of negative outcome aversion, was assessed by calculating the proportion of switches after a penalty outcome. Win-stay scores likewise reflect the choice of the same option after receiving a reward. Each behavioural measure was calculated at the beginning (first 40% of the task) and at the end of the experiment (last 40% of the task), according to previous studies (Pittaras et al., 2016).

2.8. Reward sensitivity

In humans, the Behavioural Inhibition and Activation System scales (BIS/BAS) allowed us to approach behavioural motivation (Rizvi et al., 2016). In mice, reward sensitivity was evaluated at

the end of the mGT using the sucrose preference test (adapted from Lutz et al., 2013). For details, see Supplementary data.

3. Data analysis

3.1. Whole group analyses

A population's overall performance in terms of the advantageous choices for the gambling tasks was analysed with Bayesian and frequentist approaches. To consider learning rates, the relative risk (the ratio of the 'risk' of success in both populations (humans/mice) - RR) was measured. The resulting proportions were compared by Beta laws, whose parameters are interpreted in terms of the number of successes and failures, establishing the probability law of success rates' ratio. A credibility interval, inside which a parameter has a given probability (CI), was then calculated.

Hereafter, in order to address the behavioural kinetics the performance was divided into five blocks, each representing 20% of the task. The performance of each block was compared to the chance level using Student's tests (*t*-tests). The evolution of the performance was assessed by the analysis of variance (ANOVA), with the factor being 20%-blocks. Differences between populations were analysed using a partially repeated ANOVA with species as the between-subject variable and 20%-blocks as the within-subject variable.

The evolution of choice behaviours scores was first compared for each group using a *t*-test, followed by a comparison of the overall population's scores by two-way repeated-measures ANOVA with factors species and time course (first and last 40% of the task).

BIS/BAS data in humans and sucrose preference in mice were analysed using the Kruskal-Wallis (KW) test (see Supplementary data). Correlations between the endpoint performance (percentage of advantageous choices in the last 30% of the task) and choice behaviours were also carried out. Comparisons were Bonferroni corrected when necessary to account for the multiple comparisons.

3.2. Interindividual data analysis

For each group, the evolution of the gambling performance was assessed by repeated-measures ANOVAs, with the factors being 20%-Blocks and clusters (*good*, *intermediate* and *poor DMs*), followed by Mann Whitney U (MW) tests to show subgroup differences two by two. Each interindividual distribution was also compared between mice and humans using an MW test.

We used Wilcoxon (W) tests to compare, for each subgroup, the evolution of the gambling performance relative to the chance level. The subgroup's proportions of each population were compared using the Chi-square test. Differences in the choice behaviour scores between the subgroups in mice and humans were assessed by KW tests for the beginning and the end of the task. These measures were also compared between the species using MW tests. All MW and W tests were Bonferroni corrected.

The statistical significance threshold of all the tests was set at $p < 0.05$.

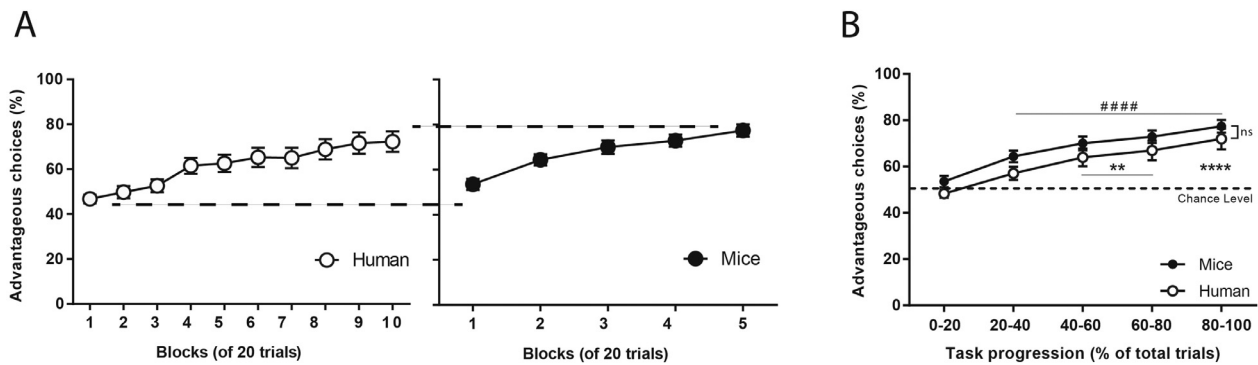


Fig. 1 Gambling performance in human and mice populations.

(A) Performances expressed as percentage of advantageous choices (mean \pm SEM) during task progression (blocks of 20 trials). After 100 trials, mice performed better than humans did (CI 95% = 1.191 to 1.277), but final performances were similar (CI 95% = 0.956 to 1.016). (B) No significant (ns) differences between species when comparing performance in 20% trial-blocks (repeated measures ANOVA). Human performance differed from chance level from the 3rd 20%-block onwards, while mice performance was already different from the 2nd block (t -test: mice: #####, $p < 0.0001$; human: **, $p < 0.01$; ***, $p < 0.0001$).

4. Results

4.1. Overall gambling performance: mice learn faster than humans (Fig. 1 and Supplementary Analysis 1)

The Bayesian analysis revealed a difference in the learning rate between the species (Fig. 1(A)), showing that mice learned faster than humans did: after 100 trials, mice had better overall gambling performance (CI 95% = 1.191 to 1.277). However, comparing the last 100 mice and human trials shows similar performances (CI 95% = 0.956 to 1.016).

In order to circumvent variations in the gambling tasks' designs and to allow for the direct comparison of data, we analysed performance as a function of task progression, expressed as percentages. Splitting all trials into five 20% trial-blocks (Fig. 1(B)) clearly shows that humans acquired the task contingencies after completion of 40% of the task, performing above the chance level from the third 20%-block onwards (t -test, $p < 0.01$). A learning effect has been evidenced as the performance gradually improved over time ($F(4, 156) = 15.4$; $p < 0.0001$) after 20% of the task was completed and till the end ($p < 0.0001$, above the behavioural output of the 1st 20%-block).

Mice, however, performed above the chance level already after the first 20% of the task, thus, orientating towards the favourable choices earlier than humans (t -test, $p < 0.0001$). The performance progressively improved over time ($F(4, 195) = 11.9$; $p < 0.0001$) from the second 20%-block (2nd block: $p < 0.05$, 3rd block: $p < 0.001$, 4th and 5th blocks: $p < 0.0001$) and compared to the beginning of the task.

The comparison of the IGT and mGT performances shows no significant effect of the species in the percentage of advantageous choices ($F(1, 78) = 3.1$; $p = 0.80$). Both populations performed similarly along the task progression, as shown by the absence of a statistical difference in the interaction between factors (species and 20%-Blocks) ($F(4, 312) = 0.06$; $p = 0.99$).

These results show that both species learn the task contingencies, however demonstrating that mice are faster

learners than humans are. When compared as function of task progression, both populations display a similar improvement in performance and reach equivalent final performance despite the design differences.

4.2. Comparable categories of good, intermediate and poor DMs with similar proportions among the species (Fig. 2 and Supplementary Fig. 1 and Analyses 2 and 3)

In Figs. 2, A1 and B1 we plot individual data showing larger dispersion in humans than in mice. Intraspecies variability was investigated using k-mean clustering stratification according to the endpoint performance, discriminating three subpopulations: good, intermediate and poor DMs. Individuals majoratively displaying the optimal strategy were referred to as good DMs and represent 42.5% of the human population (mean percentage of advantageous choices \pm SEM: 97.4 ± 0.8) and 40% of the mice population (91.3 ± 1.5). Poor DMs remained at around 50% of advantageous choices, hence showing no significant preference for advantageous nor for disadvantageous options, represent 25% of humans (34.3 ± 5.2) and 22.5% of mice (54.1 ± 2.5). The third subpopulation corresponds to individuals that developed a preference towards some options -although they did not find the optimal strategy. These intermediate DMs represent 32.5% of humans (64.5 ± 2.4) and 37.5% of mice (73.8 ± 1.0). The proportions of each subpopulation in humans and mice were compared, and no significant differences were found (Chi-square test = 0.011, $p = 0.99$; confirmed by Bayesian analysis, see Supplementary Analysis 2) (Figs. 2, A2 and B2).

In humans, good DMs performed above the chance level after 40% of the task was completed (W , $p < 0.01$), while intermediate and poor DMs differed from the chance level only in the last 20%-block ($p < 0.05$). Furthermore, the ANOVA revealed a significant interaction between the clusters and 20%-blocks ($F(8, 148) = 16.2$; $p < 0.0001$). Good DMs performed differently than poor DMs from the second

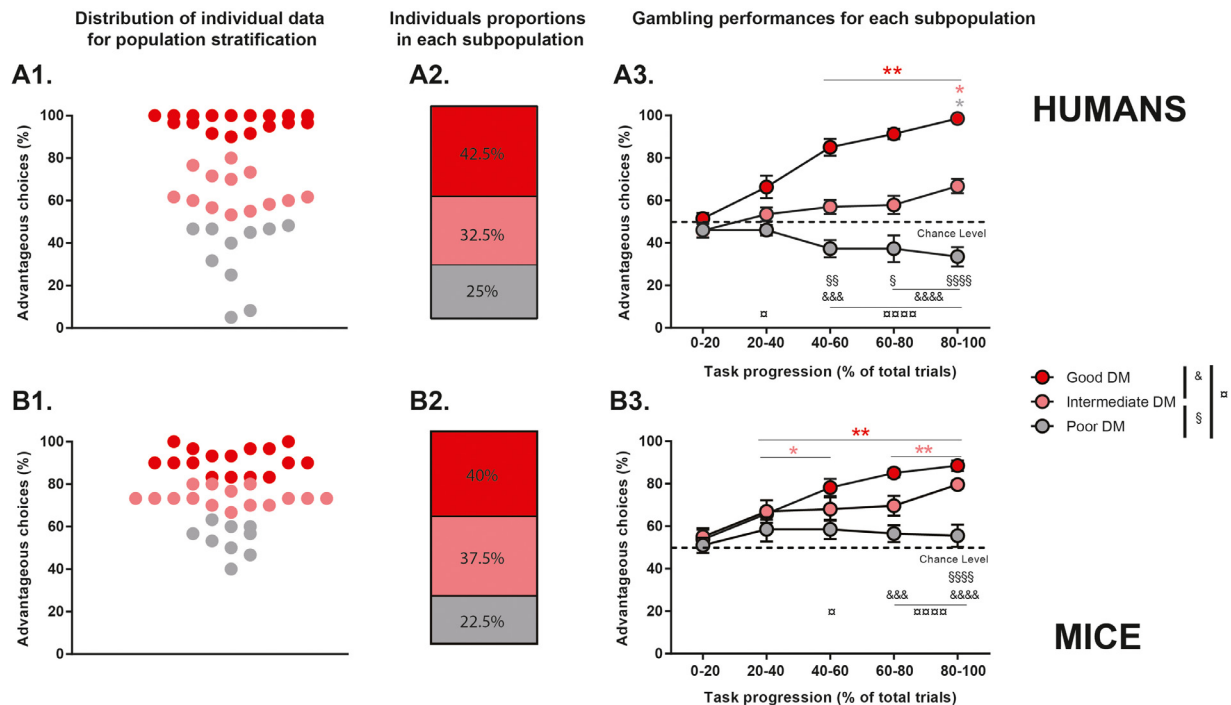


Fig. 2 Comparable categories of good, intermediate and poor DMs with similar proportions among species. Distribution in humans (A1) and mice (B1) of individual performance at the end of the task used for k-mean clustering. Proportions in humans (A2) and mice (B2) of individuals in good (red), intermediate (pink) and poor (grey) DM subpopulations (Chi², $p = 0.99$). Gambling performance in human (A3) and mice (B3) during task progression (20% trial-blocks). *W* tests to show group performance different from chance level (50%) (*, $p < 0.05$; **, $p < 0.01$). *MW* tests to show group differences (good vs intermediate: &, $p < 0.05$; &&, $p < 0.01$; &&&, $p < 0.001$; &&&&, $p < 0.0001$; good vs poor: □; intermediate vs poor: §). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

20%-block onwards (*MW*, 2nd block: $p < 0.05$, 3rd to 5th blocks: $p < 0.0001$), and differently than intermediate DMs in the last 60% of the task (3rd block: $p < 0.001$, 4th and 5th blocks: $p < 0.0001$). Intermediate and poor DMs performed differently from the third block onwards (3rd block: $p < 0.01$, 4th block: $p < 0.05$, 5th block: $p < 0.0001$) (Figs. 2 and A3).

Regarding mouse data, intermediate and good DMs performed above the chance level soon after 20% of the task was completed (*W*, good 2nd to 5th blocks: $p < 0.01$; intermediate 2nd and 3rd blocks: $p < 0.05$, 4th and 5th blocks: $p < 0.01$), while poor DMs did not differ from the chance level ($p > 0.05$). The ANOVA also revealed a significant interaction between the factors ($F(8,185) = 5.3$; $p < 0.0001$). Mice from the good DM category performed differently than poor DMs after 40% of the task was completed (*MW*, 3rd block: $p < 0.05$, 4th and 5th blocks: $p < 0.0001$), and differently from the intermediate DMs later on (4th block: $p < 0.001$; 5th block; $p < 0.0001$). Mice from the intermediate and poor DM categories performed differently only during the last 20% of the task ($p < 0.0001$).

When comparing the gambling performance in mice and humans for each subpopulation, differences were found for intermediate DMs (*MW*, $p < 0.05$), with mice making more advantageous choices than humans. On the contrary, humans from the good DM category achieved more ad-

vantageous choices than mice of the same subgroup ($p < 0.01$). Furthermore, humans from the poor DM category made worse decisions than mice ($p < 0.01$). In addition, mice from the intermediate subgroup developed a weaker preference for the most advantageous option (D') than good DMs did, whilst humans from the same category chose the best deck (D) as good DMs did (see Supplementary Fig. 1 and Analysis 3).

Collectively these data reveal that both mice and human populations clustered into three comparable DM categories with closely matching proportions. However, the performance dispersion was more pronounced in the human population.

4.3. Relationship between the DM performance and choice behaviours in humans and mice (Fig. 3 and Supplementary Figs. 2 and 3)

4.3.1. Correlations between the endpoint performance and behavioural determinants of DM (Supplementary Fig. 2)

In humans, rigidity scores significantly correlated with the final performance at the beginning ($r = 0.428$, $p < 0.05$) and at the end of the task ($r = 0.489$, $p < 0.01$). Flexibility scores showed a negative correlation with the endpoint

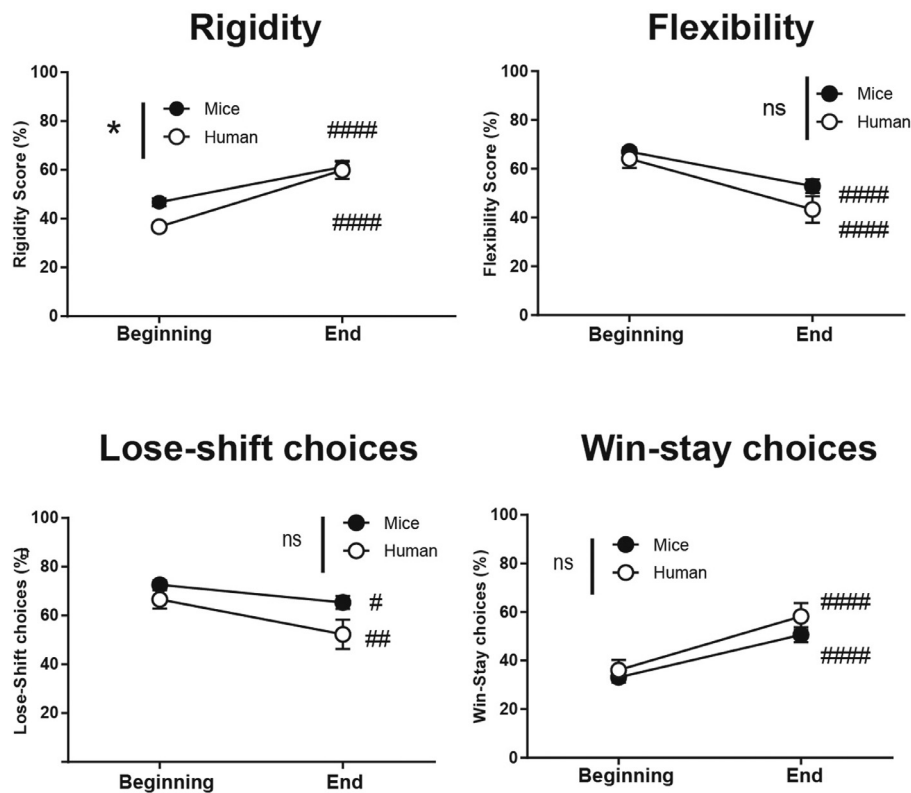


Fig. 3 Evolution of choice behaviors during task progression.

Significant progression of the rigidity, flexibility, lose-shift and win-stay choices for human and mice populations, between the beginning and the end of the task. Comparison of choice strategies at the population level by repeated measures ANOVA and post hoc *t*-tests (beginning vs end of the task: #, $p < 0.05$; ##, $p < 0.01$; ####, $p < 0.0001$; humans vs mice: *, $p < 0.05$).

performance at the beginning of the task ($r = -0.509$, $p < 0.01$), which disappeared at the end ($p > 0.05$). No significant correlation was found for the lose-shift choices and the endpoint performance, independently of the moment of the experiment. However, the win-stay choices at the beginning of the task significantly correlated with the endpoint performance ($r = 0.518$, $p < 0.01$).

In mice, like in humans, rigidity scores significantly correlated with the endpoint performance at the end of the task ($r = 0.669$, $p < 0.001$) but not at the beginning ($p > 0.05$). On the contrary, no correlation was found for flexibility and the endpoint performance at the beginning of the task ($p > 0.05$), but a negative correlation was found at the end ($r = -0.595$, $p < 0.001$). In the same way as humans, no correlation was found regarding the lose-shift choices ($p > 0.05$), but a significant correlation between the win-stay choices and endpoint performance at the end of the task ($r = 0.582$, $p < 0.001$).

In brief, DM strategies in humans and mice seem to rely on comparable adaptive choice behaviours that correlate with the endpoint performance.

4.3.2. Evolution of choice behaviours during task progression (Fig. 3 and Supplementary Fig. 3)

In both populations, a significant effect of the time course was found for flexibility ($F(1,78) = 48.4$, $p < 0.0001$), lose-shift ($F(1,78) = 15.8$; $p < 0.001$) and the win-stay scores ($F(1,78) = 51.9$, $p < 0.0001$) (Fig. 3).

Whereas no global difference among the species was found for any parameters, a significant interaction between the factors (species and time course) was found for rigidity ($F(1,78) = 4.6$, $p < 0.05$). Both populations became more rigid along the task (*t*-test, $p < 0.0001$), humans were significantly less rigid than mice at the beginning ($p < 0.05$) but not at the end of the experiment ($p = 1$) (Fig. 3).

The interspecies comparisons in all subpopulations revealed that, at the beginning of the task, humans from the intermediate and poor DM categories were significantly less rigid than the corresponding mice (MW, intermediate: $p < 0.0001$; poor: $p < 0.01$) but not good DMs ($p > 0.05$). At the end of the task, this property only remained preserved for humans from the intermediate DM subgroup ($p < 0.01$).

Flexibility scores were similar in both species for the intermediate and poor DMs at the beginning and the end of the experiment ($p > 0.05$). However, humans from the good DM category remained less flexible than mice of the same subgroup throughout the experiment (beginning: $p < 0.05$; end: $p < 0.001$). No subgroup differences in the lose-shift and win-stay choices were observed between the populations at the beginning of the task ($p > 0.05$). In the end, only mice from the good DM category were more prone to switch from an option after a penalty than humans ($p < 0.05$). However, humans from the good DM category continued choosing the same option after a positive outcome more frequently than mice ($p < 0.01$). No differences were found for the other subgroups ($p > 0.05$) (see Supplementary Fig. 3).

Overall, these data show that humans and mice do not differ in their choice strategies at the population level, but suggest there are slight differences in the intermediate and poor DMs subpopulations among the species. Remarkably, mice and humans from the good DM category behaved alike.

5. Discussion

The goal of this study was to directly compare DM under uncertainty between humans and mice using IGT adaptations according to the literature (van den Bos et al., 2006; Giustiniani et al., 2015; Pittaras et al., 2016). Our study shows closely related performance curves when comparing data as a function of task progression, suggesting that our animal model is a suitable candidate to bridge the findings between preclinical and clinical data. Therefore, our study offers a chance to get a better insight into the common conserved cognitive processes among mammals.

Humans rewarded with money and mice rewarded with food started the task with an explorative search, displaying an equal preference for either the advantageous or disadvantageous options. A preference for advantageous choices progressively emerged in both populations during what has been referred to as the exploitation phase (Daniel et al., 2017; Rivalan et al., 2013; de Visser et al., 2011). The overall performance was very similar, however, mice more promptly selected advantageous options than humans. The learning curves observed in our mice confirm previous animal results obtained in similar conditions (Pittaras, 2013; Pittaras et al., 2016) and in other variants of rodent gambling tasks (van Enkhuizen et al., 2014; de Visser et al., 2011).

Mice being faster than humans in developing a favourable strategy could be explained in terms of the rewards' nature, which is considered a major limitation for animal versions of gambling tasks. Modelling loss of reward in animals in a similar manner as in humans is a challenge. Food aids in the survival of species: it is a primary reinforcer since it strengthens behaviour and satiates the basic biological drives. Money, however, is a secondary reinforcer: its value is relative to the primary reinforcer. Hunger and satiety are factors difficult to control, which patently influence animals motivational state (van den Bos et al., 2014; Brevers et al., 2013). However, due to its subjective nature, the interest in money is also difficult to control, which raises similar concerns and can lead to similar consequences. This is the reason why the performance alignment in our mice and human participants is relevant.

In addition to the challenge associated with the reward nature and processing, the internal state (see the *somatic marker theory*, Verdejo-García and Bechara, 2009) and context also generate differences in behaviour. Animal automated operant testing would also help deepen the analysis of motivational aspects for instance (Nithianantharajah et al., 2015).

A good validity of the model should also be substantiated by similar choice strategies, which can be investigated from additional cognitive proxies sub-serving behaviour (Winstanley and Clark, 2015). A closer look at the endpoint performance revealed that they correlated with the choice behaviours similarly but not identically in both popula-

tions. In fact, as advantageous choices increased along the task progression, preference for one option progressively emerged (increased rigidity), while fewer options were explored (decreased flexibility) with individuals becoming more sensitive to the reward (increased win-stay choices) and tending to more easily cope with penalties (decreased lose-shift choices).

Our results also highlighted close common interindividual variability in mice and humans when clustered into three subgroups of individuals, ranging from good over intermediate to poor DMs. Whether this clustering reflects the speed of acquiring a favourable strategy, or actually to different behavioural strategies could be further investigated (by a computational modelling analysis, for instance). However, the similar matched proportions of these subgroups for both species strengthen the face validity of our animal model.

Good DMs composed the largest subgroup in both species. Several human studies correlate a good performance predominantly with the development of an optimal strategy, accepting risky options (Barbalat et al., 2010; Charpentier et al., 2017). In mice studies a good performance is related to a secure strategy (Pittaras et al., 2016). Interestingly, mice from this category needed less time than humans to perform above chance level. Regarding the other subgroups, humans from the good DM category developed a stronger preference for one option over the intermediate and poor DMs, whereas mice of the same category differed only from the poor DMs. The evolution of the penalty aversion was also similar for both species, suggesting that the cognitive strategies underlying the DM performance might be similar in both species, at least for the good DM subgroup.

Individuals conforming to the intermediate DM category, while selecting advantageous choices more often, maintained a high level of exploration of all the options but did not achieve the best strategy to maximise their rewards. However, IGT studies have shown that the performance can be significantly enhanced with additional trials (Overman and Pierce, 2013). Furthermore, mice from this category became more sensitive to a reward along the task progression, whereas humans did not. This suggests that intermediate categories might not completely overlap between the species.

The subgroup with the worst performance, poor DMs, maintained the exploration of all the available options, exhibiting high behavioural flexibility. Nevertheless, they did not manage to find a favourable strategy along the task, suggesting an ineffective exploration of options. However, humans from this category ended the task performing significantly below the chance level in terms of the advantageous choices, which did not happen in the animal population. This kind of deleterious preference has also been described in mice following singled-session mGT protocols (van Enkhuizen et al., 2014) and other rodent IGT versions (Rivalan et al., 2009, 2013). Mice performing poorly in the gambling tasks have been proposed as models for the vulnerability of pathological gambling or addiction (van den Bos et al., 2013; Pittaras et al., 2016), in the same line of clinical studies seeking behavioural markers of pathological predisposition or endophenotypes (Cavedini et al., 2010; Zhang et al., 2015). Indeed, these animals seem less risk-averse, a trait that has been already

interpreted as an indicator of weaker cognitive control over immediate loss (de Visser et al., 2011).

Concerning the endpoint performance, interindividual variabilities show a larger spread in the human population, accounting for better and worse decisions. The extreme upper scores in humans might be a consequence of a longer gambling design (Balodis et al., 2006; Buelow et al., 2013; Bull et al., 2015). Food restriction in our animals could also explain why they never preferred the disadvantageous options. Noticeably, very low performance more reminiscent of human data than ours (Rivalan et al., 2009, 2013), illustrates that performance output highly depends on task design.

A general common pattern between humans and mice subpopulations has not been fully revealed when the endpoint performance is correlated with choice behaviours. By themselves, rigidity, flexibility and sensitivity to positive and negative outcomes cannot explain the evolution of the performance, neither their emergence, in the three subgroups equally. The IGT alone, unfortunately, does not allow to distinguish reward maximisation from ambiguity aversion for instance; its output is insufficient to determine why a participant selected an option. In this perspective, further behavioural characterisation has been attempted. In humans, we evaluated the motivation to avoid aversive and to approach goal-oriented outcomes, respectively by the behavioural inhibition and activation system's scales (BIS/BAS). The endpoint performance did not correlate with the BIS/BAS scores in humans (data not shown). In parallel, the reward sensitivity assessed by the sucrose preference task in mice did not significantly differ between the subgroups (see Supplementary Fig. 4). These results contrast with those from Pittaras et al. (2016), who described a stronger sucrose preference for good decision-makers ("safe") compared to mice which perform poorly ("risky"). Moreover, reward sensitivity did not correlate with the endpoint performance either (data not shown), whereas poor performance was likely mediated by sensitivity to a high reward in a single session mGT (van Enkhuizen et al., 2014). These apparent discrepancies could be accounted for by protocol variations and suggest complex relationships between reward sensitivity and DM strategies (van Enkhuizen et al., 2014).

A similar overall performance and comparable gambling strategies observed between both species suggest that they share conserved cognitive processes essential for successful DM. A wide range of comparative approaches, in rodents and in humans, have been proposed to discern between these processes during cognitive tasks, both at the attentional and the mnemonic level (Steckler and Muir, 1996). The literature has also shown that there is a strikingly similar range of cognitive abilities between rodents and humans, as well as a remarkably high degree of anatomical overlap in their brain functions (Woolley et al., 2013). Rodents are even able to outperform humans in some learning tasks (Vermaercke et al., 2014), but we do believe that our slightly differing kinetics are mainly accounted for by the task design variability (including the rewards' nature, the number of trials and the tasks 'probabilistic schedule).

To conclude, our data thus far suggest that in DM, mice behave in a way similar to humans: they tend to choose the option with the best long-term payoff more often as

the task progresses. Our results point to close patterns of choice behaviours present across species, but the parameters we evaluated are insufficient to draw a firm conclusion for the relationship between reward maximisation and risk aversion. Extensive behavioural characterisations and validated animal models will be crucial to study brain regions and circuits involved in DM, and the relationships between reward and cognitive control systems. Nonetheless, our results directly support good face validity of the mouse version of IGT. Future studies including pathological animal models, manipulations with pharmacological tools or brain stimulation techniques and computational modelling should help disentangle processes sub-serving choice strategies, clarifying DM in animals and humans.

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Contributors

LC, JG, DF, DG, YP conceived and designed the study. LC, JG, TC, BR, CJ, DG, MN, LP, EH performed the experiments. LC, DG, YP wrote the draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare no conflicts of interest.

Authors' disclosures

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2019.11.005.

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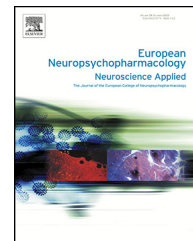
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ANNEX IV: Cabeza *et al.*, 2021



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Chronic exposure to glucocorticoids induces suboptimal decision-making in mice

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KEYWORDS

Decision-making;
Corticosterone;
Gambling task

Abstract

Anxio-depressive symptoms as well as severe cognitive dysfunction including aberrant decision-making (DM) are documented in neuropsychiatric patients with hypercortisolaemia. Yet, the influence of the hypothalamo-pituitary-adrenal (HPA) axis on DM processes remains poorly understood. As a tractable mean to approach this human condition, adult male C57BL/6JRj mice were chronically treated with corticosterone (CORT) prior to behavioural, physiological and neurobiological evaluation. The behavioural data indicate that chronic CORT delays the acquisition of contingencies required to orient responding towards optimal DM performance in a mouse Gambling Task (mGT). Specifically, CORT-treated animals show a longer exploration and a delayed onset of the optimal DM performance. Remarkably, the proportion of individuals performing suboptimally in the mGT is increased in the CORT condition. This variability seems to

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be better accounted for by variations in sensitivity to negative rather than to positive outcome. Besides, CORT-treated animals perform worse than control animals in a spatial working memory (WM) paradigm and in a motor learning task. Finally, Western blotting neurobiological analyses show that chronic CORT downregulates glucocorticoid receptor expression in the medial Prefrontal Cortex (mPFC). Besides, corticotropin-releasing factor signalling in the mPFC of CORT individuals negatively correlates with their DM performance. Collectively, this study describes how chronic exposure to glucocorticoids induces suboptimal DM under uncertainty in a mGT, hampers WM and motor learning processes, thus affecting specific emotional, motor, cognitive and neurobiological endophenotypic dimensions relevant for precision medicine in biological psychiatry.

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1. Introduction

Chronically elevated circulating glucocorticoids (GC) have been extensively shown to have detrimental physiological and cognitive effects (see for instance [Wolkowitz et al., 2009](#)). Particularly, persistent hypothalamo-pituitary-adrenal (HPA) axis dysfunction has been reported in humans upon repeated stress, with elevated levels of the endogenous GC cortisol ([Marin et al., 2011](#); [McEwen, 2017](#); [Zunszain et al., 2011](#)), but also in patients with chronic inflammatory diseases treated with exogenous GC ([Oray et al., 2016](#); [Paragliola et al., 2017](#); [Straub and Cutolo, 2016](#)). In fact, hypercortisolaemia is part of the symptomatology reported in patients with neuropsychiatric disorders afflicted with severe cognitive dysfunction ([Gomez et al., 2009](#); [Hinkelmann et al., 2009](#)). Specifically, aberrant decision-making (DM) has been described in patients suffering from depression using the Iowa Gambling Task (IGT) ([Cella et al., 2010](#)). This paradigm involves probabilistic learning via monetary rewards and penalties, and optimal task performance that leads to the maximization of gains, requires subjects to develop a preference for smaller immediate rewards in order to avoid more important losses in the long-term. Interestingly, maladaptive DM strategies have also been reported in healthy subjects ([Bechara et al., 1994](#); [Giustiniani et al., 2015](#)). Of particular interest, depressed patients show a reduced ability to detect and incorporate experience from reward-learning associations ([Pizzagalli, 2014](#)), therefore anhedonia is thought to act by modifying goal-directed behaviours when positive reinforcements are involved ([Must et al., 2013](#)). Moreover, hyposensitivity to positive outcome (reward) and maladaptive responses to negative outcome have been linked to depression ([Belzung et al., 2015](#); [Must et al., 2006](#)), suggesting a dysfunctional interaction between limbic and motor-executive regions as putative underlying mechanisms. Yet, the influence of the HPA axis on DM alterations remains poorly understood. The regulatory role of GC on HPA axis activity ([Smith and Vale, 2006](#)) has pointed to imbalances in the expression of their main receptors (glucocorticoid- GR, and mineralocorticoid receptors -MR) as biomarkers of depressive states ([de Kloet et al., 2018](#); [Zhe et al., 2008](#)). Simultaneously, the corticotropin-releasing factor (CRF), the major activator of the HPA axis, is thought a key player in stress-induced executive dysfunction ([Uribe-Mariño et al.,](#)

[2016](#)) and in mood and anxiety disorders ([Arborelious et al., 1999](#); [Binder and Nemeroff, 2010](#); [de Kloet et al., 2005](#); [Gillespie and Nemeroff, 2005](#); [Zobel et al., 2000](#)).

Chronic corticosterone (CORT) administration in rodents represents a tractable mean to address these human pathological conditions ([Darcet et al., 2016](#)). In fact, chronic CORT-treated animals exhibit a behavioural spectrum reminiscent to emotional anxio-depressive symptoms as evidenced in several conditioned and non-conditioned tasks ([Darcet et al., 2014](#); [David et al., 2009](#); [Dieterich et al., 2019](#); [Dieterich et al., 2020](#); [Gourley et al., 2008](#); [Gourley and Taylor, 2009](#)). Besides, in gambling tasks, healthy rodents efficiently explore and sample from different options prior to establish their choice strategy upon associative and reinforcement learning, showing a high inter-individual variability, probably shared with humans ([Cabeza et al., 2020](#); [Daniel et al., 2017](#); [de Visser et al., 2011](#); [Pittaras et al., 2016](#); [Rivalan et al., 2013, 2009](#); [Steingroever et al., 2013](#)).

Here, we hypothesized that chronic CORT exposure leads to suboptimal DM processing under uncertainty in a mouse Gambling Task. In line with the dimensional framework of the Research Domain Criteria Initiative (RDoC) ([Cuthbert and Insel, 2010](#)), we addressed feedback sensitivity since optimal performance in gambling tasks requires effective exploration of options in their early stages ([de Visser et al., 2011](#); [Pittaras et al., 2016](#)). Aiming to elucidate their implication in suboptimal DM, spatial working memory (WM) and psychomotricity, as cognitive and arousal-sensorimotor constructs, were also explored. Three relevant brain regions were targeted in this study given their contribution in instrumental behaviour, and mood and stress-related symptomatology: the medial prefrontal cortex (mPFC) and the dorsolateral striatum (DLS), modulators of goal-directed and habit-based learning processes respectively ([Daw et al., 2005](#); [Schwabe and Wolf, 2009](#)), and the ventral hippocampus (VH), involved in stress and emotional processing, exerting strong regulatory control on the HPA axis ([Fanselow, 2010](#)). The protein levels of GR, MR and CRF were quantified in these brain areas. As depression is associated with a high rate of pharmacological resistance ([Akil et al., 2018](#); [McIntyre et al., 2014](#)) and to a high risk of suicide ([Conejero et al., 2018](#); [Hawton et al., 2013](#)), understanding how neuronal mechanisms underlying DM processes are altered may offer insights towards the detection of predictive biomarkers for treatment selection.

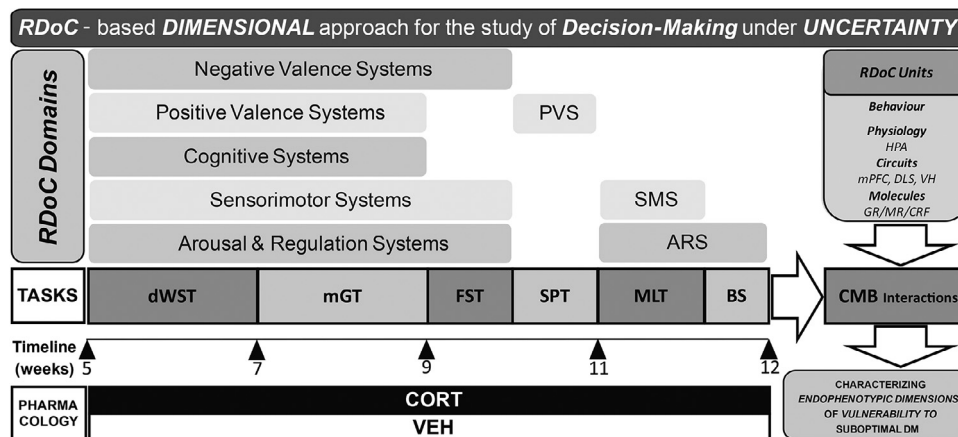


Fig. 1 Dimensional approach of the experimental design. Mice were treated with corticosterone (CORT) (35 $\mu\text{g}/\text{mL}$; $n=40$) or vehicle (VEH) (0.45% hydroxypropyl- β -cyclodextrin- β CD; $n=40$) for four weeks before behavioural screening started. Treatment was maintained throughout. Mice were tested in a series of behavioural tasks, namely delayed spatial Win Shift Task (dWST), mouse Gambling Task (mGT), Forced Swim Test (FST), Sucrose Preference Test (SPT) and Motor Learning Task (MLT) in order to assess the functioning of several *Research Domain Criteria* (RDoC) domains/systems including Negative and Positive Valence Systems (PVS), Cognitive Systems, Sensorimotor Systems (SMS) and Arousal and Regulation Systems (ARS). Five-seven days after the last behavioural test, mice were subjected to blood sampling (BS) and then sacrificed. Their brains were harvested for subsequent post-mortem analyses enabling a multilevel approach, e.g., molecules-circuits-behaviour (MCB), as biobehavioural basis of the influence of chronic CORT exposure (Hypothalamo-pituitary-adrenal axis -HPA; medial prefrontal cortex -mPFC; dorsolateral striatum -DLS; ventral hippocampus -VH; glucocorticoid receptor -GR; mineralocorticoid receptor -MR; corticotropin-releasing factor -CRF).

2. Experimental procedures

2.1. Animals

Eighty 6-8 week-old male C57BL/6J mice (*EtsJanvier Labs*, Saint-Berthevin, France) were group-housed and maintained under a normal 12-h light/dark cycle with constant temperature (22 ± 2 °C). They had access to standard chow (Kliba Nafag 3430PMS10, *Serlab*, CH-4303 Kaiserau, Germany) ad libitum for three weeks, and the fourth week onwards, under food restriction to 80-90% of their free-feeding weight (mean \pm SEM (g) = 26.20 ± 0.26). Bottles containing water and/or treatment were available at all times.

Experiments were all conducted following the standards of the Ethical Committee in Animal Experimentation from Besançon (CEBEA-58; A-25-056-2). All efforts were made to minimize animal suffering during the experiments according to the Directive from the European Council at 22nd of September 2010 (2010/63/EU).

2.2. Pharmacological treatment

Mice started being treated four weeks before the beginning of the behavioural assessment. Half the individuals received corticosterone (CORT, $-4\text{-Pregnene-}11\beta\text{-diol-}3,20\text{-dione-}21\text{-dione}$, *Sigma-Aldrich*, France) in the drinking water (35 $\mu\text{g}/\text{mL}$ mice drank around 3-4 ml/day, therefore equivalent to approximately 5 mg/kg/day, CORT group, $n=40$). CORT was freshly dissolved twice a week in vehicle (VEH, 0.45% hydroxypropyl- β -cyclodextrin- β CD, *Roquette GmbH*, France) which control animals (VEH group, $n=40$) received in the drinking water throughout the entire experiment (David et al., 2009; Mekiri et al., 2017).

2.3. Multi-domain behavioural characterization

Mice were tested behaviourally during the light phase of the cycle (from 8:00 a.m.) from the fourth week of differential treatment.

A timeline of the experiment is presented in Fig. 1, established within the framework of the RDoC to assess the functioning of several complementary systems, including Negative and Positive Valence Systems, Cognitive, Sensorimotor and Arousal and Regulation Systems.

2.3.1. Delayed spatial win-shift task (dWST)

After 5 consecutive training days, spatial WM was tested in a subset of animals (VEH, $n=22$; CORT, $n=22$) as previously described (adapted from (Ferguson et al., 2014), for details see SOM).

2.3.2. Mouse gambling task (mGT)

Decision-making was measured using the mGT task the protocol of which we have previously described (Cabeza et al., 2020). The task took place in a completely opaque 4-arm radial maze, with identical and equidistant arms, and a common central zone used as a start-point. Mice were rewarded with 20 mg grain-based pellets or punished with grain-based pellets previously treated with quinine (180 mM quinine hydrochloride, *Sigma-Aldrich*, Schnellendorf, Germany). Quinine pellets were not palatable but edible.

Mice were trained twice daily for five consecutive days with each daily session consisting of 20 choice trials (a total of 100 trials per animal). On each trial, positive and negative reinforcers were allocated to the arms following a probabilistic rule so that visiting arms A and B was overall *disadvantageous* while visiting arms C and D was overall *advantageous*. The former resulted in access to immediate larger reward, but larger cumulative negative reinforce in the long run (9 and 14 rewards per session respectively) whereas the latter resulted in access to smaller immediate reward but a smaller cumulative punishment over time (50 and 66 rewards per session). Mice were placed in their home cages for 90 s between consecutive trials. The location of advantageous and disadvantageous arms was randomized with different reward and punishment sequences for each animal.

Decision-making performance in the mGT was measured as the percentage of advantageous choices over five 20-trial sessions. Choice strategy based on 4 different behavioural dimensions

(stickiness, flexibility, lose-shift and win-stay scores), was assessed in 40-trial blocks as previously described (Cabeza et al., 2020; de Visser et al., 2011; Pittaras et al., 2016; Robbins and Cardinal, 2019). Performance during the last session was considered for the overall measure of DM performance, as previously described (Rivalan et al., 2013, 2009). Six mice displaying immediate spatial preference amongst options (choice proportion different from the expected in absence of spatial preference, thus 25% of choices for each of the 4 available options; χ^2 , $p < 0.05$) were discarded from the subsequent mGT analyses (VEH, $n=39$; CORT, $n=35$).

2.3.3. Sucrose preference test (SPT)

The individual sensitivity to reward (Fouyssac et al., 2020) was measured using the preference for a sucrose solution over water, as previously described (Cabeza et al., 2020).

2.3.4. Forced swim test (FST)

Coping strategies in the face of distressing, uncertain conditions were measured in a FST. The switch from an active to a passive coping style i.e. the acquirement of immobility after initial attempts to scape by swimming, struggling and climbing, has been directly associated to a complementary GR and MR mediated action of glucocorticoids in the behavioural adaptation to stress-coping (de Kloet et al., 2018; de Kloet and Molendijk, 2016; Molendijk and de Kloet, 2019). Mice were individually placed for 6 min in an inescapable glass cylinder filled with 20 cm of warm water (31.5 ± 0.5 °C) and the overall time during which they were immobile was recorded (Porsolt et al., 1978). Two animals were discarded from the analysis due to technical reasons.

2.3.5. Motor learning task (MLT)

Psychomotricity was measured using a rotarod task (adapted from (Le Merrer et al., 2013) and detailed in the SOM.

All procedures including food reward (20 mg Dustless Precision Pellets® Grain-Based Diet, PHYMEP s.a.r.l., Paris, France) were preceded by a habituation period inside the home cages (see Fig. 1 for the experimental design).

2.4. Physiological responses to chronic cort treatment

2.4.1. Fur coat state (FCS)

Owing to the effects of GC on epidermal homeostasis (Pérez, 2011) and hair growth initiation (Stenn et al., 1993), the state of the fur coat of each animal was evaluated weekly as an index of pharmacological efficacy and reflecting self-oriented behaviour (Nollet et al., 2013).

2.4.2. CORT plasma assays

Final trunk blood samples were collected from all animals ($n=80$) 5-7 days after the last behavioural test and directly centrifuged at 2100 g for 15 min at 20 °C. Serum was collected and stored at -80 °C until assayed. Plasma CORT concentration was measured using an immunoassay kit (DetectX Corticosterone Immunoassay kit, arbour Assays, Ann arbour, Michigan, USA). In order to measure the homeostatic stress reactivity of the HPA axis, blood samples were also collected from a subset of mice (VEH, $n=10$; CORT, $n=9$) following a gentle restraint stress (Benedetti et al., 2012) directly before sacrifice.

2.5. Western-blots

Animals were sacrificed by rapid cervical dislocation 5-7 days after the last behavioural test, in the central hours of the light cycle (from 2:00 p.m.). Brains were removed, snap-frozen and stored at

-80 °C until processed. Bilateral samples from the mPFC (from 2.3 to 1.3 mm anterior to bregma), the DLS (from 1.1 to 0.1 anterior to bregma) and the VH (from 2.8 to 3.8 posterior to bregma) were obtained from 1 mm-thick coronal sections obtained using a cryostat and stored at -80 °C.

Samples were processed as described in the SOM with anti-GR (mouse; 1:500; sc-393,232, Santa Cruz Biotechnology), anti-MR (rabbit; 1/1000; ab62532, Abcam) or anti-CRF (mouse; 1:250; sc-293187, Santa Cruz Biotechnology) primary antibodies and HRP anti-Mouse Ig (goat; 1:5000; BD Pharmingen™) or HRP anti-Rabbit (goat; 1:5000; BD Pharmingen™) secondary antibodies. Membranes were reprobated with anti- β -actin, which served as a loading control and allows normalization for sample comparison (mouse; 1:1000; sc-47778, Santa Cruz Biotechnology).

Western-blot images were acquired either with a Bio-Rad Chemi-Doc XRS+ System (Life-Science, Bio-Rad, France) or with autoradiographic films (Hyperfilm ECL, GE Healthcare, Velizy-Villacoublay, France). All quantifications were made blind to the experimental conditions using ImageJ software (National Institutes of Health, Bethesda, MD, USA) (see Fig. S1).

Due to technical issues, some samples were not included in the final analyses, so that final samples sizes were: mPFC, GR/MR $n=72$, CRF $n=53$; DLS, GR/MR $n=70$, CRF $n=55$; VH, GR/MR $n=66$, CRF $n=57$.

2.6. Data and statistical analyses

Data are presented as means \pm SEM.

Statistical analyses were conducted using STATISTICA 10 (Statsoft, Palo Alto, USA) and figures were designed using GraphPad Prism 8 software (GraphPad Inc., San Diego, USA).

The sample sizes were identified a priori by statistical power analysis (G*Power software, Heinrich Heine Universität, Dusseldorf, Germany) with a repeated measures ANOVA (RM-ANOVA) design including 3 groups (between-subject factor), 5 measurements (within-subject factor) and predicted effect size of 0.14, $1-\beta=0.8$ and $\alpha=0.05$. Our animal sample is predicted to yield highly reproducible outcomes with $1-\beta > 0.8$ and $\alpha < 0.05$.

Individuals across pharmacological conditions were clustered in three different groups, namely (1) good, (2) intermediate and (3) poor decision-makers (DMs), with distinct preference for the advantageous options: $\geq 70\%$ preference, between 70% and 50% preference, and $\leq 50\%$ preference respectively.

Assumptions for parametric analysis were verified prior to each analysis: normality of distribution with Shapiro-Wilk, homogeneity of variance with Levene's and sphericity with Mauchly's tests. Behavioural time-dependent measures assessed during the mGT, the dWST and the MLT were analysed by RM-ANOVA with session (1 to 5) or 40-trial block (beginning or end) as within-subject factors, and treatment (VEH vs CORT) or clusters (good, intermediate or poor DMs) as between-subject factors. Group's performance in the mGT was compared to chance level (50% of advantageous choices) using Student t-tests. The degradation of the coat state due to the treatment was analysed by ANOVA with factors being weeks (1 to 13) and treatment (VEH vs CORT). When datasets did not meet assumptions for parametric analyses, non-parametric analyses i.e. Kruskal-Wallis, Wilcoxon or Mann Whitney U tests, were used. Upon significant main effects, further comparisons were performed with Duncan or Bonferroni corrections.

The assumption of independent and normally distributed distribution of treatment populations within each cluster was tested with Chi-squared tests (χ^2). Dimensional relationships between behavioural markers of DM, as measured as final performance (% of advantageous choices in the last session) variable in the mGT and protein levels in the various brain structures under investigation were analysed using Pearson correlations.

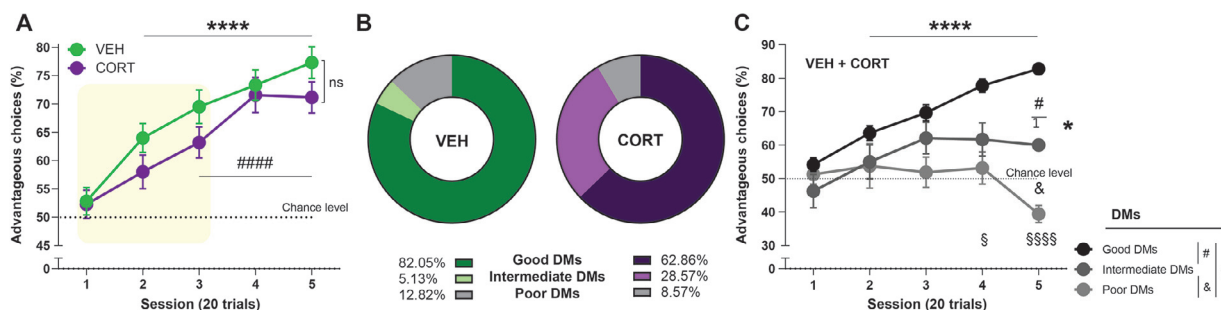


Fig. 2 Chronic CORT increases the propensity for suboptimal decision-making in the mouse Gambling Task. (A) The behavioural performance in the mGT of CORT-treated animals reveals a longer exploration of options and a delayed onset of optimal DM strategy compared to controls (VEH). CORT animals needed 40 trials to allocate their response towards advantageous options, while VEH animals required only 20 trials [% advantageous choices different from 50%: VEH, ****, $p < 0.0000$; CORT, #####, $p < 0.0000$]. (B) The frequency distribution of VEH and CORT individuals in good ($n=54$), intermediate ($n=12$) and poor ($n=8$) DM categories was significantly different [X^2 , $p < 0.0001$], with an increased proportion of treated animals among the intermediate DMs. (C) The three DM subpopulations learnt at different speeds [cluster \times session interaction: $p < 0.0000$]. Good (black) DMs improved their performance already in the second session [% advantageous choices different from 50%, ****, $p < 0.0000$], while intermediate (dark grey) DMs only significantly improved in the last session [*], $p < 0.05$. Good and intermediate DMs strategies significantly differed in the last session [#], $p < 0.05$. Poor DMs (light grey) however, never allocated their response towards the advantageous options and performed differently than good DMs from the fourth session [session 4: §, $p < 0.05$; session 5: §§§§, $p < 0.0000$], and than intermediate DMs in the last session [&], $p < 0.05$].

For all analyses, alpha was set at 0.05 and effect sizes are reported as partial η^2 ($p\eta^2$).

3. Results

At the population level, all mice showed a progressive increase in their performance in the mGT over 5 sessions [main effect of session: $F_{4,288}=31.8$, $p < 0.0000$, $p\eta^2=0.31$] but no general difference was found between groups [treatment: $F_{1,72}=2.4$, $p > 0.05$, $p\eta^2=0.03$] (Fig. 2A). However, further analyses revealed that whereas VEH mice allocated their response preferentially towards advantageous options from session 2 onwards [% advantageous choices vs chance, session 1: $t_{38}=1.2$, $p > 0.05$, sessions 2-5: $t_{38} > 2.5$, all $ps < 0.0000$], CORT mice required 20 more trials to improve DM [sessions 1&2: $t_{34} < 2.7$, $ps > 0.05$, sessions 3-5: $t_{34} > 4.8$, all $ps < 0.0000$]. Thereby this highlights that chronic CORT lengthens exploration and delays the onset of optimal DM performance.

We further explored whether chronic CORT could be considered a vulnerabilisation factor to suboptimal DM (Fig. 2B). The majority of individuals displayed the optimal strategy (good DMs). They represent 82.05% of VEH and only 62.86% of CORT animals (session 5 - mean% advantageous choices \pm SEM: 82.78 ± 1.25). Individuals from the intermediate DM subpopulation developed a delayed preference towards the advantageous options, without reaching the optimal strategy. They constitute only 5.13% of VEH whereas 28.57% of CORT mice (60.00 ± 1.38). Poor DMs failed to develop a preference for any option and correspond to 12.82% of VEH and 8.57% of CORT animals (39.38 ± 2.58). The distribution of CORT and VEH mice in the three subpopulations was compared, highlighting a significant difference (X^2 , $p < 0.0001$) which is mainly accounted for by the intermediate DMs.

In-depth analysis of interindividual variability was further performed. Decision-making subpopulations learnt at different rates [main effect of group: $F_{2,71}=21.0$, $p < 0.0000$; $p\eta^2=0.37$; session \times cluster interaction: $F_{8,284}=6.1$, $p < 0.0000$, $p\eta^2=0.15$] (Fig. 2C). Good DMs ($n=54$) needed 20 trials to orientate towards the advantageous options [% advantageous choices vs chance, session 1: $t_{53}=2.1$, $p > 0.05$, sessions 2-5: $t_{53} > 6.2$, all $ps < 0.0000$], while intermediate DMs ($n=12$) needed 80 trials [sessions 1-4: $Z < 2.05$, $p > 0.05$, session 5: $Z=7.3$, $p < 0.0001$]. Unlike the other two categories, poor DMs ($n=8$) never exhibited a preference [sessions 1-5: $Z < 2.4$, all $ps > 0.05$]. In the last session of the mGT, good DMs performed differently than intermediate DMs [$p < 0.05$, post-hoc], and the latter differently than poor DMs [$p < 0.05$, post-hoc]. Good and poor DMs performed differently from the fourth session [session 4: $p < 0.05$, session 5: $p < 0.0001$, post-hoc]. Further analyses revealed that, within the good DM cluster, whose individuals developed the optimal performance, CORT treatment delayed the onset of the strategy i.e. the allocation of the responses towards the advantageous options. Good DMs from the CORT group ($n=22$) performed differently than chance from the third session onwards [sessions 1&2: $Z < 2.4$, $p > 0.05$, sessions 3-5: $Z > 3.5$, all $ps < 0.01$], while in the VEH group ($n=32$) they differed already from the second session [session 1: $t_{31}=1.2$, $p > 0.05$, sessions 2-5: $t_{31} > 6.1$, all $ps < 0.0000$] (Figure S2). Collectively these data indicate that chronic CORT increases the propensity to suboptimal DM performance with an increased proportion of intermediate DMs compared to controls.

To infer strategies mediating mGT performance, we studied the evolution of the behavioural dimensions along task progression. Stickiness [main effect of block: $F_{1,72}=54.8$, $p < 0.0000$, $p\eta^2=0.43$] and flexibility [block: $F_{1,72}=35.4$, $p < 0.0000$, $p\eta^2=0.33$] scores changed along the task,

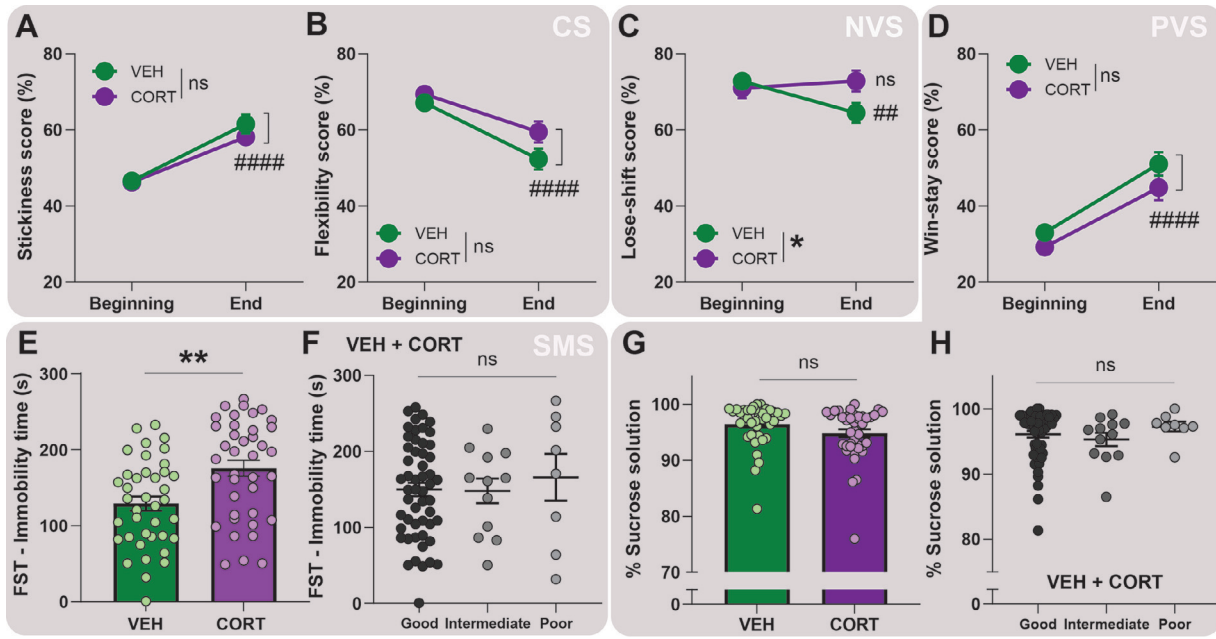


Fig. 3 Chronic CORT differentially impacts DM strategy and enhances sensitivity to negative outcome. The evolution of the behavioural dimensions along the mGT was studied in order to evaluate the influence of CORT overexposure in the development of an optimal DM strategy. Irrespective of the treatment [ns], mice became more rigid [####, $p < 0.0000$] (A) and less flexible [####, $p < 0.0000$] (B) in their choices as the task progressed (Cognitive Systems -CS). However, while VEH animals learnt to cope with penalties [##, $p < 0.01$], CORT animals continued to frequently change option after a negative outcome at the end of the task [ns]. Final lose-shift scores significantly differed between conditions [treatment: *, $p < 0.05$] (Negative Valence Systems -NVS) (C). Moreover, irrespective of the treatment, all animals more frequently selected the same option after a positive outcome with the task progression [####, $p < 0.0000$] (D) (Positive Valence Systems -PVS). Chronic CORT significantly influenced the coping style of mice when facing uncertainty in the Forced Swim Test (FST), with an increment of their immobility duration compared to VEH animals [treatment: **, $p < 0.01$] (E). However, DM clusters displayed similar coping styles [ns] (F) (Sensorimotor Systems -SMS). In terms of reward sensitivity, no differences were found between VEH and CORT animals in the preference for a sucrose solution over water [ns] (G), neither between DM subpopulations [ns] (H).

without significant effect of the CORT treatment [main effect of treatment, stickiness: $F_{1,72}=0.6$, $p > 0.05$, $p\eta^2=0.01$; flexibility: $F_{1,72}=3.4$, $p > 0.05$, $p\eta^2=0.05$] (Fig. 3A, B). Final stickiness [$r=0.669$, $p < 0.0000$] and flexibility scores [$r=-0.585$, $p < 0.0000$] significantly correlate with final mGT performance.

Remarkably, a significant effect of the treatment in interaction with the time course for the lose-shift score was evidenced [block x treatment interaction: $F_{1,72}=4.7$, $p < 0.05$; $p\eta^2=0.06$]. At the beginning of the task, all animals were prone to change option after a negative outcome (mean percentage \pm SEM of lose-shift, VEH: 72.83 ± 1.89 ; CORT: 70.97 ± 2.63). At the end of the task, VEH animals were significantly less prone to change after a penalty (64.49 ± 2.59) than CORT animals (72.85 ± 2.75) [$p < 0.01$, post-hoc] (Fig. 3C). Whereas lose-shift scores do not correlate with final mGT performance in CORT animals [$r=-0.050$, $p=0.77$], a trend was evidenced for the VEH condition [$r=-0.307$, $p=0.058$]. Concerning the win-stay score, all animals more frequently chose the same option after a reward as the task progressed [main effect of block: $F_{1,72}=52.8$, $p < 0.0000$, $p\eta^2=0.42$], irrespective of the treatment [treatment: $F_{1,72}=2.6$, $p > 0.05$, $p\eta^2=0.03$] (Fig. 3D). Final win-stay scores significantly correlate with final mGT performance [$r=0.614$, $p < 0.0000$].

At the subpopulation level, good DMs progressively developed and relied on a more rigid and less flexible strategy than intermediate and poor DMs [main effect of cluster, stickiness: $F_{2,71}=7.0$, $p < 0.01$, $p\eta^2=0.16$; flexibility: $F_{2,71}=4.3$, $p < 0.05$, $p\eta^2=0.11$]; block x cluster interaction, stickiness: $F_{2,71}=13.5$, $p < 0.0001$, $p\eta^2=0.27$; flexibility: $F_{2,71}=9.6$, $p < 0.001$, $p\eta^2=0.21$]. Intermediate and poor DMs were equally rigid and flexible in their choices [$p > 0.05$, post-hoc] (Fig. S3A, S3B). Only final stickiness [$r=0.551$, $p < 0.0001$] and flexibility [$r=-0.587$, $p < 0.0000$] scores of good DMs significantly correlate with final mGT performance. Concerning the outcome sensitivity, the three DM subpopulations behaved differently along the task [main effect of cluster, lose-shift: $F_{2,71}=0.7$, $p > 0.05$, $p\eta^2=0.02$; win-stay: $F_{2,71}=7.7$, $p < 0.001$, $p\eta^2=0.18$; block x cluster interaction, lose-shift: $F_{2,71}=6.0$, $p < 0.01$, $p\eta^2=0.14$; win-stay: $F_{2,71}=7.6$, $p < 0.01$, $p\eta^2=0.18$]. Initially, good and poor DMs more frequently shift after a penalty than intermediate DMs [$p < 0.05$, post-hoc], the latter significantly increasing their lose-shift-based strategy along the task [$p < 0.05$, post-hoc]. Final lose-shift scores were not different between subpopulations [$p > 0.05$, post-hoc] (Fig. S3C). Besides, good DMs more frequently chose the same option after a reward as the task progressed [$p < 0.001$, post-hoc], becoming significantly different from intermediate

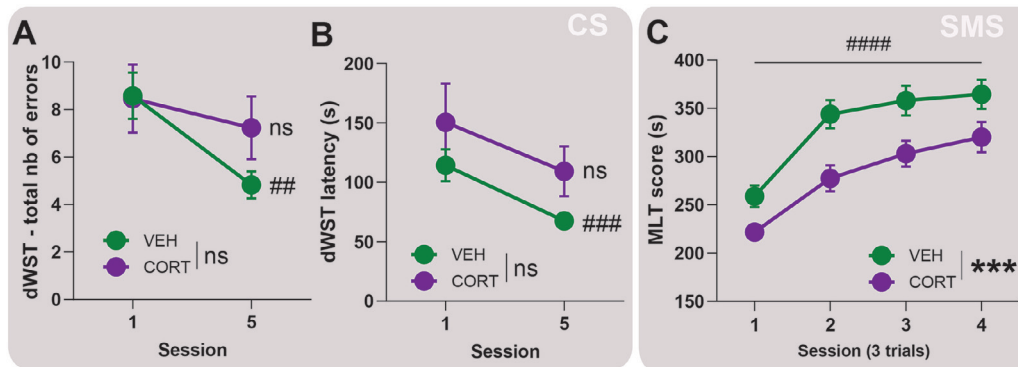


Fig. 4 Chronic CORT hampers spatial WM and motor learning processes. Irrespective of the condition [ns], the evaluation of spatial working memory (WM) capabilities in the delayed spatial Win Shift Task (dWST) revealed a general learning process, shown as a decreased in the total number of errors (A) and the time needed to finish the task (B), mainly accounted for by VEH animals [##, $p < 0.01$; ###, $p < 0.001$] (Cognitive Systems -CS). Additionally, psychomotricity (C) was found to be impaired after chronic CORT exposure [treatment: ***, $p < 0.001$], even if all animals, irrespective of the treatment, improved their performance along the Motor Learning Task (MLT) [session: ####, $p < 0.0001$]. (Sensory Motor Systems -SMS).

and poor DMs [$p < 0.01$, post-hoc] (Fig. S3D). Finally, optimal DM relies on final lose-shift [$r = -0.447$, $p < 0.001$] and win-stay scores [$r = 0.614$, $p < 0.0000$] as they correlate with final mGT performance in good DMs. No effect of the CORT treatment was evidenced for the behavioural dimensions within DM clusters, irrespective of the block [good DMs, stickiness, beginning: $U = 345.5$; end: $U = 336.0$; flexibility, beginning: $U = 302.0$; end: $U = 290.5$; lose-shift, beginning: $U = 325.0$; end: $U = 238.0$; win-stay, beginning: $U = 350.5$; end: $U = 321.5$, all $ps > 0.05$; intermediate DMs, stickiness, beginning: $U = 9.5$; end: $U = 4.5$; flexibility, beginning: $U = 9.0$; end: $U = 8.0$; lose-shift, beginning: $U = 9.0$; end: $U = 10.0$; win-stay, beginning: $U = 9.0$; end: $U = 7.0$, all $ps > 0.05$; poor DMs, stickiness, beginning: $U = 5.5$; end: $U = 3.5$; flexibility, beginning: $U = 2.0$; end: $U = 5.0$; lose-shift, beginning: $U = 2.0$; end: $U = 5.0$; win-stay, beginning: $U = 2.0$; end: $U = 5.0$, all $ps > 0.05$].

To better characterize the influence of chronic CORT on DM processes we further addressed its impact on complementary behavioural domains within the RDoC framework. CORT-treated mice displayed a more passive coping style when facing uncertainty in the FST, with a significantly longer immobility duration (total time of immobility (s) \pm SEM: 175.43 ± 10.36) as compared to VEH animals (129.07 ± 9.21) [$t_{76} = -3.3$, $p < 0.01$] (Fig. 3E). As DM subpopulations did not differ [$F_{2,70} = 0.2$, $p > 0.05$, $p\eta^2 = 0.01$] (Fig. 3F) and final mGT performance did not correlate with FST scores [$r = -0.12$, $p > 0.05$], these results suggest that the coping style does not primarily influence DM performance.

Both VEH [consumption of sucrose solution vs 50%: $t_{39} = 79.3$, $p < 0.00$] and CORT [$t_{39} = 61.6$, $p < 0.00$] mice expressed a strong preference for the sucrose solution compared to water (percentage of total sucrose consumption \pm SEM, VEH: 96.50 ± 0.59 ; CORT: 94.84 ± 0.73) (Fig. 3G), and no difference between them was evidenced for the sucrose solution consumption [$t_{78} = 1.8$, $p > 0.05$]. Moreover, DMs categories did not either differ [$F_{2,71} = 0.7$, $p > 0.05$, $p\eta^2 = 0.02$] (Fig. 3H), suggesting that DM performance does not primarily rely on reactivity to positive outcome.

We further investigated whether chronic CORT alters other dimensions required for goal-directed based DM. Chronic CORT did not impact spatial WM [mean effect of treatment, total number of errors: $F_{1,41} = 1.8$, $p > 0.05$, $p\eta^2 = 0.04$; task latency: $F_{1,41} = 3.7$, $p > 0.05$, $p\eta^2 = 0.08$]. However, a learning process was highlighted [session, total errors: $F_{1,41} = 4.8$, $p < 0.05$, $p\eta^2 = 0.10$; test latency: $F_{1,41} = 5.5$, $p < 0.05$, $p\eta^2 = 0.12$] which is accounted for by VEH animals only [total errors: $Z = 2.8$, $p < 0.01$; test latency: $Z = 3.3$, $p < 0.001$], while CORT mice did not improve along the task [total errors: $Z = 0.5$, $p > 0.05$; test latency: $Z = 1.2$, $p > 0.05$] (Fig. 4A, B). Decision-making subpopulations differed in their dWST performance [cluster \times session interaction, total number of errors: $F_{2,40} = 7.5$, $p < 0.01$, $p\eta^2 = 0.27$; task latency: $F_{2,40} = 5.0$, $p < 0.05$, $p\eta^2 = 0.20$], with poor DMs significantly improving through the task [total number of errors: $p < 0.001$; task latency: $p < 0.01$, post-hoc], unlike good [total number of errors: $p > 0.05$; task latency: $p > 0.05$, post-hoc] and intermediate DMs, the latter making more mistakes at the end of the task [total number of errors: $p < 0.05$; task latency: $p > 0.05$, post-hoc]. At the end, only intermediate and poor DMs were different in terms of total number of errors [session 5: $F_{2,40} = 3.4$, $p < 0.05$, $p\eta^2 = 0.14$; $p < 0.01$, post-hoc], but not in task latency [session 5: $F_{2,40} = 2.0$, $p > 0.05$, $p\eta^2 = 0.09$]. Final dWST scores do not correlate with final mGT performance, which questions the influence of spatial WM on DM processes.

As CORT slows down onset of optimal DM strategy, we tested whether motor performance sub-serving execution and exploitation of the mGT was also impacted. Mice improved their performance along the task [main effect of session: $F_{3,636} = 38.5$, $p < 0.0000$, $p\eta^2 = 0.15$], with chronic CORT impairing their general MLT performance [main effect of treatment: $F_{1,212} = 12.7$, $p < 0.001$; $p\eta^2 = 0.06$]. Nevertheless, no significant interaction was found between factors [treatment \times session interaction: $F_{3,636} = 0.9$, $p > 0.05$, $p\eta^2 = 0.00$]. When comparing individual sessions, CORT animals hold shorter on the rotor than VEH mice in the first three sessions, a difference that disappeared at the end of the task, thus suggesting a delayed learning process in the pathological condition (Fig. 4C). Decision-making clusters did not

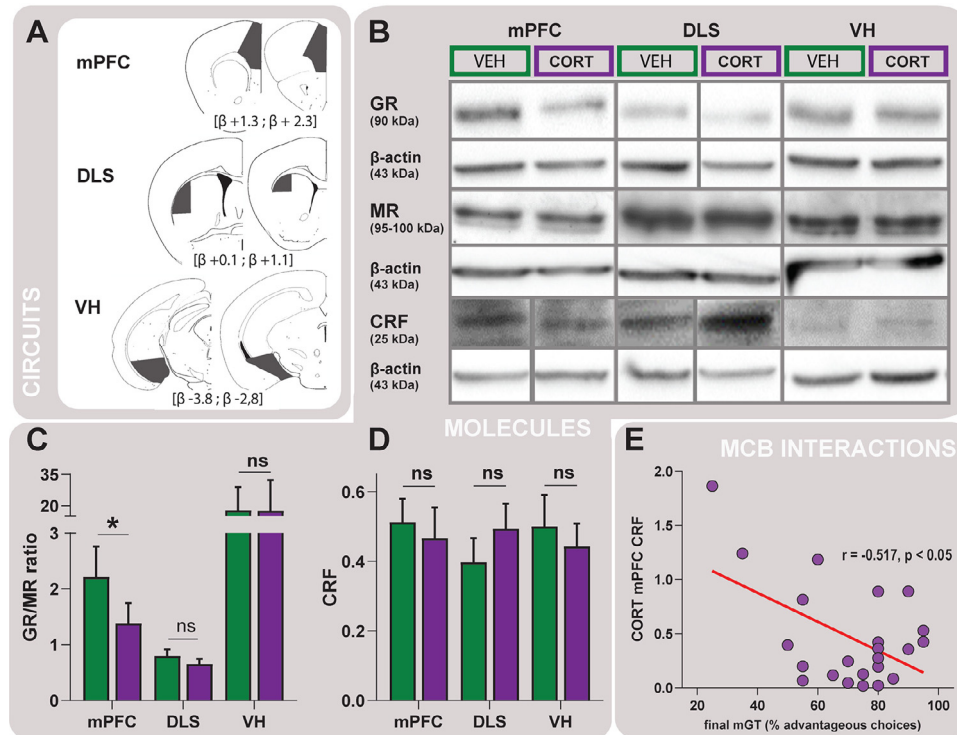


Fig. 5 HPA axis imbalance after chronic CORT. (A) Schematic representation of the sampled brain regions' location for protein quantification. Coronal sections were 1mm-thick. (B) Protein quantification by Western blotting revealed a glucocorticoid receptors (GR) downregulation in the medial prefrontal cortex (mPFC) of CORT-treated animals, which significantly diminished their GR/MR (-mineralocorticoid receptors) ratio value [*], respect to VEH animals. The protein quantification did not reveal a differential expression in the ventral hippocampus (VH), nor the dorsolateral striatum (DLS), related to the treatment [ns]. (D) Regarding the corticotropin-releasing factor (CRF), chronic CORT did not affect its expression in the brain areas investigated [ns]. However, lower CRF levels in the mPFC correlated with better final mGT performance in CORT [$p < 0.05$] but no VEH animals. (molecules-circuits-behaviours -MCB interactions).

behave differently during the task [main effect of cluster: $F_{2,211}=0.1, p > 0.05$; $p\eta^2=0.00$] and MLT scores do not correlate with final mGT performance [$r=0.0497, p > 0.05$]. These results show that the CORT treatment interferes with motor learning processes, which could impact exploration in the mGT.

We next investigated whether physiological adaptations to chronic CORT could account for differential DM performance. The FCS appeared significantly degraded in CORT animals from the third week of treatment [main effect of treatment: $F_{1,890}=448.1, p > 0.0000, p\eta^2=0.33$; week: $F_{13,890}=20.1, p < 0.0000, p\eta^2=0.23$; treatment x week interaction: $F_{13,890}=11.5, p < 0.0000, p\eta^2=0.14$; from third week of treatment: all $ps < 0.01$, post-hoc]. Decision-making categories did not differ though, neither in VEH [main effect of cluster: $F_{2,36}=1.7, p > 0.05, p\eta^2=0.08$] nor in CORT animals [cluster: $F_{2,32}=0.2, p > 0.05, p\eta^2=0.01$]. Moreover, FCS scores do not correlate with final mGT performance [$r=0.0205, p > 0.05$].

As expected, terminal blood sample analyses showed significantly higher basal plasma CORT levels in treated animals (mean CORT level (ng/mL) \pm SEM; VEH: 28.18 ± 4.97 ; CORT: 79.02 ± 12.41) [$t_{78}=-3.8, p < 0.001$], and their HPA axis reactivity to a novel acute stress was significantly blunted (VEH: 224.02 ± 35.21 ; CORT: 70.18 ± 29.97) [post-

stress plasma CORT levels, $U=10.0, p < 0.01$]. No significant differences in CORT levels were evidenced between DM clusters [main effect of cluster: $F_{2,71}=0.3, p > 0.05, p\eta^2=0.01$]. CORT levels do not correlate with final mGT performance [$r=-0.1491, p > 0.05$].

Finally, we focused on the key players GR/MR ratio and CRF from the regions of interest. The mPFC GR/MR ratio value of CORT animals (mean \pm SEM: 1.55 ± 0.37) was significantly decreased compared to control animals (2.30 ± 0.55) [$U=451.0, p < 0.05$] (Fig. 5B, C). To disentangle the origin of this difference, GR and MR levels were compared separately. While MR levels did not differ between conditions [$U=673.0, p > 0.05$], GR levels were decreased in CORT animals [$U=547.0, p < 0.05$]. No differences were found in the VH, nor the DLS between conditions [effect of treatment, VH: $U=447.0$; DLS: $U=523.0$, all $ps > 0.05$]. The three DM clusters do not either differ in their GR/MR ratio, irrespective of the region of interest [cluster, mPFC: $H_{2,68}=1.3$; DLS: $H_{2,65}=0.3$; HV: $H_{2,61}=0.6$, all $ps > 0.05$], suggesting that DM performance do not primarily depend on homeostatic HPA deregulation at the GR level.

Concerning CRF levels and regardless of the brain area, no significant differences between conditions [effect of treatment, mPFC: $U=301.0$; DLS: $U=311.0$; HV:

$U=405.0$, all $ps>0.05$], nor between DM clusters [cluster, mPFC: $H_{2,49}=1.0$; DLS: $H_{2,52}=0.3$; HV: $H_{2,52}=4.0$, all $ps>0.05$] were evidenced (Fig. 5D). Nonetheless, a significant correlation between the mPFC CRF levels and the final mGT performance of CORT animals was observed [$r=-0.5166$, $p<0.05$] (Fig. 5E), suggesting that vulnerability to suboptimal DM induced by chronic CORT relates more to CRF signalling deregulation than to GR *per se* (Figure S4).

4. Discussion

The results of this study reveal that several weeks of CORT exposure delays the encoding of the contingencies required to select responding towards optimal DM in a probabilistic gambling task. Inter-individual differences in the capability to develop an optimal DM strategy were evidenced in the global mouse population and remarkably, the proportion of individuals displaying suboptimal DM performance is enhanced upon chronic CORT.

The identified chronic CORT-induced suboptimal spatial WM, which seems to be more detrimental to the learning rate than to memory load, could somehow hamper early exploration in the mGT, impeding integration of task contingencies, in line with preclinical (Bagneux et al., 2013; Hinson et al., 2002; Jameson et al., 2004; Turnbull et al., 2005) and clinical reports (Bourke et al., 2012). Besides, chronic CORT, instead of hindering final performance, slows-down the learning rate in the MLT, a DLS-dependant motor task that does not rely on positive valence systems (Harl et al., 2017), thus evoking clinical psychomotor retardation (Bennabi et al., 2013). Additionally, though reward sensitivity does not directly account for differential DM performance, learning to cope with negative outcomes is impaired upon chronic CORT. Taken together, these results suggest a CORT-induced hedonic misbalance with differential DM performance better accounted for by a dysregulated negative (lose-shift dimension) rather than positive (win-stay dimension and sucrose preference) valence system.

This study disclosed a GR downregulation in the mPFC of treated animals, suggesting that chronic CORT exposure may disturb reflective behaviour for optimal planning, and favour suboptimal habit formation, in line with previous studies addressing the role of the mPFC in instrumental behaviour (Schwabe et al., 2008, 2007; Schwabe and Wolf, 2009). Of particular interest, CRF signalling in the mPFC of CORT individuals was found to negatively correlate with their final DM performance, underlining synergetic effects of CRF and CORT in stress-induced cognitive alterations (in line with Chen et al., 2016).

Experimental studies in humans have proposed that IGT performance relies on reward sensitivity (Must et al., 2006) though anhedonia measures do not always correlate with task performance (Rizvi et al., 2016). This is the case for our data in reward sensitivity upon chronic CORT. An efficient exploration phase in the IGT and therefore in its rodent adaptations, would guide the behaviour to a faster stickiness to the optimal choice strategy, that would become more independent of the outcome, i.e. more habitual (Balleine and O'Doherty, 2010; Schwabe and Wolf, 2009). The spatial WM and psychomotor deficits observed in our

CORT-treated mice could affect primarily action-outcome learning crucial for optimal DM strategies, rather than solely cue processing, and even if they do not directly predict differential DM, they may compromise the transition from goal-directed to habit-based behaviour. Taking in consideration the complementary roles of the studied brain structures in instrumental learning (Daw et al., 2005; Hinson et al., 2002; Schwabe and Wolf, 2009), we suggest that the GR downregulation observed in the mPFC of treated animals would primarily entail suboptimal action-outcome encoding over cue processing, yielding action-outcome consolidation especially vulnerable to chronic CORT. This interpretation is in agreement with previous studies reporting negative consequences on cognition upon chronic stress and anxiety (McEwen, 2017; Park and Moghaddam, 2017; Reul and Kloet, 1985).

Stress has been shown to affect *crf* signalling, compromising the positive valence system (Birnie et al., 2020) and disrupting fronto-striatal cognition (Hupalo et al., 2019; Uribe-Mariño et al., 2016). Together with the present study, these results suggest that high mPFC CRF levels can be considered a neurobiological endophenotype of vulnerability to suboptimal DM under chronic stress. Corticotropin-releasing factor signalling disruption may hinder mPFC computations supporting optimal fronto-striatal cognitive functioning, and specifically goal-directed behaviours, and contribute to overreliance on the negative valence system to form suboptimal action-outcome learning. This interpretation further support the *somatic marker hypothesis* (Bechara et al., 2005, 2000) and point towards an integral role of CRF. However, previous studies have warned about the dissociable roles of the ventral and dorsal mPFC in DM, especially when behaviour is reward-guided and sensitive to negative feedback (van Holstein and Floresco, 2020). Since we jointly processed ventral and dorsal mPFC structures, further investigations will be necessary to establish the exact contribution of the prelimbic and infralimbic areas of the mPFC in the CRF signalling upon chronic CORT.

The results presented here demonstrate that chronic CORT exposure impedes optimal DM under uncertainty in male mice, impacting mPFC GR and CRF signalling. Manipulating the latter to counterbalance overreliance on the negative valence system with suboptimal habit formation, could prove useful to improve coping with risk aversion towards rigidification of optimal choices when DM involves overcoming a conflict. In our study, we chose male individuals since they have been described as less risky than females, choosing the advantageous options more frequently in other rodent gambling paradigms (van den Bos et al., 2006). Besides this point, published data on females indicate that chronic CORT administration is not equally effective in altering their behaviour (Mekiri et al., 2017; Yohn et al., 2019). Future studies will examine the chronic CORT-induced effect in the mGT in females.

In sum, this study provides novel insight into the mechanisms of maladaptive value-based DM caused by chronic exposure to GC, and have important implications for understanding pathophysiological mechanisms in a transdiagnostic perspective and for identifying alternative pharmacological targets towards precision medicine in biological psychiatry.

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Author contribution

LC, YP and DF conceived the experimental design with input from DB. LC, BR and CH contributed to the data acquisition. LC and YP analysed the data. LC, YP and DB wrote the manuscript. All authors critically revised the work and approved the version to be published. LC, YP and DF agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflicts of Interest

All authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.euroneuro.2021.01.094](https://doi.org/10.1016/j.euroneuro.2021.01.094).

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SHORT FRENCH VERSION

A. Introduction

a.1. La prise de décisions

La prise de décision (PD) est un processus cognitif complexe qui aboutit à la sélection d'un plan d'action parmi plusieurs alternatives possibles. Ainsi, le mécanisme de prise de décision fait référence à un processus délibéré dont la finalité est l'engagement dans une option précise. La PD décrit un comportement motivé où des objectifs spécifiques guideront les individus pour atteindre les résultats souhaités, tout en évitant ceux qui ne le sont pas. Ainsi, une PD optimale avec des objectifs particuliers conduirait à des comportements maximisant la précision afin d'atteindre les résultats recherchés.

Dans toute sa complexité, le processus adaptatif de la PD est généralement décomposé en quatre étapes séquentielles (Doya, 2008 ; Gold et Shadlen, 2007 ; Husain et Roiser, 2018) (voir **Figure 1**).

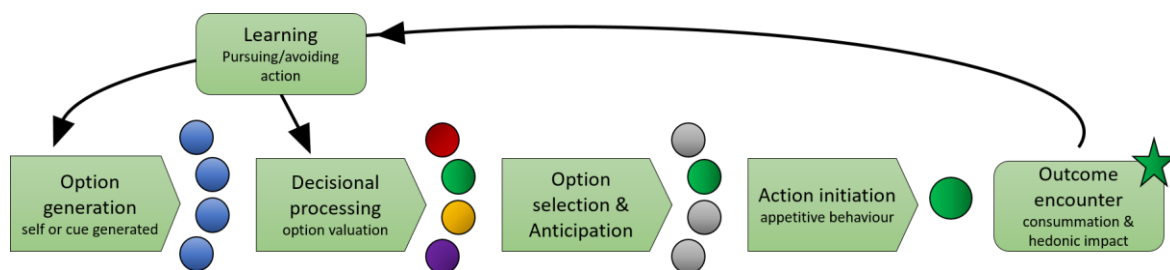


Figure 1. Étapes séquentielles de la prise de décision. Avant l'initiation d'une action, les options potentielles de comportement (disques bleus) peuvent être élaborées soit par des états internes (auto-générés), soit par les possibilités offertes par l'environnement. Une fois que les options potentielles ont été générées, elles doivent être évaluées en termes d'avantages ou de coûts avant qu'une option de comportement ne soit sélectionnée. Le fait de choisir une option potentiellement gratifiante (disque vert) induit souvent une augmentation de la motivation, appelée effet d'anticipation. Ensuite, l'initiation de l'action est favorisée par un comportement d'approche, qui conduit à la rencontre du résultat (étoile verte). A la fin de l'accomplissement du choix, les résultats exercent un impact hédonique sur l'individu ce qui influencera le processus d'apprentissage afin d'ajuster le comportement de manière adaptative. Par la suite, les options sont réévaluées avant d'en sélectionner une nouvelle. Le processus d'apprentissage peut également motiver la génération de nouvelles options.

- La génération d'options.** Le processus de PD implique l'énonciation d'hypothèses concurrentes qui représentent un état de la réalité de l'individu. Les options peuvent être générées de façon interne (e.g., sous l'effet de pulsions physiologiques ou émotionnelles), ou sous l'effet de stimuli environnementaux, comme la présentation de stimuli perceptifs associatifs.
- Traitement décisionnel pour la sélection des options.** Chaque hypothèse générée sera traitée avant l'initiation d'une action et sera probablement affectée par de nombreux facteurs, tels que la valeur subjective du résultat attendu. En conséquence, elles auront une probabilité associée d'être vraies avant de recevoir toute preuve connexe (i.e., toute information renforçant l'engagement envers une hypothèse).
- Choix de l'option.** Les éléments décisionnels précédents sont interprétés collectivement pour produire un choix discret et lancer l'action (comportement appétitif), qui est normalement nécessaire pour la réattribution des valeurs et l'évaluation des conséquences des résultats. Une fois le choix effectué, et avant de découvrir le résultat, les individus éprouvent généralement une réaction émotionnelle liée à l'attente du résultat prévu (anticipation).

- d. Impact hédonique et apprentissage. La relation entre les hypothèses et leurs actions associées est utile si des interprétations sur la probabilité sont possibles (e.g., si les individus sont capables de suivre les actions et leur impact sur les probabilités des hypothèses). Ensuite, l'évaluation subjective des résultats (e.g., l'attribution de coûts et de bénéfices subjectifs) est un processus dynamique, car leurs valeurs changent continuellement par rétroaction, conformément à leurs capacités hédoniques. De plus, le feedback peut être explicite (e.g., s'il implique la présentation physique du résultat à l'individu) ou implicite (e.g., si les coûts et les bénéfices reflètent des facteurs tacites). Finalement, les décisions sont classées comme étant plus ou moins adaptées, en fonction de l'intention de poursuivre ou d'éviter le plan d'action précédemment choisi (apprentissage).

Selon l'*Hypothèse des marqueurs somatiques* (Damasio et Everitt, 1996 ; Damasio et *al.*, 1994), la PD dépend de plusieurs niveaux d'opérations neuronales dont les opérations cognitives (dépendant elles-mêmes de processus de soutien tels que la mémoire de travail, l'attention et l'émotion). En outre, la PD dépend aussi, de manière plus ou moins importante, de l'accessibilité à la connaissance du contexte spatio-temporel des individus, des possibilités de choix et des résultats potentiels. Cette complexité intrinsèque de la PD a motivé un nombre croissant d'études en neurosciences cognitives à démêler les mécanismes neuronaux sous-jacents.

a.2. Bases anatomiques de la prise de décision

La neurobiologie sous-tendant la PD a été largement étudiée au cours de ces dernières années (voir notamment Khani et Rainer, 2016 ; O'Doherty et *al.*, 2017). Des observations comportementales, associées à des mesures physiologiques et à des techniques d'imagerie cérébrale fonctionnelle, ont été utilisées pour évaluer la PD, non seulement chez l'Homme, mais aussi chez des rongeurs et des primates non humains par la biais d'études précliniques.

Dans le processus de PD adaptative, des multiples informations somato-sensorielles sont intégrées pour sélectionner efficacement les options les plus avantageuses en fonction de leurs coûts et bénéfices. Les performances comportementales dans les tâches décisionnelles humaines dépendraient des processus d'apprentissage contrôlant (1) le comportement dirigé vers un but précis et (2) le comportement habituel. Autrement dit, elles dépendent respectivement de l'encodage de la relation entre les actions et leurs conséquences, et de la formation d'associations stimulus-réponse. A leur tour, ces processus d'apprentissage semblent régis par des réseaux cortico-striataux. Plus précisément, le comportement humain dirigé vers un but a été décrit comme reposant principalement sur l'activité du cortex préfrontal (CPF) et du noyau caudé antérieur (striatum dorsal), tandis que l'émergence et le maintien des habitudes dépendent du fonctionnement du putamen latéral (Balleine et O'Doherty, 2010 ; Walton et *al.*, 2004). Ces processus, qui façonnent les décisions, sont vraisemblablement en concurrence pour contrôler les performances et sont modulés par d'autres opérateurs, comme le réseau cortico-sous-thalamique.

D'autres systèmes de réseaux ont également été décrits comme acteurs des processus décisionnels. Le CPF, intimement lié aux noyaux thalamiques, agit tel une porte d'entrée des informations sensorielles venant de l'environnement externe (Wolff et *al.*, 2021). Un réseau comprenant le cortex orbitofrontal (COF), le CPF ventromédian (CPFvm) et le système limbique, a été lié à la récompense et au traitement des émotions qui surviennent pendant la PD. Aussi, le CPF dorso-latéral (CPFdl) intégrerait principalement les informations contextuelles, tandis que le cortex cingulaire antérieur (CCA) gérerait plutôt les informations conflictuelles et traiterait les informations de retour pendant l'apprentissage par renforcement (Khani et Rainer, 2016).

a.3. Etude chez l'Homme de la prise de décision en cas d'incertitude

Parmi les tâches de PD, l'*Iowa Gambling Task* (IGT), l'un des tests neuropsychologiques les plus fréquemment utilisés aujourd'hui, permet de mettre en œuvre un paradigme comportemental impliquant l'incertitude et la possibilité de prendre des risques.

Cette tâche a été développée par Damasio et Bechara (Bechara et *al.*, 1994) dans le but de mieux comprendre le dysfonctionnement comportemental qu'ils avaient observés chez des patients présentant des lésions du CPFvm. En effet, ils ont constaté que ces patients présentaient des difficultés au niveau de la PD émotionnelle et de l'évaluation des risques, alors que leurs performances étaient normales dans d'autres tests évaluant les processus décisionnels. Dans l'IGT, les participants doivent faire des choix répétés parmi plusieurs tas de cartes associés à des récompenses et des punitions pécuniaires, et optimiser leurs bénéfices. Ils réussissent lorsqu'ils apprennent à s'abstenir de choisir des récompenses immédiates élevées et à préférer les récompenses immédiates plus petites mais sûres à long-terme. Selon l'*Hypothèse des marqueurs somatiques*, les participants s'appuient sur des indices émotionnels pour anticiper les états corporels associés aux résultats potentiels de l'IGT, et utilisent ces attentes pour réorienter leurs choix vers les options associées à un retour affectif positif.

Les adaptations de l'IGT se sont avérées utiles pour mettre en évidence des déficiences décisionnelles et des comportements à risque dans de nombreuses pathologies neuropsychiatriques telles que la psychopathie (van Honk et *al.*, 2002), les troubles liés à l'abus de substances (Bechara et Damasio, 2002) et la dépression (Must et *al.*, 2013).

a.4. Modèles précliniques

Les modèles animaux sont essentiels pour comprendre en détail la neurobiologie des processus de PD, lui-même constitutif du comportement adaptatif humain et non-humain. Parmi les mammifères, l'utilisation de rongeurs offre des avantages dans les approches précliniques comportementales : (i) ils permettent de disséquer des mécanismes neuronaux spécifiques afin de déchiffrer les substrats et les réseaux cérébraux impliqués dans la PD ; (ii) comparé aux études chez l'Homme, l'expérimentation chez les rongeurs réduit efficacement les contraintes de temps dans les approches longitudinales ; (iii) ils permettent l'étude de la variabilité interindividuelle ; et (iv) leurs conditions environnementales, tout comme (v) leur fond génétique, peuvent être contrôlés. Puisque l'IGT capture des caractéristiques essentielles de la PD dans la vie quotidienne, le développement de modèles animaux de cette tâche était une étape judicieuse et bien fondée dans l'étude de cette fonction cognitive. Ainsi, pour des raisons éthiques et méthodologiques, les adaptations de l'IGT chez les rongeurs ont été privilégiées.

Après la première adaptation de l'IGT chez les rongeurs (van den Bos et *al.*, 2006), de nombreuses autres versions de la tâche ont été conçues et ont contribué de manière significative à la compréhension des fondements de la PD dans plusieurs domaines. Par exemple, des versions de l'IGT chez le rongeur ont été utilisées pour aborder le dimorphisme de genre (Orsini et Setlow, 2017 ; van den Bos et *al.*, 2013), les aspects physiologiques de la PD (de Visser et *al.*, 2011 ; Koot et *al.*, 2013), la variabilité interindividuelle (Pittaras et *al.*, 2016 ; Rivalan et *al.*, 2013, 2009), les modulateurs environnementaux (Zeeb et Winstanley, 2013), ainsi que les corrélats neurobiologiques (Daniel et *al.*, 2017 ; de Visser et *al.*, 2011 ; Fitoussi et *al.*, 2015 ; Pushparaj et *al.*, 2015 ; Rivalan et *al.*, 2013, 2011 ; van Enkhuizen et *al.*, 2014 ; Zeeb et *al.*, 2009).

Au cours des dernières années, des progrès considérables ont été réalisés dans la compréhension des réseaux neuronaux et des mécanismes qui sous-tendent la PD, mais beaucoup reste encore à être exploré. Plus particulièrement, il a été démontré que les capacités décisionnelles sont compromises en présence de facteurs de stress, particulièrement suite à une exposition prolongée. Le présent projet de doctorat vise donc à contribuer à la compréhension de la manière complexe dont la PD et le stress chronique sont liés, non seulement au niveau comportemental, mais aussi au niveau neuronal.

B. Stress chronique

Au sens général, le terme "stress" désigne un événement ou une série d'événements perçus comme une menace pour l'intégrité d'un individu. Ces événements, qui vont de la maladie à la prédation, en passant par des changements de contexte imprévus (environnements incertains, risqués et ambigus), provoquent des ajustements comportementaux, structurels et physiologiques dans les organismes, nécessaires pour maintenir la stabilité physiologique interne (par exemple, le stress incite à l'allostase pour préserver l'homéostasie biologique) (Houweling et *al.*, 2005 ; McEwen, 2017). Cet ajustement physiologique et cette adaptation continue face aux facteurs de stress sont certainement de la plus haute importance pour assurer la survie. Communément appelés *eustress*, certains événements conduisent initialement à des expériences difficiles qui, grâce à l'évolution de l'adaptation et de la résilience, deviennent finalement gratifiants. La *détresse*, à l'inverse, fait référence à des événements liés à des sentiments inconfortables qui conduisent principalement à des résultats négatifs. Lorsque les individus ne disposent pas du soutien physiologique, comportemental, psychologique et/ou cognitif nécessaire pour contrôler la détresse, les événements deviennent préjudiciables en surchargeant leur pression allostatique. Cette situation est, en effet, typique des facteurs de stress ayant des effets durables, communément appelés facteurs de stress chroniques.

Les réponses au stress chronique contribuent à la charge allostatique cumulative et peuvent impliquer des voies biologiques différentes de celles activées lors d'un stress aigu. Lorsque la charge allostatique augmente, elle entraîne des conséquences physiologiques résultant de réponses neuronales et neuroendocriniennes accrues. Ainsi, un couplage étroit entre les ajustements physiologiques et comportementaux qui sous-tendent les réponses au stress, est crucial sur le plan adaptatif lorsque l'individu est confronté à des menaces environnementales persistantes. Lorsqu'il est perturbé, plusieurs conditions neuropsychiatriques peuvent être favorisées.

Le système biologique de réponse au stress, en présence de facteurs de stress, fonctionne par l'intermédiaire de ses deux principaux effecteurs : (i) le système nerveux autonome (SNA), qui fournit une réponse immédiate à un événement stressant (phase d'alarme) par l'intermédiaire de ses branches sympathiques et parasympathiques, entraînant des ajustements rapides de l'état physiologique de l'organisme ; et (ii) l'axe hypothalamo-hypophyso-surrénalien (HPA) dont l'activation favorise l'augmentation des taux de glucocorticoïdes (GC) circulants qui agissent comme modulateurs directs ou indirects de l'activité de régions cérébrales spécifiques impliquées dans le comportement dépendant du stress (phase de maintien) (**Figure 2**). Lorsque les ajustements de ces deux effecteurs parviennent à réduire la charge allostatique, les organismes atteignent l'homéostasie ou le soulagement homéostatique (résilience). Cependant, lorsque ces effecteurs dépassent les limites des mécanismes homéostatiques et se déséquilibrent, les organismes ne sont capables de maintenir leur état actuel sans subir d'effets négatifs, que pendant une période de temps limitée. Ainsi, un

déséquilibre prolongé de la réponse biologique au stress entraîne une surcharge allostatique (épuisement) (McEwen, 2017 ; Selye, 1950).

Les régions et réseaux cérébraux principalement activés par des événements stressants sont

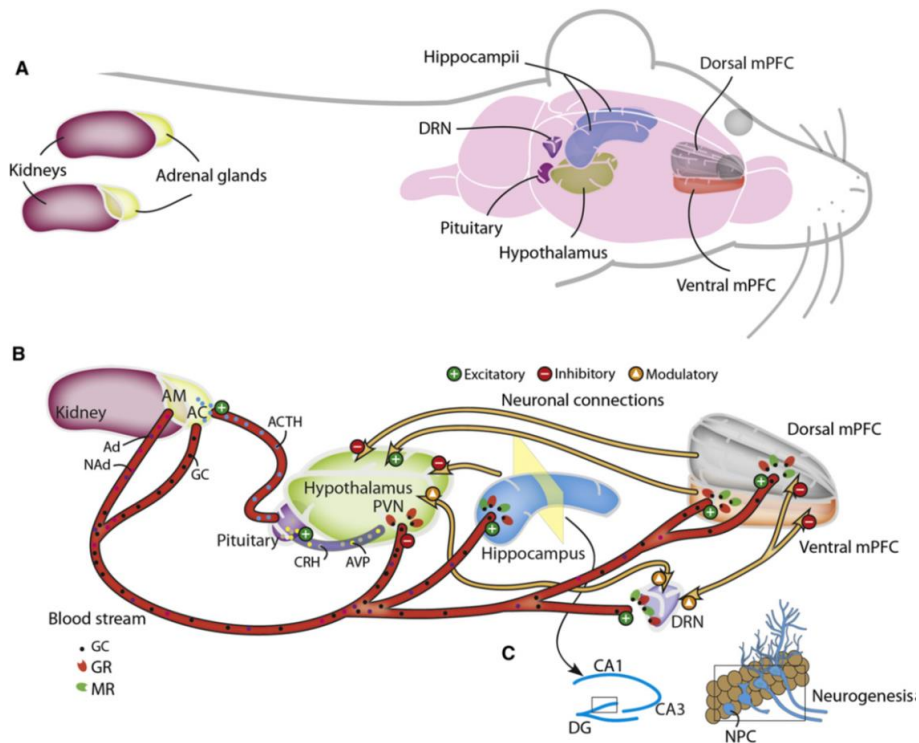


Figure 2. Connectivité anatomique et fonctionnelle de l'axe HPA et des substrats cérébraux sensibles au stress. (A) Composants principaux de l'axe HPA et régions cérébrales sensibles au stress. (B) L'activation de l'axe HPA est initiée par la stimulation des neurones parvocellulaires du noyau paraventriculaire (PVN) de l'hypothalamus et la sécrétion ultérieure de corticolibérine (CRF) et de vasopressine (AVP) dans la circulation portale ou dans la circulation générale par le système porte (hypothalamo-hypophysaire). Il est important de noter que l'AVP amplifie les effets du CRF. L'hypophyse sécrète de l'hormone adrénocorticotrope (ACTH). A son tour, celle-ci stimule la libération de GC par le cortex surrénalien (AC), et d'adrénaline (EPI) et de noradrénaline (NA) par la médullosurrénale (AM) dans la circulation sanguine. Lorsque l'événement stressant prend fin, l'axe HPA revient à son état basal par l'action de systèmes régulateurs (rétroaction négative). Les GC sont directement impliqués dans la suppression de la réponse hypothalamique et hypophysaire au stress, et contribuent donc à stopper la libération de CRF et d'ACTH. Indirectement, ils régulent la réponse au stress en activant les récepteurs au GC (GR) dans l'hippocampe et les zones corticales qui projettent vers l'hypothalamus. Les récepteurs minéralocorticoïdes (MR) sont également activés par les GC et participent de manière coordonnée avec les GR, à la réponse au stress. Les signes + et - indiquent respectivement une régulation positive et négative, et les triangles, une modulation excitatrice/inhibitrice. Image tirée de Franklin et al. 2012.

intégrés dans les circuits limbiques et comprennent le CPF, l'amygdale et l'hippocampe (Godoy et al., 2018). Il est important de noter que ces réseaux sensibles au stress reçoivent des informations associatives des zones sensorielles d'ordre supérieur (e.g., l'insula), et des régions participant au traitement de la mémoire (par exemple, le cortex cingulaire) ou/et au traitement de l'éveil et de l'attention (par exemple, le locus cœruleus –LC, et les noyaux du raphé -RN). A leur tour, les réseaux neuronaux activés pendant le stress ciblent des sites sous-corticaux nécessaires au traitement limbique. Ces réseaux de régions cérébrales en interaction coopèrent donc dans les émotions et la cognition, et soutiennent les réponses neuroendocriniennes.

En cas de détresse chronique, la structure et le fonctionnement de ces régions cérébrales sensibles au stress se modifient. Il est important de noter que le profil d'expression des composants de l'axe HPA (CRF, GR), ainsi que des catécholamines, est également modifié après une détresse chronique. Une action inefficace des GCs résultant d'une exposition chronique au stress (résistance cérébrale aux GCs) pourrait alors refléter une hyperactivité de l'axe HPA (Pariante et Lightman, 2008). Par la suite, la réactivité à de nouveaux facteurs de stress aigus diffère généralement après une détresse chronique, entraînant une facilitation ou un affaiblissement de la réponse au stress.

Il existe des preuves scientifiques que la détresse chronique est régulée de manière significative par l'amygdale centrale (ACe), très sensible aux GCs. En fait, les niveaux élevés de GCs induits par la détresse chronique entraînent une augmentation de l'expression de CRF dans l'ACe, ce qui augmente la réactivité du PVN par le biais de projections indirectes. D'autres régions du cerveau pourraient également contribuer à l'atténuation de l'activité de l'axe HPA induite par la détresse chronique, comme c'est probablement le cas du CPF et, dans une moindre mesure, de l'hippocampe. Des taux circulants élevés et persistants de GCs peuvent entraîner une régulation négative de l'expression des récepteurs aux GCs (Mizoguchi et *al.*, 2003). En ce qui concerne la réponse du système nerveux autonome, la détresse chronique favorise généralement des ajustements physiologiques à long-terme, comme l'augmentation de la fréquence cardiaque. Pour autant, les mécanismes neuronaux qui sous-tendent ces adaptations n'ont pas encore été entièrement décrits.

b.1. Stress chronique et conditions pathologiques

Bien que les troubles consécutifs à une détresse durable ne comprennent pas seulement la santé mentale, une des relations les plus explorées et les plus affirmées entre la détresse chronique et la santé est illustrée par les troubles de l'humeur (American Psychiatric Association, 2013).

L'implication du stress dans le développement des troubles de l'humeur, et en particulier de l'anxiété et de la dépression, a été largement documentée (pour une revue, voir Pêgo et *al.*, 2009). En effet, dans une proportion significative de cas, les épisodes dépressifs sont précédés d'événements stressants majeurs, et l'exposition chronique au stress a été liée à la détérioration de la symptomatologie et à la résistance au traitement (Pizzagalli, 2014). Bien que les réponses anxieuses ne puissent pas être liées à la présence discrète de facteurs de stress spécifiques, leur corrélation avec la détresse chronique a également été démontrée (Pêgo et *al.*, 2009).

Les troubles liés à l'anxiété représentent un groupe hétérogène d'affections neuropsychiatriques généralement caractérisées par des sensations psychophysiques d'inconfort et d'appréhension en réponse à des signaux diffus non conditionnés. La dépression, quant à elle, est un état psychiatrique caractérisé par une gamme complexe de symptômes comportementaux, émotionnels et cognitifs, notamment une diminution de l'intérêt pour le plaisir (anhédonie), une diminution de la motivation pour l'activité physique, cognitive et/ou émotionnelle (apathie), et un dysfonctionnement exécutif. Les étiologies de l'anxiété et de la dépression se chevauchent souvent et sont liées à des modèles altérés différents du système de réponse au stress. L'anxiété et la dépression peuvent également être comprises comme des phénotypes consécutifs et connectés dans un continuum physiologique à pathologique, où le premier précéderait le second (Cuthbert, 2014). Pour la présente étude doctorale cependant, la neurobiologie sous-jacente aux phénotypes dépressifs est d'un plus grand intérêt.

De nombreux réseaux cérébraux semblent contribuer au dysfonctionnement émotionnel et cognitif qui caractérise la dépression. Les preuves humaines concernent principalement l'imagerie cérébrale et les études post-mortem, qui ont permis de mettre en évidence des modifications de la

circulation sanguine et des altérations anatomiques dans plusieurs zones cérébrales, notamment le cortex frontaux, l'hippocampe, le striatum, l'insula, l'amygdale et les zones thalamiques (Belzung et *al.*, 2015 ; Griffiths et *al.*, 2014 ; Krishnan et Nestler, 2008 ; Nestler et *al.*, 2002). Ces altérations anatomo-fonctionnelles affectent simultanément des capacités distinctes et contribuent à l'émergence de différents symptômes cognitivo-émotionnels. Par conséquent, elles sont généralement décrites en fonction des symptômes centraux du trouble.

- a. Anhédonie, apathie et sous-appréciation de la récompense. La perte générale d'intérêt et de plaisir pour les activités ou les objets gratifiants, ou anhédonie, est considérée comme l'un des principaux symptômes de la dépression. En effet, les patients négligent souvent les stimuli agréables tout en réagissant de manière atténuée aux expériences gratifiantes. Les patients dépressifs manquent de motivation pour poursuivre de nouveaux objectifs, de sorte que leur capacité à former de nouvelles associations stimulus-réponse est diminuée. Cette perte de motivation générale, également appelée apathie, est probablement à l'origine de leurs fréquents états d'avolition et d'aboulie (e.g., un état de comportement spontané réduit). La neurobiologie qui sous-tend l'anhédonie, l'apathie et la sous-appréciation hédonique correspond donc à celle de l'intégration du plaisir, de l'évaluation de la récompense et de la motivation. Une activité neuronale altérée du COF, y compris une connectivité réduite avec l'amygdale basolatérale (BLA), est probablement le principal orchestrateur des réponses émoussées aux stimuli agréables chez les patients déprimés. Ces derniers présentent également des schémas d'activité réduite dans le striatum ventral en réponse à des stimuli positifs. Aussi, une réduction de l'activité de l'aire tegmentale ventrale (VTA) face à des stimuli agréables est signalée chez les patients déprimés. Le CPFvm et le CPFdl, acteurs du réseau médiateur de l'évaluation de la récompense, sont également impliqués dans les états liés à l'anhédonie et à l'apathie, et le niveau d'hypofonctionnalité de ces zones a été associé à la sévérité de la symptomatologie (Alloy et *al.*, 2016 ; Der-Avakian et Markou, 2012).
- b. Sur-appréciation des pertes/punitions. Contribuant à la mauvaise appréciation hédonique générale, les individus souffrant de dépression répondent de manière excessive aux stimuli de valence négative, ce qui indique une évaluation et un traitement aberrants des stimuli généraux. Ces altérations favorisent l'orientation attentionnelle vers des états émotionnels négatifs, un mécanisme qui devient une habitude par le biais du renforcement et qui est appelé *verrouillage cognitif*. Au sein du réseau qui sous-tend ce traitement de l'information biaisé par des stimuli de valence négative, le COF et le CCA sont des acteurs majeurs, mais d'autres régions cérébrales intéroceptives participantes, comme l'insula, sont également dysfonctionnelles dans la dépression (Belzung et *al.*, 2015).
- c. Biais cognitifs. L'altération de l'appréciation hédonique engendre finalement des biais cognitifs chez les patients déprimés, et en particulier des biais mnésiques et attentionnels. L'amygdale a été suggérée comme étant le principal substrat cérébral de ces biais cognitifs. En effet, les personnes déprimées présentent des schémas accrus d'activation neuronale dans l'amygdale en réponse à des stimuli négatifs (Victor et *al.*, 2010). Des schémas d'activité altérés dans le CCA pourraient également contribuer à ces biais cognitifs, puisque cette région agit en modulant l'activité des régions sous-corticales impliquées dans le traitement des informations négatives. Concernant spécifiquement les biais mnésiques, la connectivité amygdale-hippocampe apparaît renforcée chez les patients déprimés, suggérant son implication dans le renforcement des souvenirs à valeur affective.
- d. Rumination. La focalisation de l'attention sur les symptômes de détresse est typique des patients déprimés. En relation avec les biais cognitifs, la rumination dans la dépression provient de l'incapacité à détourner l'attention des informations négatives. Cette altération du

fonctionnement exécutif conduit finalement les patients à des troubles de la mémoire et à des erreurs de jugement qui affectent directement leur conscience des contingences. La neurobiologie de la rumination se superpose aux réseaux cérébraux opérant dans les biais cognitifs, avec une contribution potentiellement particulière du CCA, du CPFvm et de l'hippocampe (Belzung et *al.*, 2015).

Concernant la neuromodulation, l'activité neuronale sérotoninergique jouerait un rôle important dans la dépression. L'*hypothèse monoaminergique de la dépression* postule que ce trouble est associé à une transmission déficiente au sein des systèmes monoaminergiques, et en particulier à de faibles niveaux cérébraux de sérotonine (5-HT) (Cosci et Chouinard, 2019). De fait, les inhibiteurs sélectifs de la recapture de la sérotonine (ISRS) constituent le traitement pharmacologique privilégié de la dépression, notamment dans les cas graves. Les agents pharmacologiques à base de monoamines ont des effets antidépresseurs puissants, mais ils nécessitent de longues périodes de temps pour commencer à être efficaces et leurs taux de rémission sont considérablement faibles (Krishnan et Nestler, 2008). Ainsi, les agents monoaminergiques ont permis de mettre en évidence la contribution des monoamines dans l'étiologie de la dépression. Pour autant, ils sont au centre des discussions concernant le traitement de la dépression, révélant l'existence de mécanismes plus complexes à la base de sa symptomatologie.

b.2. Prise de décision en situation de détresse

Les effets de la détresse chronique sur les capacités décisionnelles humaines ont été principalement étudiés à travers les patients souffrant de troubles liés au stress, en particulier d'anxiété et de dépression. Ces personnes obtiennent généralement de mauvaises performances dans les tâches de PD, et montrent une activation neuronale correspondante plus faible que les individus sains (Hartley et Phelps, 2012 ; Lenow et *al.*, 2017 ; Treadway et *al.*, 2012). Par exemple, ils passent moins de temps à délibérer avant de prendre une décision, mais se sentent plus en conflit pendant le processus, ce qui se traduit par des sentiments plus fréquents de regret post-décisionnel. À leur origine, une sous-appréciation typique de la récompense et une sur-appréciation de la perte ont été proposées (Cella et *al.*, 2010 ; Giustiniani et *al.*, 2020). Différentes études utilisant le paradigme de l'IGT ont mis en évidence des altérations de la PD chez les patients déprimés (Baeza-Velasco et *al.*, 2020 ; Must et *al.*, 2013), mais des performances normales sont également rapportées dans une proportion non négligeable de cas. La sévérité et l'état de la maladie sont vraisemblablement des déterminants clés dans l'émergence des altérations décisionnelles, avec des probabilités plus élevées de dysfonctionnement cognitif en corrélation avec l'accumulation d'épisodes dépressifs.

Les études précliniques ont également fourni des informations précieuses sur les mécanismes neuronaux qui orchestrent les changements décisionnels en cas de stress. L'effet néfaste d'événements stressants répétés sur la cognition dépendante du CPF médian (CPFm) a été démontré, notamment, chez des souris exposées à un bruit persistant et chez des souris qui ont été injectées avec du CRF par voie intracérébro-ventriculaire. Ces animaux présentent un traitement de l'information dégradé dans des tâches évaluant des stratégies comportementales dirigées vers un but, concomitant à une évaluation perturbée des résultats (Devilbiss et *al.*, 2016). Puisqu'il est prouvé que le comportement dirigé vers un but dépendant du CPFm est nécessaire pour une PD optimale dans des environnements dynamiques, ces résultats indiquent que la signalisation CRF préfrontale est un facteur potentiel déterminant de la qualité de la PD, en accord avec des preuves supplémentaires lors d'un stress aigu (Bryce et Floresco, 2016). Dans une version de l'IGT chez le rongeur, une mauvaise performance décisionnelle a également été associée à des individus présentant des niveaux d'anxiété plus élevés dans des conditions physiologiques (de Visser et *al.*, 2011). De plus, des injections

répétitives de GC dans le cortex IL de rats réduisent la réactivité à la récompense par rapport aux animaux contrôles (Koot et *al.*, 2014). Un grand nombre d'études soutiennent davantage la notion de détresse, et en particulier de détresse chronique, comme déterminant clé de la valeur adaptative des processus décisionnels. Pour autant, les mécanismes neuronaux sous-jacents restent encore à être élucidés. Les hypothèses expérimentales formulées dans ce projet doctoral visent à contribuer à la compréhension générale de ces mécanismes physiopathologiques, pour finalement tenter d'identifier des cibles biologiques pour le développement de nouvelles approches thérapeutiques en psychiatrie biologique.

C. Hypothèses de travail

L'objectif principal de cette recherche doctorale est d'obtenir une meilleure compréhension des fondements neurobiologiques de la PD dans l'incertitude. Chez l'Homme et la souris, les paradigmes de PD associés à des récompenses pécuniaires ou alimentaires contribuent à mieux comprendre le comportement adaptatif dirigé vers un but, acquis par apprentissage par renforcement. Le comportement des individus s'inscrit dans un continuum comportemental adaptatif-maladaptatif dans des conditions physiologiques et pathologiques. Par conséquent, les évaluations comportementales permettant de caractériser la variabilité interindividuelle sont essentielles pour déchiffrer la stœchiométrie des différentes dimensions comportementales contribuant à la PD adaptative dans l'incertitude. Cette étude a donc été fondée sur les hypothèses de travail suivantes :

Première hypothèse de travail - La prise de décision dans l'incertitude caractérisant une population humaine saine peut être modélisée chez la souris à l'aide d'un paradigme de pari spécifiquement adapté pour faciliter les comportements dépendant de la motivation.

La prise de décision dans l'incertitude a ensuite été évaluée au cours d'une détresse chronique.

Deuxième hypothèse de travail - Les déficiences du processus décisionnel induites par la détresse humaine peuvent être modélisées chez la souris grâce à un paradigme d'administration chronique de corticostérone (CORT).

Une caractérisation comportementale approfondie des effets d'une exposition prolongée à des niveaux élevés de GC chez la souris (modèle CORT) a été réalisée afin de comprendre la contribution et l'interaction de multiples domaines biologiques dans les processus adaptatifs de la PD.

D'autres caractérisations physiologiques et neurobiologiques ont ensuite été réalisées.

Troisième hypothèse de travail – La PD sous-optimale induite par la détresse chez les souris est fondée sur l'altération des composants de l'axe HPA dans des zones télencéphaliques sensibles au stress et pertinentes pour le traitement décisionnel : la signalisation GR, MR et CRF dans le CPFm, le striatum dorsolatéral (SDL) et l'hippocampe ventral (HV).

Pour mieux démêler la relation complexe entre motivation et PD, les conséquences d'une détresse durable sur les dimensions du traitement de l'effort et de la récompense, ainsi que leurs corrélats neurobiologiques, ont été étudiés par la suite.

Quatrième hypothèse de travail – La PD sous-optimale induite par la détresse chez les souris est liée à des déficits motivationnels d'approche (appétitif).

Cinquième hypothèse de travail – La PD sous-optimale induite par la détresse chez les souris est liée à des déficits motivationnels intervenant au moment des comportements de consommation.

Sixième hypothèse de travail - Les déficits motivationnels induits par la détresse chez la souris sont attribués aux réseaux cérébraux du système de récompense, notamment le noyau accumbens (NAc), l'amygdale, le cortex insulaire (CI), le noyau parasubthalamique (PSTN) et la VTA.

Enfin, les dimensions sensorimotrices et cognitives impliquées dans l'apprentissage, et pertinentes pour la PD dans l'incertitude, ainsi que leurs corrélats neurobiologiques, ont été étudiés.

Septième hypothèse de travail - La PD sous-optimale induite par la détresse chez les souris est liée à des déficits d'apprentissage.

Huitième hypothèse de travail - Les déficits d'apprentissage induits par la détresse chez la souris sont attribués aux réseaux corticostriataux, notamment le CPFm, le COF et le SDL.

D. Discussion des principaux résultats

Les résultats obtenus au cours de cette étude ont démontré que les déficits décisionnels observés chez les individus ayant été exposés à une détresse prolongée peuvent être modélisés chez des souris traitées de façon chronique à la CORT. Ce paradigme permet de reproduire l'hypercortisolémie humaine et l'activité dysfonctionnelle de l'axe HPA, deux symptômes rapportés chez les patients souffrant de troubles induits par le stress chronique. Plus intéressant encore, les déficits décisionnels observés dans le modèle CORT résultent probablement d'une perturbation des processus d'apprentissage et de motivation reposant sur l'activité du CPFm et du système de récompense. Dans le cadre de la recherche de biomarqueurs potentiels des déficits décisionnels induits par le stress chronique, et sur la base des résultats obtenus, la signalisation *cfr* perturbée du CPFm a été proposée, ici, comme endophénotype de vulnérabilité.

Modélisation de la PD dans l'incertitude

De bons modèles animaux sont essentiels pour étudier les bases comportementales et neurobiologiques des pathologies humaines. Une correspondance entre les données humaines et animales est alors essentielle pour énoncer des conclusions pertinentes et prédire les risques et vulnérabilités à ces pathologies.

Dans cette perspective, le premier objectif de ce projet doctoral était d'adapter un protocole murin de pari afin de limiter le biais de satiété. Les modifications apportées au protocole original ont permis de faciliter les comportements d'approche chez les souris et d'assurer une consommation significative de récompenses dans les deux conditions pharmacologiques (**Figure 3**). En effet, ce premier objectif était essentiel pour la réussite du projet de doctorat, qui repose sur l'adéquation du modèle animal afin d'aborder la PD dans l'incertitude en situation de détresse.

L'étape logique suivante consistait à étudier la validité translationnelle du modèle. Une étude comparative directe entre les données comportementales chez l'Homme et chez la souris a démontré que l^AmGT a une validité d'aspect remarquable lors de la comparaison des populations saines. En effet, il a été démontré que les performances de jeu sont similaires d'une espèce à l'autre malgré les

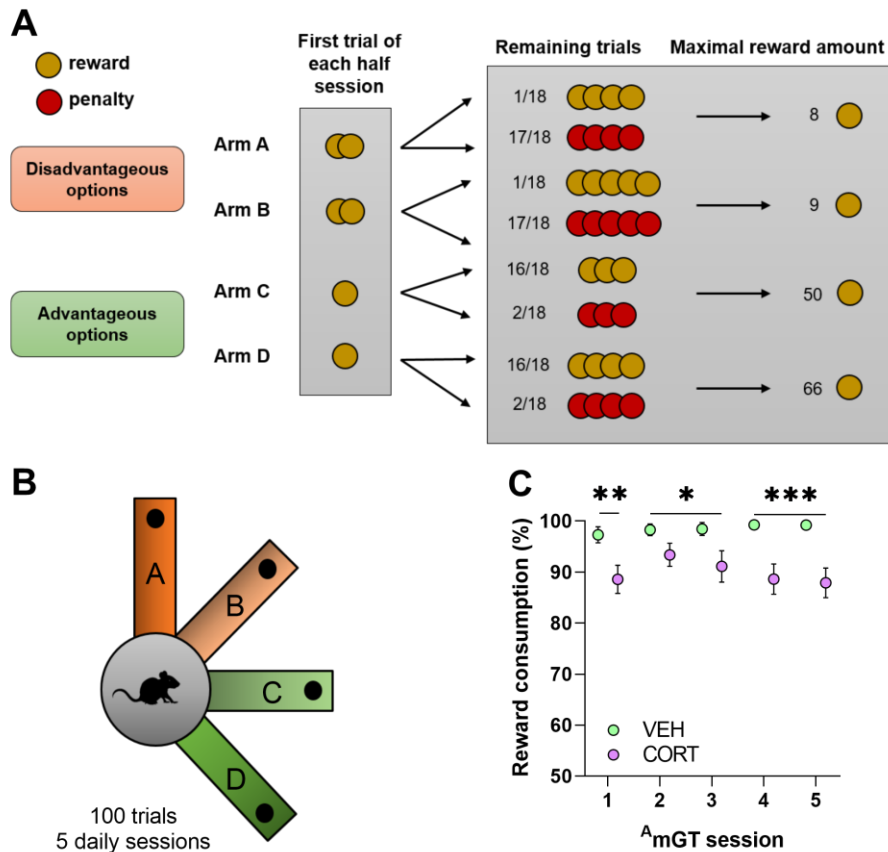


Figure 3. L'adaptation de la tâche murine de pari (^AmGT) minimise la satiété intra-session. (A) Représentation schématisée du plan expérimental de l'^AmGT. Pendant une minute, dans un labyrinthe radial à quatre bras, les souris pouvaient choisir d'entrer dans l'un des bras, de l'explorer puis de découvrir la récompense ou la punition associée. Les animaux ont pu choisir 20 fois à la suite (10 fois le matin et 10 fois l'après-midi), pendant 5 jours consécutifs. Les bras avantageux (C et D) donnaient accès à un granulé de nourriture (récompense) lors du premier essai de chaque demi-session, puis à 3 ou 4 granulés lors des 18 autres choix. Ces bras étaient associés à une faible probabilité de présenter la même quantité de granulés de quinine (pénalité ; 2 fois sur 18 choix). Les bras désavantageux (A et B) donnaient accès à 2 récompenses dans le premier essai de chaque demi-session, mais à 4 ou 5 granulés de quinine dans les 18 autres choix. Ces bras désavantageux étaient toutefois associés à une faible probabilité de présenter la même quantité de récompense (1 fois sur 18 choix). (B) Représentation schématisée du labyrinthe radial à quatre bras. L'image représente une possible localisation des bras avantageux (vert, C et D) et désavantageux (orange, A et B). (C) Satiété minimisée chez les souris pendant l'^AmGT. Le protocole de jeu a été adapté à partir du protocole original de Pittaras et al. 2016, afin de minimiser la satiété intrasession. L'adaptation du protocole à cet effet était essentielle. En effet, des altérations significatives des comportements appétitifs et de consommation sont rapportées dans le modèle animal sélectionné pour étudier les altérations liées à la PD, à la suite d'une exposition chronique au stress (modèle CORT). Les résultats sur le comportement de consommation pendant l'^AmGT ont montré que tous les animaux, indépendamment de la condition pharmacologique, ont consommés la majorité des récompenses gagnées dans chaque session de tâche. Cependant, les animaux traités avec la CORT ont consommé significativement moins de nourriture que les individus témoins, quelle que soit la session (t-test : *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

variations de protocole (Figure 4). Des comparaisons directes de données humaines et animales utilisant une méthodologie analytique commune n'ont pas été rapportées auparavant. Ainsi, les résultats présentés ici ont montré que cette approche peut aider à faire le lien entre les résultats des investigations cliniques et précliniques.

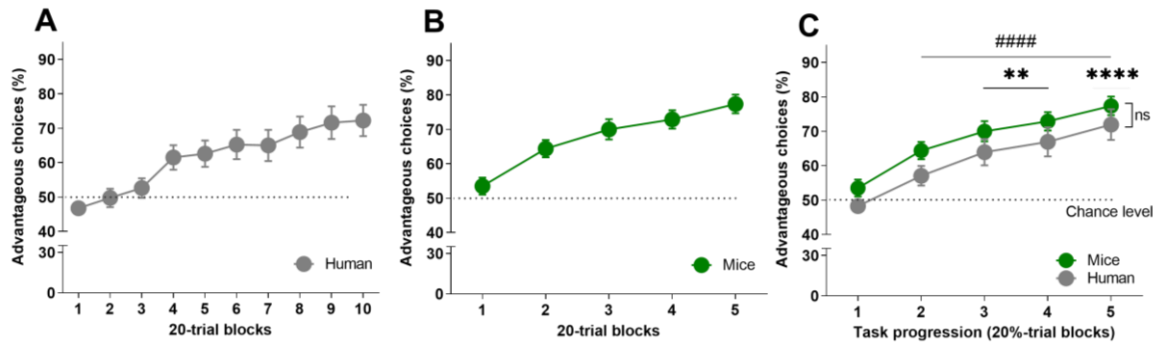


Figure 4. Performances dans les tâches de pari des populations humaine et murine. (A-B) Performances exprimées en pourcentage de choix avantageux pendant la progression de la tâche (blocs de 20 essais). Après 100 essais, les souris ont obtenu de meilleures performances que les humains (Bayesian : IC 95%=1.191 à 1.277), mais les performances finales étaient similaires (Bayesian : IC 95%=0,956 à 1,016). **(C)** Aucune différence significative (ns) entre les espèces lors de la comparaison des performances en fonction de la progression de la tâche (blocs de 20% d'essais) n'a pu être mise en évidence. Les performances humaines différaient du niveau de chance à partir du troisième bloc, tandis que les performances des souris étaient différentes à partir du deuxième bloc (t-test : souris : ####, $p < 0.0001$; humain : **, $p < 0.01$; ****, $p < 0.0001$).

Ce projet de doctorat était fondé sur la conviction que des comparaisons directes entre les données humaines et animales sont possibles lorsque les protocoles sont optimisés pour répondre aux besoins des espèces. Alors que les études sur l'IGT chez l'Homme utilisent le plus souvent des versions automatisées de la tâche en une seule session, avec des gains et des pertes d'argent comme résultats potentiels, les versions pour rongeurs reposent de préférence sur des tâches en plusieurs sessions, avec des gains et des pertes liés à de la nourriture. La modélisation des gains et des pertes d'argent est un objectif compliqué dans les versions chez le rongeur, mais il est admis que l'argent motive profondément les humains à agir et à prendre des décisions. Ainsi, la nourriture est le moteur naturel du comportement des rongeurs, en particulier dans les situations de privation alimentaire. Ce type de variation de protocole dépend de la nature de l'espèce et est malheureusement difficile à résoudre, voire impossible. Dès lors, ce projet a privilégié la stratégie de comparaison directe entre les espèces en utilisant une méthodologie analytique commune.

Fait intéressant, l'étude a révélé une différence dans l'apparition de la *réactivité à la récompense* entre les espèces (i.e., biais comportemental vers les options avantageuses). En effet, les souris ont appris les contingences de la tâche plus rapidement que les humains. Ce résultat intéressant a été interprété, ici, en fonction de la nature différente des récompenses et des punitions utilisées dans les tâches chez l'Homme et chez la souris. Il s'agit probablement de l'explication la plus plausible pour cette différence, puisque la nature des récompenses/punitions a été largement identifiée et interprétée comme une limitation majeure de ces tâches décisionnelles. Une autre explication possible repose sur l'assomption que les humains et les souris sont motivés de façon égale. Dans ce cas, l'émergence plus précoce de la réactivité à la récompense chez la souris par rapport à l'Homme pourrait refléter un comportement lié à la sécurité plutôt qu'un taux d'apprentissage plus rapide. Les humains ont consenti à leur participation à la tâche et étaient conscients du fait que la tâche ne comportait qu'une seule session. Ils ont obtenu ces informations *a priori* "pas si précieuses" en interagissant personnellement avec les chercheurs. Les souris, en revanche, ont été placées individuellement dans un labyrinthe et laissées libres d'agir. Même si elles étaient habituées au dispositif et à la manipulation du chercheur, les souris ont été retirées de leur cage d'hébergement et isolées alors qu'elles étaient privées de nourriture. Par conséquent, il est également plausible d'émettre l'hypothèse que les différences dans l'apparition de la réactivité à la récompense observées

entre les espèces pourraient refléter une stratégie comportementale plus "sécure" des souris visant à échapper aux signaux de stress internes dérivés de la nature intrinsèque incertaine de la tâche.

La valeur translationnelle du modèle murin a été confirmée par les résultats de l'étude de la variabilité interindividuelle entre les espèces. En effet, les humains et les souris se répartissent de manière similaire dans les trois profils décisionnels identifiés. Ici, une approche de regroupement de type *k-mean* a été choisie pour identifier les différentes stratégies comportementales. D'autres méthodes de regroupement auraient également pu servir cet objectif, mais il est intéressant de noter qu'aucune étude sur l'IGT chez l'Homme n'avait encore utilisé l'approche *k-mean* pour la caractérisation interindividuelle. C'est la raison pour laquelle cette méthode a été sélectionnée, même si elle comporte d'autres limites. Par exemple, le regroupement par *k-mean* ne permet pas de définir ce qu'est une bonne ou une mauvaise performance. Quoi qu'il en soit, les humains et les souris partagent des stratégies décisionnelles similaires lorsque sont considérés les clusters basés sur la méthode *k-mean*, ce qui confirme la pertinence du modèle animal pour reproduire le comportement humain (Figure 5).

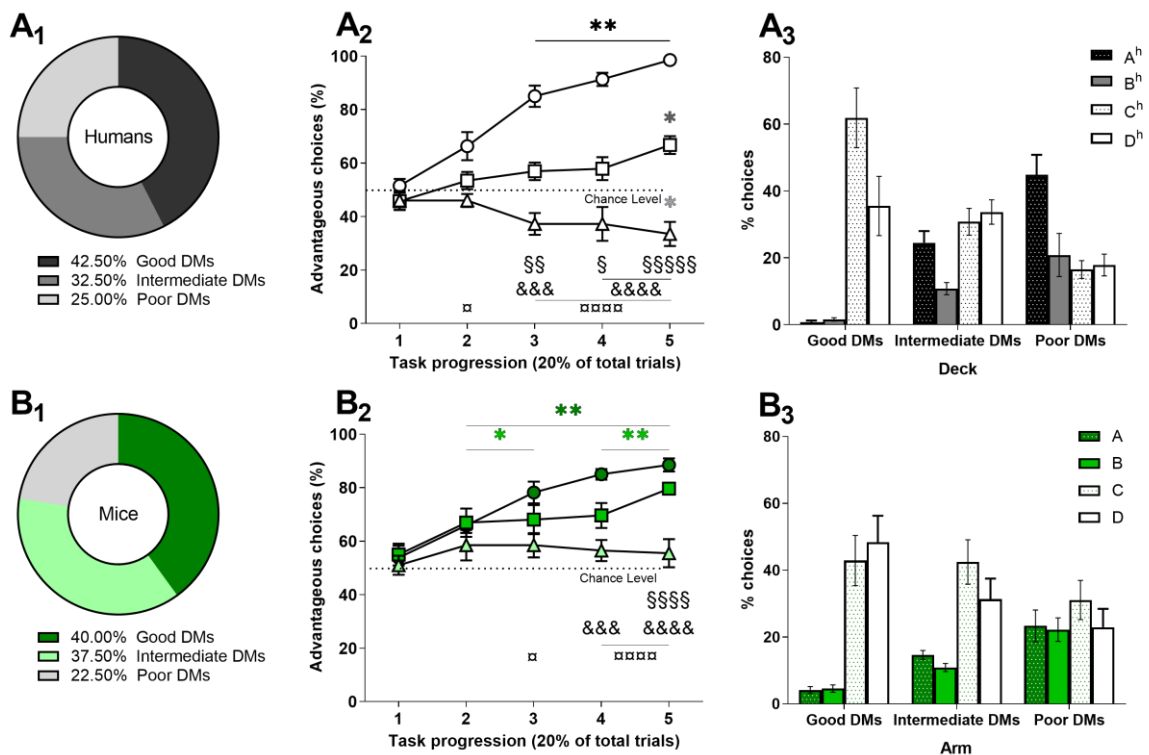


Figure 5. Catégories comparables de PD optimale, intermédiaire et médiocre avec des proportions similaires entre les espèces. Proportions d'individus (A1) chez l'Homme et (B1) chez la souris dans les sous-populations identifiées (X^2 , $p=0.99$). Performance chez l'Homme (A2) et chez la souris (B2) pendant la progression de la tâche. Performance du groupe différent du niveau de chance (Wilcoxon : *, $p<0.05$; **, $p<0.01$). Différences entre les groupes (optimale cercles ; intermédiaire carrés ; médiocre triangles) : Mann Whitney U, optimale vs intermédiaire : &, $p<0.05$; &&, $p<0.01$; &&&, $p<0.001$; &&&&, $p<0.0001$; optimale vs médiocre : †, intermédiaire vs médiocre : §. Pourcentage moyen de choix pour chaque option dans les 30 derniers essais chez (A3) l'Homme et (B3) chez la souris.

L'étude des dimensions comportementales au sein des tâches de jeu a été très instructive pour la caractérisation des stratégies chez l'Homme et la souris. Bien que similaires, les groupes décisionnels ne se comportent pas exactement de la même manière chez les deux espèces, et ces différences pourraient résulter à nouveau des limitations du protocole ou des ajustements comportementaux liés

au stress. Malheureusement, les dimensions comportementales utilisées sont insuffisantes pour évaluer la relation entre la maximisation du gain et l'aversion aux pertes. Dans ce contexte, les modèles computationnels de PD peuvent être très utiles pour mieux comprendre les tendances comportementales "discrètes" qui modulent les performances décisionnelles chez l'Homme et la souris. Notamment, un projet en cours de modélisation computationnelle de ces données, en collaboration interne avec PhD. M. Servant, vise à élucider les facteurs comportementaux qui n'ont pas pu être identifiés avec les approches statistiques classiques, en particulier pendant les premières phases des tâches.

Depuis son origine, les études sur l'IGT font référence aux performances désavantageuses comme à des comportements risqués. Dans le protocole original de Bechara et de ses collègues, les options désavantageuses sont qualifiées de "risquées" par rapport aux avantageuses décrites comme "sécures" (Bechara et *al.*, 1994). Par inertie, cette interprétation a été adoptée également dans les études précliniques. Cependant, le raisonnement qui la sous-tend est controversé et n'est pas toujours partagé par la communauté scientifique. Dans l'^AmGT par exemple, les individus du profil décisionnel intermédiaire ont développé une préférence pour les options avantageuses, mais ils n'ont pas réussi à maximiser leurs gains. Par conséquent, la performance des individus à PD intermédiaire est interprétée comme étant moins bonne que celle des individus à PD optimale, mais cela pourrait résulter du nombre limité de choix que les animaux pouvaient faire. Les individus à PD intermédiaire sont-ils moins bons que les individus à PD optimale, ou simplement des apprenants plus lents ? Il serait intéressant de savoir s'ils atteindraient la performance finale des individus à PD optimale s'ils avaient la possibilité de continuer à choisir. En effet, une phase d'exploration prolongée chez ces individus intermédiaires pourrait entraîner une meilleure reconnaissance des options et, par la suite, une meilleure gestion des indices disponibles pendant la phase d'exploitation. Les interprétations concernant le risque dans les adaptations chez les rongeurs, doivent donc être circonspectes et corroborées par des données supplémentaires provenant de tâches évaluant spécifiquement le risque.

Modélisation des altérations de la PD induites par le stress chronique

Les organismes vivants font face à des situations stressantes par des ajustements comportementaux et physiologiques leur permettant de retrouver un état homéostatique. Cependant, lorsque ces ajustements ne sont pas suffisants, la charge allostatique a un impact sur leur capacité d'adaptation à l'environnement et peut entraîner des états d'épuisement. Ceci est courant dans les situations de détresse prolongée, qui ont été associées à des augmentations des niveaux plasmatiques circulants de GC. Ainsi, le présent projet de doctorat a utilisé un protocole d'administration chronique de CORT pour modéliser la symptomatologie liée à la détresse chez les souris.

L'étude des effets d'une exposition prolongée aux GC sur la PD en situation d'incertitude a été très fructueuse. En effet, l'administration chronique de CORT a induit une PD sous-optimale chez les souris pendant l'^AmGT. D'une part, le retard de la réactivité à la récompense induit par la CORT reflète une altération du compromis exploration-exploitation, qui pourrait être la conséquence d'un ralentissement de l'apprentissage. D'autre part, l'étude de la variabilité comportementale interindividuelle de la population de souris a révélé une distribution différente des individus dans les trois profils décisionnels identifiés. En particulier, l'exposition chronique à la CORT a induit une PD sous-optimale en favorisant la stratégie décisionnelle intermédiaire (**Figure 6**). A ce stade, il est nécessaire de noter que la méthode de regroupement utilisée dans cette approche est différente de celle utilisée dans l'évaluation de la valeur translationnelle de l'^AmGT. En effet, les limites de bonnes et mauvaises performances ont été définies dès le départ afin de faciliter les comparaisons ultérieures

avec d'autres études précliniques. Par conséquent, la distribution des fréquences pour les trois groupes dans les conditions physiologiques diffère entre les études.

Le succès de la modélisation des altérations liées à la PD renforce la validité du traitement pharmacologique comme moyen de reproduire la symptomatologie humaine liée à la détresse. Les résultats confirment donc que le modèle CORT est un outil utile pour les études précliniques sur la pathologie humaine résultant d'une mauvaise adaptation à un stress prolongé. Cependant, les études futures devraient inclure des femelles afin d'évaluer les effets de la CORT sur les performances de l^AmGT. Des preuves scientifiques ont démontré que les souris femelles sont moins sensibles à l'administration chronique de CORT en ce qui concerne l'émergence de comportements à valence négative (Mekiri et al., 2017). Cependant, il faudrait prouver que ce serait également le cas pour la PD en situation d'incertitude.

Les approches opérantes de l^AmGT pourraient être utiles pour affiner les limites du protocole. Au cours de ces recherches, la PD dans l'incertitude a été évaluée dans un paradigme basé sur un labyrinthe et sa validité translationnelle a été démontrée. Cependant, les approches opérantes, avec enregistrement automatisé des paramètres offrent certainement plusieurs avantages par rapport aux versions en labyrinthe. Une limitation majeure est la participation active du chercheur, qui déplace individuellement les animaux à l'intérieur du labyrinthe. Cela augmente considérablement le temps nécessaire à la réalisation de l'expérience et ne permet pas de travailler avec plusieurs animaux en parallèle, contrairement aux approches opérantes. Quoiqu'il en soit, l^AmGT basée sur le labyrinthe était appropriée sur le plan expérimental, puisque le dispositif a été utilisé pour l'évaluation de différentes tâches comportementales, diminuant ainsi le comportement lié au stress dû à l'exposition des animaux au dispositif.

La PD sous-optimale induite par un stress chronique repose sur l'altération des composants de l'axe HPA dans le CPFm.

L'administration chronique de CORT permet de modéliser l'activité altérée de l'axe HPA à la suite d'une détresse durable. Pour contrebalancer la charge allostatique, plusieurs mécanismes physiologiques de l'axe HPA agissent de manière coordonnée pour retrouver l'état de pré-stress. Cependant, ces mécanismes de régulation pourraient être inefficaces dans le modèle CORT puisque l'hormone est administrée de façon ininterrompue. Cette recherche doctorale s'est concentrée sur trois acteurs clés impliqués dans ces mécanismes de régulation : les récepteurs aux GCs GR et MR, et le principal activateur de l'axe HPA, le neuropeptide CRF. Leur contribution a été étudiée dans trois zones télencéphaliques impliquées dans l'apprentissage associatif et le traitement des émotions : le CPFm, le SDL et le HV.

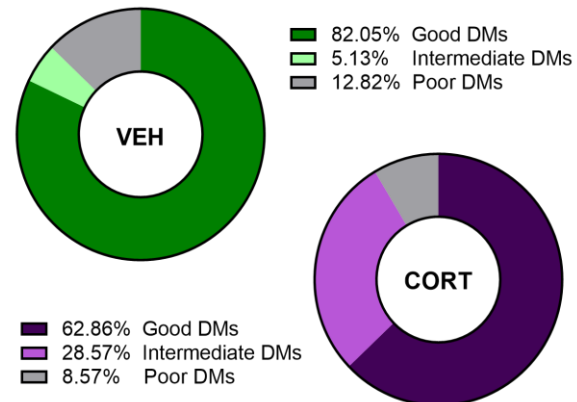


Figure 6. L'exposition chronique à la CORT augmente la propension à une PD sous-optimale dans l^AmGT. Distribution de fréquences différente des individus control (VEH) et traités à la CORT dans les groupes à stratégie décisionnelle optimale (n=54), intermédiaire (n=12) et médiocre (n=8), avec une proportion accrue d'animaux traités par CORT parmi les intermédiaires.

Cette étude a mis en évidence une diminution, induite par la CORT, du rapport d'expression GR/MR dans le CPFm des souris, résultant d'une régulation négative de l'expression des GR dans cette zone du cerveau. Cette altération découle probablement d'une sensibilisation locale suite à une surstimulation hormonale (voir par exemple de Kloet et *al.*, 2018). Le CPFm est une région télencéphalique essentielle pour le comportement réfléchi précédant la planification adaptative. Ainsi, l'expression altérée des GR induite par la CORT pourrait être à l'origine d'un encodage sous-optimal de l'information liée aux résultats des actions. Malheureusement, l'expression différentielle des GR ne prédit pas directement la performance de l'^AmGT, que ce soit dans des conditions pathologiques ou dans des conditions physiologiques. De manière inattendue, cette étude n'a pas révélé d'altération du rapport d'expression GR/MR dans les deux autres régions d'intérêt (**Figure 7**). Un rapport d'expression GR/MR différentiel dans le SDL était attendu en relation avec le renforcement des actions, pour la transition exploration-exploitation (i.e., l'émergence d'un comportement habituel) dépendant en grande partie de l'activité striatale. De la même manière, le rapport d'expression GR/MR dans le HV n'indique ni un dysfonctionnement de l'axe HPA, ni une altération du traitement des émotions. Quoi qu'il en soit, avant de rejeter complètement ces possibilités, les études futures devront comparer les rapports GR/MR d'échantillons provenant d'un moment plus précis. En effet, les extraits de protéines dans cette étude correspondent à 13 semaines de traitement cumulatif de CORT, alors que les performances de l'^AmGT correspondent à 7-8 semaines. Une variation de l'expression de ces éléments de l'axe HPA a pu se produire, ce qui pourrait biaiser les conclusions de cette étude.

Dans l'étude de la contribution de l'activité du CRF à la PD sous-optimale, il a été montré que l'expression du CRF dans le CPFm prédit la performance de l'^AmGT en situation de détresse (**Figure 7**). En effet, la corrélation négative trouvée entre ces paramètres a motivé la proposition que des niveaux élevés de CRF dans le CPFm pouvaient être un endophénotype neurobiologique de la vulnérabilité à la PD sous-optimale dans l'incertitude après une détresse continue. Les computations du CPFm à l'origine d'un fonctionnement cognitif frontostriatal sous-optimal, et notamment d'un comportement dirigé vers un but, pourraient donc résulter d'une perturbation locale de la signalisation du CRF. Cependant, cette étude n'a pas permis de révéler de différences dans les expressions du CRF dans le SDL et le HV. De plus, les études futures devront envisager l'échantillonnage à des moments correspondants plus précis afin d'étayer davantage ces résultats. De manière optimale, elles devraient également aborder la contribution de ces éléments de l'axe HPA dans d'autres zones cérébrales pertinentes pour la PD, telles que le CI.

Au cours de l'étude de l'expression des trois éléments identifiés de l'axe HPA, les échantillons de CPFm ont été traités intégralement, sans discerner les parties dorsales et ventrales. Cependant, les cortex prélimbique (PL) et infralimbique (IL) sont connus pour exercer des rôles complémentaires mais dissociables dans la PD (van Holstein et Floresco, 2020). Afin d'obtenir un meilleur aperçu de la contribution de ces sous-régions du CPFm et de la signalisation du CRF pendant le comportement dirigé vers un but, cette recherche a initié une série d'expériences causales. À partir d'une approche chemogénétique, la signalisation CRF dans le cortex IL a été modulée au cours de deux tâches opérantes différentes portant spécifiquement sur l'allocation de l'effort et la flexibilité comportementale. Les données préliminaires ont montré que l'activation neuronale liée au CRF dans le cortex IL pourrait réduire l'allocation de l'effort dans des contextes de récompense. En outre, l'activité neuronale basale liée au CRF dans le cortex IL n'est apparemment pas impliquée dans l'allocation de l'effort, mais pourrait être requise pour l'ajustement comportemental et l'apprentissage dans des tâches opérantes. Ainsi, si ces résultats se confirment, des niveaux élevés de CRF dans le cortex IL pourraient être considérés comme étant à l'origine de l'obstruction de l'apprentissage associatif, impactant potentiellement la PD. Malgré leur nature préliminaire, ces résultats convergent avec les résultats précédents dans le modèle CORT et soutiennent davantage l'hypothèse des niveaux

élevés de CRF dans le CPFm comme endophénotype neurobiologique de vulnérabilité à une PD sous-optimale en cas d'incertitude pendant la détresse. Les expériences en cours visent à compléter ces résultats et à englober l'activité du CRF dans le cortex PL.

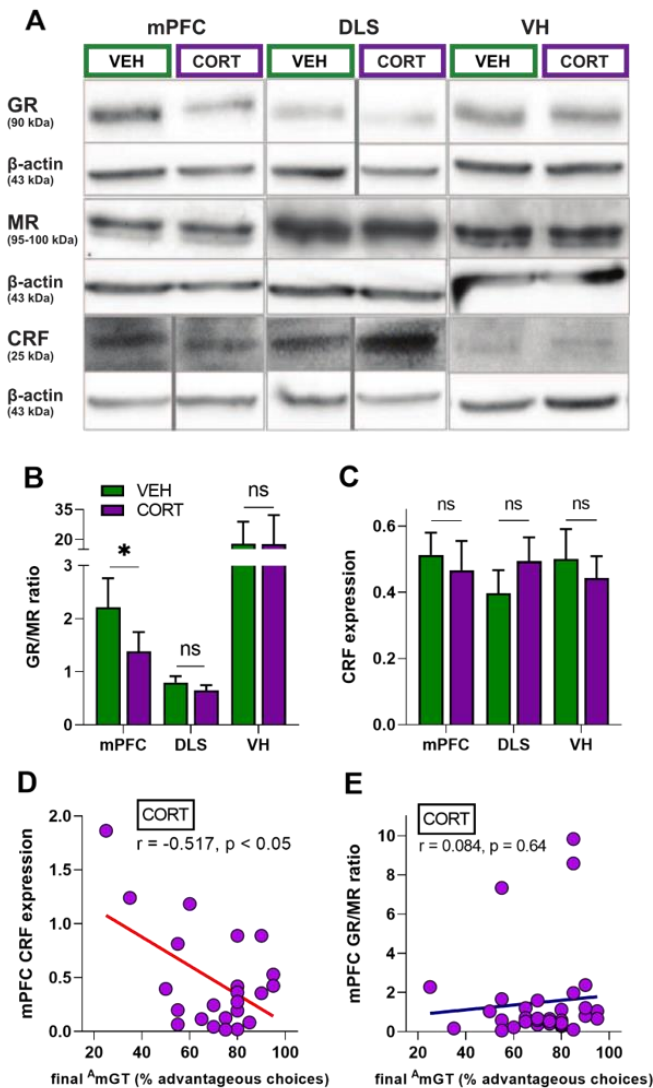


Figure 7. Déséquilibre de l'axe HPA chez la souris après administration chronique de CORT. (A,B) La quantification des protéines par Western Blot a révélé une régulation négative des GR dans le CPFm des animaux traités à la CORT qui présentent des valeurs significativement réduites du rapport GR/MR en comparaison des animaux témoins (*, $p < 0,05$). La quantification des protéines n'a pas révélé d'expression différentielle dans le HV, ni dans le SDL, liée au traitement (ns, non significatif). **(C)** Egalement, l'administration chronique de CORT n'a pas affecté l'expression du CRF dans les régions cérébrales étudiées (ns). **(D)** Cependant, des niveaux plus faibles de CRF dans le CPFm sont corrélés à une meilleure performance finale au Δ mGT chez les souris traitées à la CORT, mais pas chez celles traitées au VEH. **(E)** Les valeurs du rapport GR/MR dans le CPFm ne sont pas corrélés avec la performance finale chez les animaux traités à la CORT.

La PD sous-optimale induite par le stress chronique relaie des déficits motivationnels liés aux comportements d'approche.

Il a été démontré que les dimensions motivationnelles sont des modulateurs majeurs de la PD en situation d'incertitude. Dans le processus de PD, la génération d'options et leur évaluation dépendent de la rencontre des résultats des actions. Pour cela, les individus doivent initier un plan d'action, qui peut ensuite être maintenu et renforcé, ou ajusté. Ainsi, les comportements appétitifs constituent une dimension motivationnelle clé pendant la PD, en particulier dans les environnements dynamiques et pendant les phases exploratoires.

L'étude des effets d'une exposition prolongée au GC sur les comportements appétitifs, requis pour une PD optimale en cas d'incertitude, a montré des déficits non équivoques chez les individus traités à la CORT. Il est important de noter que les souris en détresse présentaient des déficits comportementaux auto-orientés, comme l'indique l'évaluation de l'état de leur pelage, et une

difficulté particulière dans l'initiation des actions, comme le montre l'augmentation des latences d'alimentation dans l'évaluation de la consommation supprimée par la nouveauté (NSF) et de tâche d'alimentation libre (FFT). De plus, cet état de vacillation ou de difficulté à initier un plan d'action était accentué dans des situations comprenant des récompenses non familières, comme le démontrait l'évaluation de la néophobie dans le FFT. Pourtant, le traitement pharmacologique n'a pas eu d'impact sur le choix des animaux entre les récompenses lorsque celles-ci étaient familières, contrairement à celles qui ne l'étaient pas. Ces derniers résultats confirment que les situations incertaines ont un impact sur la capacité des animaux traités à la CORT à faire des choix. En outre, l'évaluation de l'allocation de l'effort a démontré que l'administration chronique de CORT entraîne des déficits dans le maintien et la dynamisation des actions ou des choix récompensés pendant des paradigmes

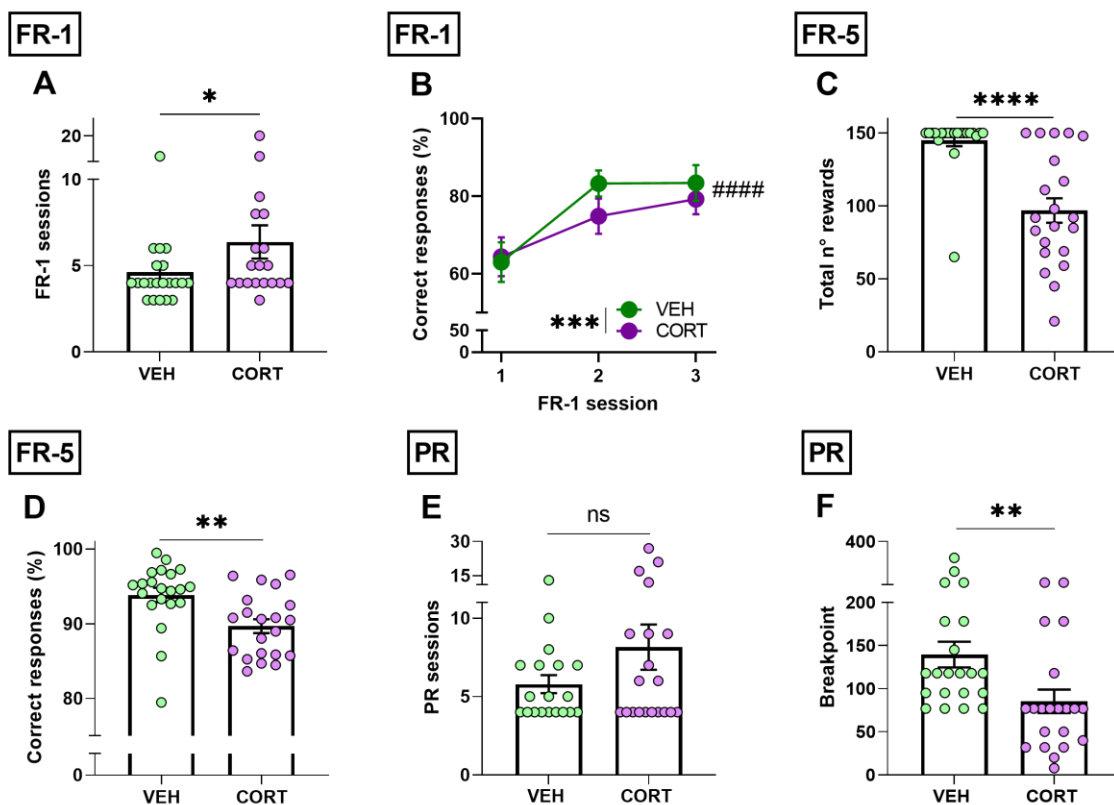


Figure 8. Réduction de la réponse instrumentale conditionnée chez la souris après administration chronique de CORT. (A) Par rapport aux animaux témoins, les souris traitées à la CORT ont eu besoin de plus de sessions pour atteindre les critères d'acquisition dans la phase d'entraînement opérant (FR-1), indiquant un effet négatif du traitement pharmacologique dans l'association action-résultat (Mann Whitney U : *, $p < 0.05$). (B) Les animaux des deux conditions ont amélioré la proportion de réponses correctes au cours des 3 premières sessions du FR-1 (ANOVA à mesures répétées : ####, $p = 0.0000$), mais la performance globale des animaux traités à la CORT était moins précise que celle des animaux témoins (ANOVA à mesures répétées : ***, $p < 0.001$). (C) Pendant la phase transitoire (FR-5), les animaux traités à la CORT ont obtenu beaucoup moins de récompenses alimentaires que les animaux témoins (ANOVA à mesures répétées : ****, $p = 0.0000$), et (D) même s'ils répondent majoritairement correctement, leur performance était moins bonne que celle des animaux VEH (ANOVA à mesures répétées : **, $p < 0.01$). (E) Tous les animaux, quelle que soit leur condition, ont eu besoin d'un nombre similaire de séances pour stabiliser leurs performances dans le test à ratio progressif (PR) (Mann Whitney U : ns, non significatif). (F) Cependant, les animaux traités à la CORT ont alloué significativement moins d'efforts pour obtenir une récompense alimentaire, indiquant un effet délétère du traitement pharmacologique sur l'évaluation et le traitement de la récompense et de l'effort (Mann Whitney U : **, $p < 0.01$).

opérants (**Figure 8**). Ce résultat est également corroboré par une altération du conditionnement opérant dans l'évaluation de l'apprentissage par inversion des règles (RL), où les souris traitées à la CORT ont été significativement moins récompensées que les souris témoins, même si la proportion de réponses correctes ne différait pas entre les groupes. Ainsi, ces déficits reflètent une perturbation induite par la CORT du maintien et de la poursuite du cours des actions, qui, en combinaison avec les difficultés d'initiation comportementale, pourrait expliquer la réactivité retardée à la récompense pendant l^AmGT. Ces déficits étaient également prédictifs de l'altération du traitement de la récompense et de l'effort. Cette dernière peut probablement perturber l'évaluation subjective des options, dont la valeur incitative de l'allocation d'efforts pour la récompense, nécessaires à l'apprentissage avant la génération d'options.

Les comportements appétitifs renvoient à la dimension motivationnelle de ce qui est appelé "vouloir" les résultats. Parmi les déficits mis en évidence dans le comportement appétitif, les latences accrues au NSF et au FFT pourraient illustrer un phénotype de type *verrouillage cognitif* induit par la CORT chez la souris, similaire à celui décrit chez l'Homme avec des humeurs dépressives (Belzung et al., 2015). En effet, il est plausible qu'une émotion à valence négative, similaire à la frustration humaine, apparaisse parallèlement à l'augmentation du temps nécessaire pour entamer une action. Cela contribuerait donc à la charge allostatique, obstruant la capacité des individus à prendre de nouvelles décisions. Cette interprétation est à nouveau en accord avec les observations cliniques incluant des patients souffrant de dépression, dont les états liés à l'avolition et à l'aboulie les bloquaient dans le processus de prise de nouvelles décisions (Husain et Roiser, 2018).

La diminution de l'allocation d'effort dans la tâche de PR après une exposition prolongée à la CORT pourrait également refléter des déficits énergétiques. En effet, les GC sont directement impliqués dans le métabolisme du glucose et participent à différents processus servant à la modulation de la concentration de glucose dans le sang. Par exemple, ils stimulent la gluconéogenèse dans le foie et fournissent ses substrats par la mobilisation des acides aminés des tissus extra-hépatiques, ils augmentent la rétention de sodium et l'excrétion de potassium, etc. Malheureusement, les mesures liées au métabolisme énergétique ne sont pas disponibles ici, et des études futures permettront de mieux discerner les effets induits par la CORT sur la motivation et l'énergie dans le paradigme du PR. Quoi qu'il en soit, les symptômes liés à l'avolition/aboulie et à la perte d'énergie/fatigue trouvent leur correspondance dans la pathologie humaine, ce qui confirme l'adéquation du modèle CORT dans les recherches translationnelles.

Enfin, il est intéressant de confronter les différents scores d'évaluation comportementale aux performances de l^AmGT afin de clarifier comment les déficits appétitifs induits par la CORT impactent le traitement décisionnel. Malheureusement, les scores du FFT n'ont pas offert d'explication causale pour la PD sous-optimale dans l^AmGT, et les scores du NSF et des tâches opérantes sont liés à différentes cohortes d'animaux. Pourtant, la présente étude offre des preuves substantielles du rôle des déficits comportementaux appétitifs dans le modèle CORT, et de leur contribution potentielle au traitement sous-optimale de la PD dans des conditions dynamiques.

La PD sous-optimale induite par le stress chronique relaie des déficits motivationnels liés aux comportements de consommation.

Une autre dimension motivationnelle qui influence le processus de PD est l'impact hédonique des récompenses et, par la suite, des pertes. Dans le cas des tâches reposant sur des récompenses et des pertes alimentaires, chez le rongeur, il faut non seulement rencontrer mais aussi consommer activement le résultat pour que l'impact hédonique se produise. Ainsi, l'étude des comportements de

consommation est importante, voire essentielle, pour aborder l'appréciation des récompenses et des pertes par les animaux, et pour étudier la dynamique de l'évaluation des valeurs subjectives.

L'étude sur les effets d'une exposition de longue durée à des niveaux élevés de GC circulants a mis en évidence des altérations du comportement de consommation des animaux traités à la CORT. Dans le cadre de l^AmGT, la stagnation de la dynamique de la dimension comportementale « perdre et changer » est interprétée comme une appréciation excessive de la perte. L'appréciation de la récompense, qui se traduit par l'évaluation de la dimension comportementale « gagner et rester », n'a cependant pas été influencée par l'exposition chronique à la CORT. Encore une fois, la complexité des résultats obtenus pour les profils décisionnels, combinée à la nature intrinsèque de ces mesures au sein de l^AmGT, ne permet pas de formuler une causalité directe de l'impact hédonique sur la stratégie de choix.

Des modifications de l'appréciation de la récompense n'ont pas été mis en évidence avec le test de la préférence de sucrose (SPT), tandis que la consommation alimentaire des animaux traités à la CORT était significativement inférieure au FFT. Cependant, les scores de consommation dans ces deux tests sont significativement corrélés, ce qui suggère que la différence négligeable de consommation de sucrose entre les conditions pharmacologiques pourrait être attribuable à des variations de protocole. En effet, la littérature scientifique rapporte fréquemment une diminution de la consommation de sucrose dans les modèles animaux liés au stress, mais aussi dans des conditions physiologiques (voir par exemple Pittaras et *al.*, 2016). Pourtant, différentes concentrations de solution de sucrose et différents plans expérimentaux sont rapportés, empêchant des comparaisons objectives. Les comportements de consommation divergents observés lors des évaluations SPT et FFT pourraient également refléter les effets putatifs de l'environnement. Au cours du SPT, par exemple, les animaux ont été isolés pendant le cycle d'obscurité et pendant une période prolongée, alors que le FFT n'a duré que cinq minutes et a été réalisé pendant le cycle de lumière en présence du chercheur. Ainsi, l'optimisation des protocoles sera essentielle pour démêler ces divergences et pourrait permettre d'aborder d'autres aspects motivationnels des comportements des animaux.

La sur-appréciation de la pénalité et la sous-appréciation de la récompense observées chez les souris traitées de façon chronique à la CORT convergent respectivement avec les déficits d'adaptation mis en évidence pendant le test de la nage forcée (FST), et avec la diminution de la prise alimentaire après le NSF. Bien que cela ne soit pas direct, les déficits d'adaptation pourraient témoigner d'un impact accru des émotions négatives sur le comportement des animaux, ainsi que de l'influence d'une situation à haut risque pour la sécurité des animaux. L'altération de l'appréciation hédonique pourrait influencer le traitement et la réévaluation des récompenses nécessaires à l'apprentissage adaptatif du renforcement, et donc influencer non seulement le début de la réactivité aux récompenses, mais aussi le compromis exploration-exploitation pendant l^AmGT. Par conséquent, cette étude confirme que la détresse soutenue chez les souris modifie l'impact que les résultats peuvent avoir sur la PD en cas d'incertitude en modifiant la valeur incitative des gains et des pertes de la même manière que l'anhédonie et l'apathie perturbent la qualité de la PD chez l'Homme.

Les déficits motivationnels induits par le stress chronique s'inscrivent dans les réseaux cérébraux du système de récompense et impliquent principalement la BLA, le CI antérieur, la strie terminale (BNST) et la VTA.

Les déficits motivationnels, dont l'aboulie, l'anhédonie et l'apathie, sont inscrits dans la symptomatologie de diverses pathologies résultant de l'exposition à une détresse chronique, comme la dépression et la schizophrénie. Les bases neurales de ces symptômes sont liées au système de

récompense, et donc principalement situées dans la boucle cortico-ganglions de la base-thalamo-corticale.

Cette étude a démontré que les déficits comportementaux liés à la motivation résultant d'une détresse durable chez la souris sont liés à des schémas d'activité aberrants dans plusieurs structures du système de récompense. En particulier, les déficits appétitifs liés à une diminution de l'allocation d'effort chez les souris CORT sont concomitants avec une diminution de l'activité neuronale dans la BLA et le CI antérieur. Même sans être significatifs, les schémas d'activation de la BNST et de la VTA ont également été diminués chez les souris après une exposition chronique à la CORT. En outre, la performance finale dans la tâche de RL, qui implique une diminution des réponses récompensées chez les animaux CORT, est liée à une diminution de l'activité neuronale dans la VTA, le BNST dorsale et le CI antérieur, ce dernier sans atteindre la significativité. Ainsi, l'activité neuronale aberrante faisant suite à une exposition chronique à la CORT affecte des substrats neuronaux au sein des réseaux neuronaux participant à l'apprentissage associatif et au traitement de la récompense et de l'effort. Plus précisément, l'activité aberrante de la BLA et du CI antérieure pourrait avoir un impact sur l'encodage des valeurs des résultats principalement par l'obstruction de l'évaluation de la récompense, et du traitement intéroceptif et lié à l'effort. En parallèle, l'activité neuronale aberrante de la VTA et du BNST pourrait expliquer pourquoi les animaux traités à la CORT s'engagent dans une moindre mesure dans la réalisation de la tâche. L'activité de la VTA est essentielle pour la motivation du comportement dans des contextes dirigés vers un but, tandis que le BNST joue un rôle dans la traduction des informations émotionnelles et dans l'ajustement des comportements ultérieurs.

Malgré le fait que le COF soit important pour la PD basée sur l'effort, cette recherche n'a pas réussi à démontrer son implication directe dans les altérations du comportement opérant induites par la CORT. De même, il était inattendu de ne pas trouver un effet plus fort de la détresse sur l'activité de la VTA liée à l'allocation de l'effort, puisque le PR est classiquement utilisé pour aborder les déficits motivationnels. Étant donné que la diminution de l'activation neuronale de la VTA a été constatée comme étant liée aux aspects motivationnels de la tâche de RL, il sera intéressant d'aborder la performance finale du PR d'une manière similaire. En d'autres termes, pour mieux comprendre l'implication de l'activité de la VTA dans les déficits comportementaux révélés par l'évaluation du PR, l'expression locale de cFos devrait être liée à la performance finale. Cela pourrait aider à discerner l'implication de l'activité de la VTA dans les déficits liés à la motivation d'état ou de trait.

De la même manière, il a été surprenant de ne pas trouver d'effet de l'exposition chronique à la CORT sur l'activité du NAc. La connectivité intime entre le NAc et la BLA est reconnue comme étant cruciale dans la médiation du conditionnement appétitif et des comportements liés à la récompense. En effet, les projections glutamatergiques de la BLA vers le NAc potentialisent le renforcement instrumental (Amir et al., 2015) et peuvent augmenter la recherche en réponse à des indices prédictifs de récompense (Namburi et al., 2016). De plus, des investigations récentes ont démontré de manière causale l'implication des connexions NAc-BLA dans la PD en cas de risque (Bercovici et al., 2018) et, d'un intérêt particulier pour la présente étude doctorale, dans les déficits appétitifs induits par la CORT (Dieterich et al., 2021). Cette étude a montré que l'expression de cFos, mais pas celle de FosB, dans le *core* du NAc prédit la performance finale globale dans le RL. Cependant, des modèles similaires d'expression de cFos dans le *core* du NAc ont été trouvés dans les deux conditions pharmacologiques. Compte tenu de la pertinence du NAc dans le système de récompense et des déficits motivationnels sans équivoque dans le modèle CORT, les futures études devront examiner les relations causales entre la connectivité BLA-NAc et la PD sous-optimale dans l'incertitude.

L'étude sur les fondements neuronaux des déficits motivationnels dans le modèle CORT devait également mettre en évidence un schéma aberrant d'activité neuronale dans le réseau CC-ACe-PSTN.

La connectivité de ce réseau participe au contrôle du comportement alimentaire, et pourrait donc réguler les comportements appétitifs chez la souris. Concernant la contribution particulière du PSTN au sein du réseau, Barbier et ses collaborateurs ont expliqué son rôle comme suit : " Je ne veux pas consommer cet aliment, reconnu comme bon, car je ne suis pas dans un état émotionnel ou physiologique propice " (Barbier et *al.*, 2020). Ainsi, le PSTN module le comportement appétitif dans des contextes liés à la récompense, et influence par la suite l'impact hédonique des récompenses. Malheureusement, cette étude n'a pas révélé d'altération des schémas d'activation neuronale dans cette région, ni dans l'ACe. Cependant, que ce soit en raison de la petite taille de l'échantillon ou d'autres limitations méthodologiques, les valeurs SEM liées à l'expression de FosB dans l'ACeA sont probablement trop larges pour être considérées comme précises. Par conséquent, avant de rejeter la possibilité de l'implication du réseau CI-ACe-PSTN dans l'altération des comportements appétitifs induits par la CORT, les approches immunohistologiques devraient être répliquées.

La PD sous-optimale induite par la détresse résultent de déficits d'apprentissage

L'apprentissage par l'expérience est nécessaire pour que les individus puissent s'adapter avec succès à de nouvelles situations. Différentes fonctions cognitives participent au processus de PD,

depuis les processus de mémoire permettant le rappel d'informations pertinentes pour guider le comportement futur, jusqu'à la pensée flexible permettant se désengager des règles dépassées.

Cette étude est concluante et démontre les effets négatifs d'une exposition prolongée à la CORT sur les capacités d'apprentissage des souris. En relation avec la réactivité retardée à la récompense dans l'AmGT, l'étude sur les domaines comportementaux ciblant la stratégie de choix a montré que l'apprentissage devenait plus sensible à la perte qu'à la récompense après une exposition chronique à la CORT. En outre, le traitement pharmacologique a eu un impact sur l'apprentissage reposant sur les capacités mnésiques spatiales pendant une tâche de mémoire de travail (Figure 9). Bien que les scores mnésiques ne prédisent pas directement les

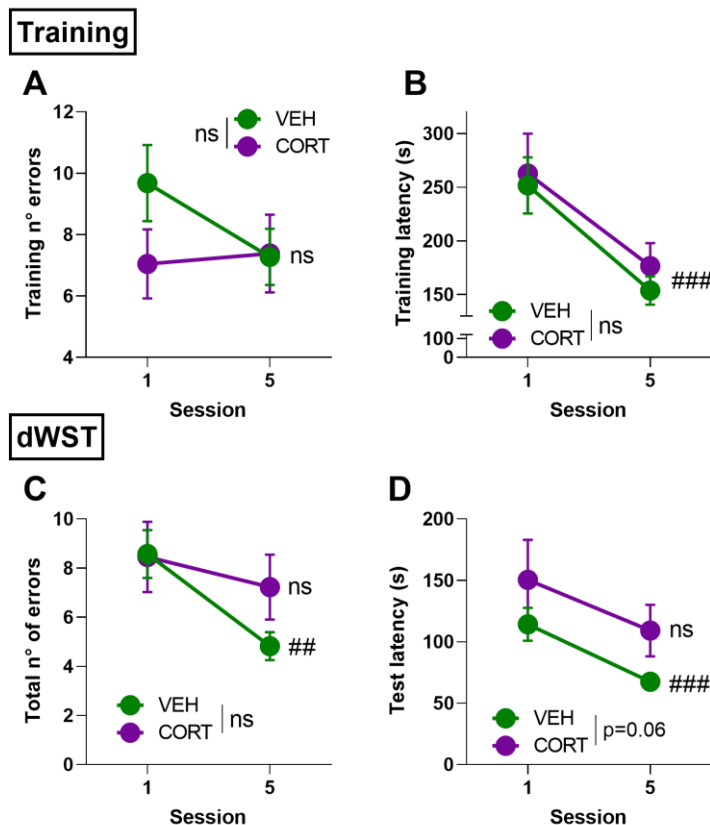


Figure 9. L'administration chronique de CORT entrave la MT spatiale chez les souris. (A,B) Les souris apprennent à trouver une récompense alimentaire grâce à entraînement dans un labyrinthe radial à 8 bras, indépendamment de leur traitement. L'évaluation des capacités de la mémoire spatiale a révélé un processus d'apprentissage général, principalement chez les animaux traités au VEH plutôt que par les souris CORT, comme le montre, (C), une diminution du nombre total d'erreurs et, (D), le temps nécessaire pour terminer la tâche (ANOVA à mesures répétées : ###, $p < 0.001$; ##, $p < 0.01$; ns, non significatif).

performances différentielles à l'^AmGT, les déficits mnésiques pourraient compromettre plusieurs processus impliqués dans la PD. La réévaluation continue des options nécessite un rappel de la mémoire afin de juxtaposer des informations avant la génération de nouvelles options. Ainsi, la transition d'un comportement dirigé vers un but à un comportement basé sur l'habitude dans l'^AmGT (i.e., de l'exploration à l'exploitation) pourrait être compromise chez les animaux CORT.

Cette étude a aussi démontré une perturbation des capacités d'apprentissage des animaux traités à la CORT lors de tâches opérantes. En effet, l'acquisition instrumentale était altérée après une exposition hormonale prolongée, comme le montre le retard dans l'atteinte des critères d'acquisition (**Figure 8**). Ainsi, les processus d'apprentissage menant à la formation d'associations action-résultat ont été perturbés. De plus, l'administration chronique de CORT a provoqué une inflexibilité comportementale chez les animaux en retardant leur ajustement comportemental face à une nouvelle règle dans les premières phases du RL. Ces déficits d'apprentissage sont susceptibles d'affecter la capacité des animaux à réévaluer les options en fonction de leur expérience, et donc d'avoir un impact sur la génération de nouvelles options. Suivant un raisonnement similaire à celui utilisé pour les aspects motivationnels, les déficits d'apprentissage chez les souris pourraient provoquer des émotions négatives additives contribuant potentiellement à un état général de frustration. Cet état émotionnel complexe pourrait conduire les animaux à se comporter de manière non adaptative, en supprimant l'initiation d'actions, par exemple, ou même en facilitant les actions persévérantes ou impulsives. Le déficit d'apprentissage lui-même pourrait donc contribuer à l'émergence d'états rappelant la rumination humaine ou le déséquilibre comportemental. Malheureusement, il n'est pas possible ici de formuler des relations de causalité directe puisque différentes cohortes d'animaux ont été utilisées au cours de cette étude. Des projets en cours permettraient d'élucider la contribution particulière de ces déficits d'apprentissage dans la PD sous-optimale induite par la CORT. En particulier, des plans expérimentaux plus complexes de RL, ainsi que des tâches de changement de stratégie, compléteront les résultats de la présente étude sur la flexibilité comportementale. En outre, les approches opérantes des performances de jeu offriront des informations sur d'autres dimensions comportementales susceptibles d'affecter la PD en cas d'incertitude, comme la tendance des animaux à choisir de manière impulsive.

Il est intéressant de noter que les déficits d'apprentissage dans le modèle CORT englobent également des composantes psychomotrices. En effet, l'ajustement comportemental dans une tâche de rotarod était retardé après l'administration chronique de CORT. Pourtant, les scores moteurs ne prédisent pas directement les performances des souris à l'^AmGT. Les études futures devraient également inclure des investigations supplémentaires afin de discerner les effets cognitifs des effets énergétiques de l'administration de CORT sur les performances psychomotrices. En conclusion, les retards dans l'encodage des contingences et les taux d'apprentissage plus lent qui en découlent dans le contexte de la PD sont susceptibles de réduire le succès dans l'^AmGT en empêchant la réactivité à la récompense aux premiers stades de l'apprentissage. Cette obstruction de l'apprentissage associatif sur le traitement des indices va donc contrarier la maximisation des gains.

Les déficits d'apprentissage induits par la détresse ne sont pas attribuables aux réseaux cérébraux cortico-striataux impliquant principalement le CPFm, le COF et le SDL.

Les troubles de l'apprentissage sont généralement signalés chez les personnes ayant subi durablement des événements pénibles. Les patients souffrant de dépression, par exemple, ont des difficultés à former de nouvelles associations instrumentales, et leur capacité à adapter leur comportement dans des environnements dynamiques est donc compromise. Ainsi, les régions du CPF pourraient jouer un rôle clé dans ces troubles de l'apprentissage. De plus, les patients souffrant de

dépression et d'autres troubles neuropsychiatriques se retrouvent souvent submergés par l'effet des stimuli négatifs, et se retrouvent dans un blocage cognitif qui peut biaiser leurs capacités cognitives (Belzung et al., 2015). Des zones corticales telles que le CPFm et le COF, ainsi que des régions intéroceptives comme l'insula, ont été proposées comme participant à cet état d'esprit négatif.

Cette recherche doctorale a démontré que des niveaux élevés de CRF dans le CPFm prédisent une performance à l'^AmGT sous-optimale chez les souris en détresse, comme mentionné précédemment. Cependant, l'expression du CRF dans le CPFm n'était pas prédictive de déficits de la MT spatiale ou de l'apprentissage psychomoteur. Des résultats similaires ont été obtenus pour le rapport d'expression GR/MR dans les autres régions télencéphaliques d'intérêt. De manière inattendue, l'activité striatale après une détresse chronique n'a pas été trouvée altérée au cours de cette étude. De même, les données neurobiologiques liées au striatum n'étaient pas prédictives de déficits d'apprentissage. Compte tenu de ces résultats et des preuves scientifiques dans la littérature, des études futures devraient interroger le rôle de ces éléments de l'axe HPA dans d'autres régions corticales, telles que le COF et le CCA, dans les déficits d'apprentissage induits par la CORT et, en particulier, dans la performance sous-optimale à l'^AmGT.

Des schémas différentiels d'activation neuronale dans les zones corticales liées aux déficits d'apprentissage opérant étaient également attendus. En particulier, cette étude attendait des variations au niveau des cortex PL et IL expliquant le retard induit par la CORT dans l'acquisition instrumentale, puisque ces sous-régions du CPFm agissent de manière complémentaire pendant le processus de formation des associations action-résultat. En revanche, les schémas d'activation neuronale des cortex PL et IL étaient toujours similaires chez les souris, indépendamment des conditions pharmacologiques. L'étude n'a pas non plus permis de démontrer une implication directe du COF dans les déficits d'apprentissage opérants, malgré le fait que le schéma d'activation de la BLA se soit révélé aberrant et que la connectivité BLA-COF soit considérée comme essentielle à l'acquisition instrumentale (Zeeb et Winstanley, 2013). Les déficits d'apprentissage induits par la CORT ont été bien établis après avoir étudié comment les animaux adaptent individuellement leur comportement dans les premières phases de la tâche de RL. Il est intéressant de noter que les animaux qui mettent le plus de temps à apprendre la nouvelle règle de la tâche, les individus nommés *peu flexibles*, présentent des schémas aberrants d'activité neuronale dans la BLA et la VTA par rapport aux individus *très flexibles*. Ces résultats sont cohérents avec les preuves scientifiques soutenant le rôle de la BLA dans la médiation de l'ajustement comportemental à la suite de résultats conflictuels (Wassum et Izquierdo, 2015). En outre, la diminution de l'activité dans la VTA pourrait refléter des déficits motivationnels chez les individus *peu flexibles*. Il est donc plausible que la capacité à apprendre la nouvelle règle de la tâche soit compromise chez les individus *peu flexibles* : d'abord parce qu'une activité aberrante de la BLA pourrait empêcher le désengagement de l'ancienne règle pour former une nouvelle association action-résultat, et ensuite parce qu'ils étaient intrinsèquement moins motivés, comme l'indique la diminution de l'activité dans VTA. Un manque de motivation chez ces individus *peu flexibles* (qui sont principalement des individus en détresse) pourrait bien dériver de leur difficulté à apprendre la nouvelle règle. Malheureusement, le plan expérimental utilisé au cours de cette étude ne permet pas de discerner ces deux possibilités. Les projets en cours aideront à répondre à ces questions en étudiant les schémas d'activation neuronale chez les individus *très* et *peu flexibles*, directement liés aux degrés d'apprentissage dans les premières étapes de la tâche de RL.

Dans la recherche de marqueurs biologiques de la vulnérabilité à la PD sous-optimale, cette étude a abordé l'activation neuronale dans des conditions physiologiques et pathologiques à partir d'une stratégie localisationniste. Cependant, les données obtenues au cours de cette recherche sont en cours d'être abordées du point de vue du réseau fonctionnel, ce qui s'ajuste probablement mieux

à la fonctionnalité réelle du cerveau. Cette approche permettra également le regroupement hiérarchique des modules fonctionnels, ce qui aidera à mieux comprendre les fondements du processus décisionnel sous-optimal induit par la détresse en situation d'incertitude.

Cette étude a démontré que l'administration chronique de CORT est un paradigme efficace pour reproduire les déficiences décisionnelles humaines dans des environnements dynamiques à la suite d'une détresse prolongée, et que ces déficiences sont principalement médiées par des déficits d'apprentissage et de motivation. Divers corrélats neuronaux ont été proposés comme étant à l'origine de ces déficits. Tout d'abord, la signalisation aberrante du *crf* dans le CPFm pourrait entraver le fonctionnement fronto-striatal optimal, et ainsi avoir un impact sur le comportement orienté vers un but et l'apprentissage ultérieur nécessaire pour la PD optimale. Deuxièmement, les déficits motivationnels pourraient provenir principalement d'une activité aberrante de la BLA, du CI antérieur et de la VTA, qui auront un impact sur la PD en perturbant l'évaluation et le traitement de la récompense et de l'effort.

E. Conclusions et perspectives

La compréhension des fondements neurobiologiques de la PD a considérablement progressé au cours des dernières années, mais il reste encore beaucoup à faire. La PD est un processus extrêmement complexe, qui se produit presque constamment au cours de la vie des individus. Les recherches sur la PD reposent sur un grand nombre de tâches qui interrogent des composantes spécifiques de cette fonction en faisant appel à des circuits sous-jacents particuliers. Bien que ce vaste ensemble de tâches ait contribué de manière significative à la compréhension générale de la neurobiologie de la PD, les résultats sont souvent controversés et l'interprétation basée sur leur comparaison reste prudente. L'étude de la PD bénéficie de la recherche translationnelle, qui inclut de nombreuses espèces et des paradigmes comportementaux non humains. En effet, le développement et le perfectionnement des paradigmes animaux fournissent constamment des outils supplémentaires pour la dissection des substrats et des réseaux neuronaux qui sous-tendent les phénotypes dans un continuum physiologique-pathologique. Ainsi, l'application systématique de paradigmes translationnels pourrait contribuer à l'identification d'éléments biologiques particuliers affectant la PD. Parallèlement, les études cliniques pourraient alimenter la recherche translationnelle par l'incorporation d'interventions basées sur les spécificités du phénotype, puisque les définitions cliniques pourraient ne pas être suffisantes pour englober la variabilité interindividuelle, que ce soit dans la santé ou dans la maladie.

Dans ce cadre, le modèle CORT offre la possibilité d'étudier si les altérations majeures identifiées dans le CPFm, la BLA, le CI antérieur et la VTA sous-tendent les perturbations cognitives et motivationnelles observées dans des conditions neuropsychiatriques et d'autres conditions cliniques faisant suite à une détresse prolongée. Le modèle CORT, par exemple, reproduit avec succès un phénotype de type aboulie, qui est souvent au centre de la symptomatologie liée à l'amotivation dans différentes conditions cliniques, notamment la dépression, la schizophrénie et la fibromyalgie. Cependant, une compréhension détaillée de la biologie des GC et de leurs récepteurs est essentielle pour l'interprétation de résultats qui, autrement, pourraient conduire à des conclusions erronées. Les processus épigénétiques induits par des événements négatifs qui peuvent avoir un impact sur l'expression des GR et des MR, qui à leur tour interféreraient sur les mécanismes de réponse au stress, sont d'un grand intérêt. Les récepteurs des GC sont des médiateurs primordiaux de la réponse au stress et influent sur la plasticité des synapses et l'activité énergétique cellulaire par le biais de mécanismes

mitochondriaux. Ainsi, les réseaux neuronaux qui réagissent à la détresse dépendent de la disponibilité temporelle et de l'activité des récepteurs GC, du moins dans une certaine mesure (Hollis et *al.*, 2015). Comprendre comment la signalisation et les voies moléculaires des GC modulent les ajustements neuronaux et comportementaux dans un environnement dynamique est donc indispensable pour aborder la physiopathologie des troubles liés au stress chronique.