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# Emotional biases in mood disorders: study of olfaction in mice models of depression and mania

Mathilde Bigot

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# SORBONNE UNIVERSITÉ

École doctorale Cerveau, Cognition et Comportement

Laboratoire Gènes, Synapses et Cognition, UMR 3571, CNRS

Équipe Perception et Mémoire, Institut Pasteur

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## Emotional biases in mood disorders

*Study of olfaction in mice models of depression and mania*

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## Les biais émotionnels dans les troubles de l'humeur

*Étude de l'olfaction dans des modèles murins de dépression et de manie*

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**Mathilde Bigot**

THÈSE DE DOCTORAT EN NEUROSCIENCES

Dirigée par Chantal HENRY et Mariana ALONSO

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# RÉSUMÉ

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Parmi les troubles de l'humeur, le trouble dépressif caractérisé et les troubles bipolaires sont des maladies psychiatriques relativement fréquentes, affectant respectivement 15% et 2% de la population mondiale. Un délai de plusieurs semaines est nécessaire avant d'observer les effets thérapeutiques des antidépresseurs, et 30% des dépressions sont résistantes à ces traitements. La dépression devrait devenir la principale source de charge mondiale de morbidité d'ici à 2030 d'après les estimations de l'Organisation Mondiale de la Santé. En conséquence, une meilleure compréhension de la pathophysiologie de ces maladies mentales est cruciale pour espérer développer de nouveaux outils diagnostiques et proposer de thérapies plus efficaces.

La classification actuelle des maladies psychiatriques définit des entités cliniques très hétérogènes ne reposant probablement pas sur des pathophysiologies communes, ce qui représente un frein à la recherche clinique comme préclinique. Afin d'y remédier, une nouvelle approche dite dimensionnelle réunit psychiatres et neuroscientifiques et s'attache à la description de composantes essentielles du comportement telles que la cognition, les émotions, la motivation ou les interactions sociales, pour les étudier notamment sous un angle neurobiologique. Au-delà de l'humeur, les épisodes thymiques peuvent être différenciés par des biais émotionnels associés. Les perturbations des processus émotionnels sont un élément essentiel des troubles de l'humeur, mais restent pourtant exclues des critères diagnostiques. Les épisodes maniques sont associés à un biais positif, c'est-à-dire que les stimuli émotionnels prennent une teinte plus plaisante et moins désagréable. À l'inverse, un biais négatif représenté par une augmentation du ressenti déplaisant des stimuli émotionnels caractérise les états dépressifs.

Le but de ce travail de thèse a été d'utiliser les réponses spontanées comportementales face à des stimuli olfactifs appétitifs et aversifs chez la souris comme mesures indirectes de la valence hédonique olfactive attribuée à ces stimuli. Par cette approche, nous proposons ainsi d'identifier des biais hédoniques dans des modèles murins de dépression et de manie, et d'étudier les mécanismes neuraux sous-jacents.

En particulier, le noyau basolatéral de l'amygdale (BLA) a récemment reçu beaucoup d'attention pour son rôle dans l'encodage de la valence, cette valeur hédonique que nous attribuons aux perceptions de notre environnement. Deux populations neuronales distinctes de la BLA seraient impliquées dans l'encodage des valences positive et négative. Des altérations de l'amygdale tant sur le plan structurel que fonctionnel sont bien établies chez les patients atteints de troubles de l'humeur. Notre hypothèse est donc que des modifications d'activité des deux sous-populations neuronales de la BLA codant des valences opposées, définies notamment par leurs projections vers d'autres aires cérébrales aussi impliquées dans le traitement des émotions, seraient à l'origine des biais émotionnels associés aux troubles de l'humeur.



Pour tester cette hypothèse, nous nous sommes tout d'abord intéressés à un modèle murin de manie. Utilisant le modèle d'administration de GBR 12909, inhibiteur de la recapture de dopamine, nous avons observé des phénotypes classiques dits-maniaques tels qu'une hyperlocomotion et une augmentation de la combativité. Quant à l'attribution de la valence hédonique, des tests de préférence olfactive et gustative ont en revanche révélé un important biais négatif, à l'opposé de ce que nous avions prédit. En effet, des odeurs et goûts considérés comme plaisants pour des souris contrôles perdaient de leur attractivité chez les souris recevant du GBR 12909, tandis que des odeurs et goûts déplaisants l'étaient encore plus. Nous avons ainsi conclu que ce modèle ne semblait pas approprié pour l'étude des perturbations émotionnelles observées dans les états maniaques.

Au contraire, nous avons pu mettre au jour un biais hédonique olfactif négatif conforme à nos hypothèses dans un modèle murin de dépression induite par administration chronique de corticostérone. Ce biais s'accompagnait de modifications spécifiques dans l'activité de circuits impliquant les neurones de la BLA. Nous avons ensuite manipulé l'activité de ces circuits de la BLA à l'aide de la chimiogénétique. Cela a révélé qu'une activation spécifique de certains neurones de la BLA était suffisante pour augmenter l'attractivité d'odeurs plaisantes chez des souris recevant de la corticostérone, sans toutefois affecter le biais hédonique négatif touchant les odeurs aversives.

L'ensemble de ces résultats souligne l'intérêt d'évaluer les biais hédoniques pour mieux décrire les modèles murins de dépression et de manie. Ils démontrent également l'implication causale de la BLA dans une partie du biais hédonique négatif associé aux états dépressifs.

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# ABSTRACT

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Mood disorders, including major depressive disorder and bipolar disorders, are heterogeneous psychiatric diseases that affect around 15% and 2% of the general population, respectively. Treatments work with a delayed onset of action and approximately 30% of patients do not respond to any therapy. Depression should become the leading cause of global burden disease by 2030 according to the World Health Organization. Therefore, a better understanding of the pathophysiology of these diseases would be of first help to propose new diagnostic tools and to move towards more effective therapeutic strategies. Validity of our classification system in psychiatry is questioned, because of heterogeneity and great overlap between mental illnesses, impeding preclinical and clinical research. To face these issues, a new research area based on dimensions has emerged. It consists of exploring domains derived from fundamental behavioral components to link them to neurobiological systems. Beyond mood, emotional biases differentiate mood states in patients. Mania episodes are associated with positive biases, *i.e.* emotional stimuli become more rewarding and less aversive, while the opposite characterizes depression.

The objective of this thesis was to assess behavioral responses to innately appetitive and aversive olfactory cues in mice as a proxy for assigned olfactory hedonic valence. Such approach offers the possibility to identify hedonic bias in mouse models of depression and mania, and to study the underlying neural mechanisms.

Recently, the basolateral nucleus of the amygdala (BLA) has been shown to encode valence, namely the value we assign to the perceptions of our external and internal environments, with distinct neuronal subpopulations encoding either positive or negative valence. It is well established that the amygdala activity is altered in mood disorders. Then, we hypothesize that modifications in the activity of the BLA and its connections to other cortico-limbic areas could be responsible for the emotional biases observed in mood disorders.

To test this hypothesis, we first assessed hedonic valence assignment in the GBR 12909-induced mouse model of mania. Surprisingly, apart from the classical mania-like phenotype characterized by hyperlocomotion, GBR 12909-treated mice exhibited strong negative olfactory and gustatory hedonic biases. In other words, appetitive odors and tastants were less appetitive and aversive ones were more aversive, as opposed to our expectations. On the other side, we uncovered a negative olfactory hedonic bias in the corticosterone-induced mouse model of depression, as we predicted. This bias was accompanied by specific BLA circuits activity disturbances. Furthermore, manipulating some of these BLA circuits activity thanks to chemogenetics was sufficient to partially improve the negative olfactory hedonic bias induced by chronic corticosterone administration.

Taken together, our results highlight the interest of olfactory hedonic evaluation in mouse models of depression and mania, and demonstrate the causal role of BLA circuits in hedonic biases associated with depressive-like states.



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# FOREWORD

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## **The neglected mental health : a "failure of humanity" ?**

Psychiatric disorders have a huge impact on global health and quality of life. More than one billion people were affected by mental or addictive disorders in the world in 2016, representing about 16% of the human population (Rehm and Shield, 2019). This same year, the global burden of mental and addictive diseases, as quantified by disability-adjusted life years (DALYs, combining lost years due to premature mortality and to disability) stood at 162.5 million DALYs (6.8% of all the DALYs). However, most of the lost years measured by DALYs for psychiatric diseases refers to non-fatal disease burden. Indeed, the World Health Organization (WHO) cause of death definition only includes the disease or injury that initiated the train of morbid events leading directly to death, or the circumstances of the accident or violence that produced the injury. Suicides represent about 800,000 deaths annually (1.49% in 2016), and the vast majority of suicides occur in the context of psychiatric disorders (Naghavi, 2019; Bachmann, 2018). Yet, the overall mortality due to mental condition is still tough to estimate. Walker et al. (2015) calculated in a meta-analysis a death rate 2.22 times higher compared to general population, with a decade life expectancy lost. When examining only disability, mental and addictive disorders represented the first cause of years lived with disability (YLDs) worldwide in 2016, with 18.7% of all global YLDs (Rehm and Shield, 2019). Despite this knowledge, investing to improve mental health and evidence-based care is challenging.

Scepticism about epidemiological data for mental disorders is nourished by the confusion around their clinical definition in general population, the lack of specific biological correlates, the idea that psychiatric illnesses are culturally defined and then not universal, and finally

maybe because they question our intimate beliefs about mind, self-control, normal suffering and human nature (Summergrad, 2016). The ignorance about mental illnesses across the world lead to dramatic consequences, among which the human rights denial of people suffering psychiatric diseases. Kleinman ('Global mental health : A failure of humanity', 2009) describes, for instance, his personal observations in east and southeast Asian countries of abuses, discrimination, rejection and generally stigma making "individuals with severe psychiatric disorders as virtually non-human". Globally, the stigma towards patients with psychiatric diseases is both cross-cultural and cross-diagnostic, and exposes them to discriminative behaviors from the society, including from their relatives (Mannarini and Rossi, 2019). Such stigma prevents them from using the healthcare system, aggravating the burden and making epidemiological data inaccurate (Herrman et al., 2019).

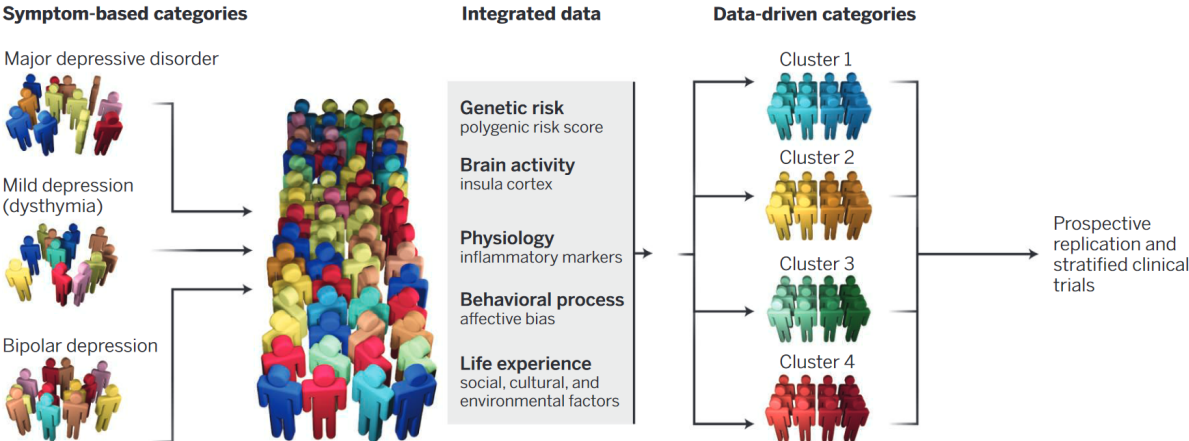
Studies suggested that people were more likely to attribute psychological causes than biological ones to mental illnesses, and that these etiological beliefs would be associated with discriminative attitudes (Mannarini and Rossi, 2019). However, even though a biological explanation of the disorder was correlated to higher recommendation of treatment endorsement, a study reported that it did not affect social rejection and perceived danger from the peers (Pescosolido et al., 2010). Furthermore, campaigns asserting "mental illness is an illness like any other and should be treated with medical treatments" did not prevent stigmatizing attitudes, possibly because it did not target the very anchored stereotype of dangerousness, uncontrollability and antisocial features of people with psychiatric condition (Angermeyer et al., 2011).

In low-income as in high-income countries, the investment in mental health programs is never proportionate to the burden of these disorders. Low-income countries invest less than 1% of their health budget in mental health services, while this proportion reach 5% in high-income countries (World Health Organization, 2011). Beds for psychiatric patients are mostly found in dedicated mental hospitals (62%). The world median is estimated at 7.5 beds for 100,000 population in mental hospitals, varying from 1.3 beds for 100,000 population in low-income countries, to 30.9 in high-income countries. In addition, almost half the world population lives in countries where, on average, there is one psychiatrist to serve 100 000 or more people ; other mental health care providers are mainly nurses. As a consequence, about two-thirds of people suffering from mental illnesses receive no treatment, even in high-income countries (Thorncroft, 2007; Wang et al., 2007).

To improve our understanding of the diseases and the patients care, several initiatives have

been launched. The WHO, stating that there is “no health or sustainable development [possible] without mental health” developed an action plan whose objectives are to strengthen effective leadership and governance for mental health; to provide comprehensive, integrated and responsive mental health and social care services in community-based settings; to implement strategies for promotion and prevention in mental health; and to strengthen information systems, evidence and research for mental health (World Health Organization, 2013). It also explicitly included mental health in the targets of the Sustainable Development Goals (SDG). Scientific journals like *the Lancet* and *Nature* promoted commissions and publication series to foster mental health knowledge and research and to call for action (Collins et al., 2011; Horton, 2007; Patel et al., 2018).

For instance, the last *Lancet* commission (Patel et al., 2018) proposed few principles for re-framing of mental health, among which a staged approach to understanding and responding to mental health problems, as opposed to the binary approach of current classifications. Indeed, current classifications in psychiatry, based on the Diagnostic and Statistical Manual of Mental Disorders 5th revision (DSM-5, American Psychiatric Association, 2013) and International Classification of Diseases 11th revision (ICD-11, World Health Organization, 2018) were successively revised to improve reliability of psychiatric diagnoses, meaning that one patient would receive the same diagnosis from different clinicians. However, reliability does not implicate validity of diagnoses, represented by the accuracy with which clinical criteria define and differentiate a disease from other diseases (Feighner and Herbstein, 1987).



**FIGURE 1. The dimensional approach in psychiatry.** From Insel and Cuthbert (2015). “A hypothetical example how precision medicine might deconstruct traditional symptom-based categories. Patients with a range of mood disorders are studied across several analytical platforms to parse current heterogeneous syndromes into homogeneous clusters.”

Diagnosis of mental illnesses, in contrast to most of other medical fields remains restricted



to a list of criteria corresponding to subjective self-reported and observed symptoms. The lack of biomarkers helping the diagnosis impedes the validity of diagnoses, since current classifications suffer from heterogeneity within disorders (*i.e.* two patients diagnosed with the same psychiatric disease can have no symptom in common), overlap and blurred boundaries between disorders and high prevalence of comorbidities (*i.e.* one patient is diagnosed with more than one psychiatric disease) (Keshavan and Ongur, 2014). For example, genomics studies have revealed many risk variants shared across clinically discrete phenotypes among which autism spectrum disorder, schizophrenia, bipolar disorder, depression and alcoholism (Gandal et al., 2018). Kapur et al. (2012) denounced the often vain quest for biomarkers matching DSM-defined disorders. Instead, they and others propose to focus on identifying subtypes of patients biologically homogeneous (Figure 1, Insel and Cuthbert, 2015). In that line, the Research Domain Criteria framework intends to rethink research on psychiatric pathophysiology by describing mental illnesses as alteration of general dimensions of behavior and brain function such as cognition, emotion, motivation, arousal, *etc.* (Cuthbert, 2014). These dimensions are already heavily studied by neuroscientists from different disciplines, gathering data to uncover mechanisms underlying cognitive, affective and social functioning, from the behavior to the molecule (Insel and Cuthbert, 2015). Hopes are raising around the resulting multilevel biomarker set to better understand causes of disturbances, eventually providing a more accurate description and classification of mental health conditions (Cuthbert, 2014). Such prospective progress would open easier diagnosis based on biomarkers, and precision medicine therapies adapted to each individual pathophysiology. Great advances have been made in neuroscience over the last two decades about the neural mechanisms involved in major brain functions, and now is the time to find ways to translate it to the psychiatric field.

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## LIST OF ABBREVIATIONS

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- 2-AG 2-arachidonoylglycerol
- 5-HTT Serotonin transporter
- 5HT Serotonin
- Ach Acetylcholine
- ADHD Attention Deficit Hyperactivity Disorders
- BA Basal nucleus of the amygdala
- BD Bipolar disorders
- BDNF Brain-derived neurotrophic factor
- BLA Basolateral nucleus of the amygdala
- CBD Cannabidiol
- CCK Cholecystokinin
- CeA Central nucleus of the amygdala
- CeL Centrolateral nucleus of the amygdala
- CeM Centromedial nucleus of the amygdala
- CNO Clozapine N-oxide
- COX-2 Cyclooxygenase 2
- CRF Corticotropin-releasing factor
- CRP C-Reactive protein

CSF Cerebro-spinal fluid

DA Dopamine

DALYs Disability-Adjusted Life Years

DAT Dopamine transporter

DBS Deep Brain Stimulation

dmPFC Dorsomedial prefrontal cortex

DREADD Designer receptor exclusively activated by designer drugs

DRN Dorsal raphé nucleus

DSM Diagnostic and Statistical Manual of Mental Disorders

ECT Electroconvulsive therapy

EPSC Excitatory post-synaptic current

FDA Food and Drug Administration

GABA Gamma amino butyric acid

GPCR G-protein-coupled receptor

GR Glucocorticoid receptor

GWAS Genone Wide Association Study

HPA Hypothalamic-Pituitary-Adrenal axis

IL Infralimbic part of the prefrontal cortex

IPSC Inhibitory post-synaptic current

iPSC induced Pluripotent stem cell

LA Lateral nucleus of the amygdala

LC Locus coeruleus

LHb Lateral habenula

LPS Lipopolysaccharide

MAO Monoamine oxidase

MAOI Monoamine oxidase inhibitor

MDD Major Depressive Disorder

mPFC Medial prefrontal cortex

NA Noradrenalin

NAc Nucleus accumbens

NOL Novel object location test

NOR Novel object recognition test

NRI Noradrenaline Reuptake Inhibitor

NTS Nucleus of the tractus solitarius

PFC Prefrontal cortex

PL Prelimbic part of the prefrontal cortex

SGD Sustainable Development Goals

SI Substantia innominata

SK Small-conductance calcium-activated potassium channels

SN Substantia nigra

SNP Single-Nucleotide Polymorphism

SNRI Serotonin-Noradrenaline Reuptake Inhibitor

SSRI Selective Serotonin Reuptake Inhibitor

TCA Tricyclic antidepressant

TMS Transcranial Magnetic Stimulation

UCMS Unpredictable chronic mild stress

vHPC Ventral hippocampus

VNS Vagus Nerve Stimulation

VP Ventral pallidum

VTA Ventral tegmental area

WHO World Health Organization

YLDs Years Lived with Disability





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# INTRODUCTION

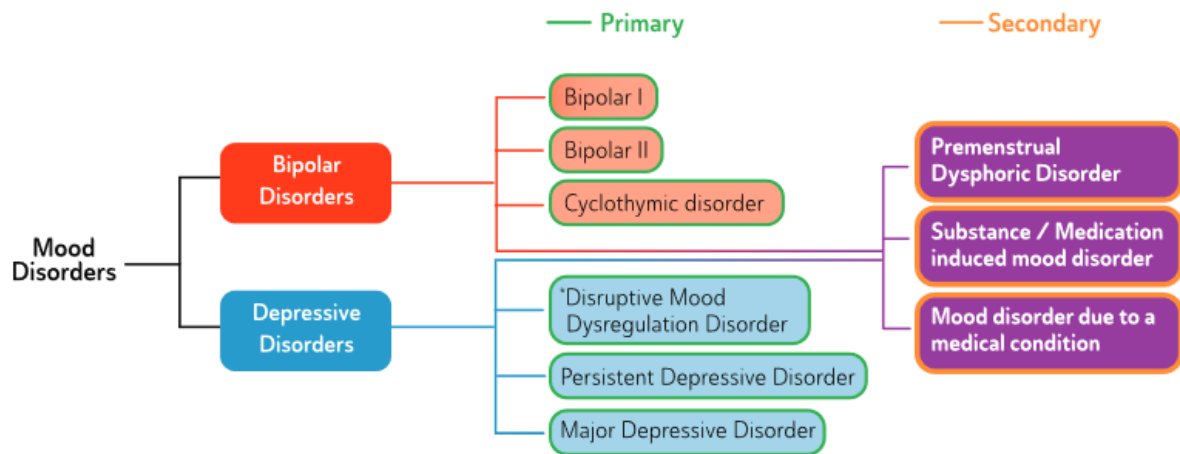
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## 1.1 Mood disorders

### *1.1.1 Clinical description and current classifications*

First descriptions of depression date back to antiquity (Davison, 2006; Angst and Marneros, 2001). Hippocrates, who identified “melancholia” and “mania” syndromes, is thought to be the first stating that these mental disorders emanated from dysfunction of the brain. Two thousand years later, Emil Kraepelin aggregated all types of affective disorders under the term of “manic-depressive insanity”, the idea of one unique disease dominating the first half of the 20<sup>th</sup> century. In opposition, Kleist introduced in 1911 the concepts of “unipolar” and “bipolar”, considering mania and depression on their own, and Wernicke recognized five different types of melancholia (1900). Further publications from Angst (1966), Perris (1966) and Winokur et al. (1969) demonstrated clinical, familial and course characteristics individuating unipolar and bipolar disorders (BD). Thus, BD appeared in the third edition of the diagnostic and statistical manual of mental disorders (DSM-III-R, American Psychiatric Association, 1987), establishing the ground for the current unified classification.

Among the psychiatric diseases, the broad group of mood disorders then refers to both bipolar and depressive disorders (Figure 2, Malhi et al., 2015, ICD-11, World Health Organization, 2018). The concept of mood is not clearly defined in the diagnostic classifications for psychiatric disorders, rather referring to examples, such as “elevated, expansive or irritable mood” or “depressed mood” (see below, DSM-5, American Psychiatric Association,



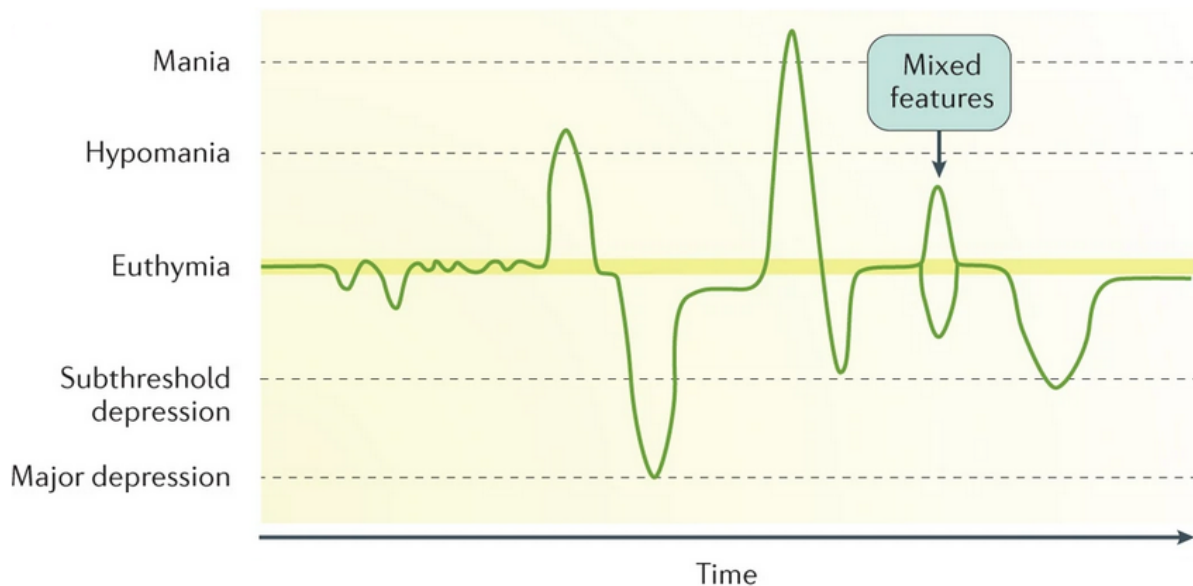
**Figure 2. Classification of mood disorders.** From Malhi et al. (2015). “Mood disorders can be divided in two ways: either as bipolar and depressive disorders, or as primary and secondary disorders. This creates four types of mood disorder diagnoses; some of which can be further divided into subtypes. For the purposes of classification these are regarded as discrete, non-overlapping entities, but in reality some diagnoses readily transmute into other forms of mood disorders. \*Disruptive Mood Dysregulation Disorder is a new diagnosis, which in DSM-5 is grouped with depressive disorders but clinically overlaps considerably with bipolar disorders.”

2013). It corresponds to a persistent state of mind or feeling which can be good or bad, *e.g.* sad, hopeless, happy, angry, *etc.* Mood disorders are characterized by specific types of mood episodes and their pattern over time. Mood episodes in psychiatric classifications mostly fall in four categories: depressive episode, manic episode, hypomanic episode and mixed episode.

A depressive episode can be identified upon the presence of the following symptoms: depressed mood (*i.e.* sad, empty or hopeless feelings), loss of interest or pleasure, weight and appetite disruptions, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue, excessive guilt, poor concentration, thoughts of death (DSM-5). The symptoms impair daily-life functioning, namely in social and professional areas.

On the contrary, a manic episode is characterized by abnormally and persistently elevated, expansive or irritable mood associated with increased goal-directed activities and energy, inflated self-esteem, decreased need for sleep, pressure to keep talking, racing thoughts, distractibility, psychomotor agitation, risk-taking behaviors. Again, the symptoms impair social or occupational functioning. The same features of manic episodes are found in hypomanic episodes, albeit their less marked severity do not usually disrupt functioning. Finally, mixed episodes require the patient to express both depressive and manic features (DSM-IV, the DSM-5 replaced them by the specification of mood episode “with mixed features”).

Based on these definitions, depressive disorders are made of one or several recurrent



**Figure 3. Example of clinical course during bipolar disorder type I (at least one mania episode).** Adapted from Vieta et al. (2018).

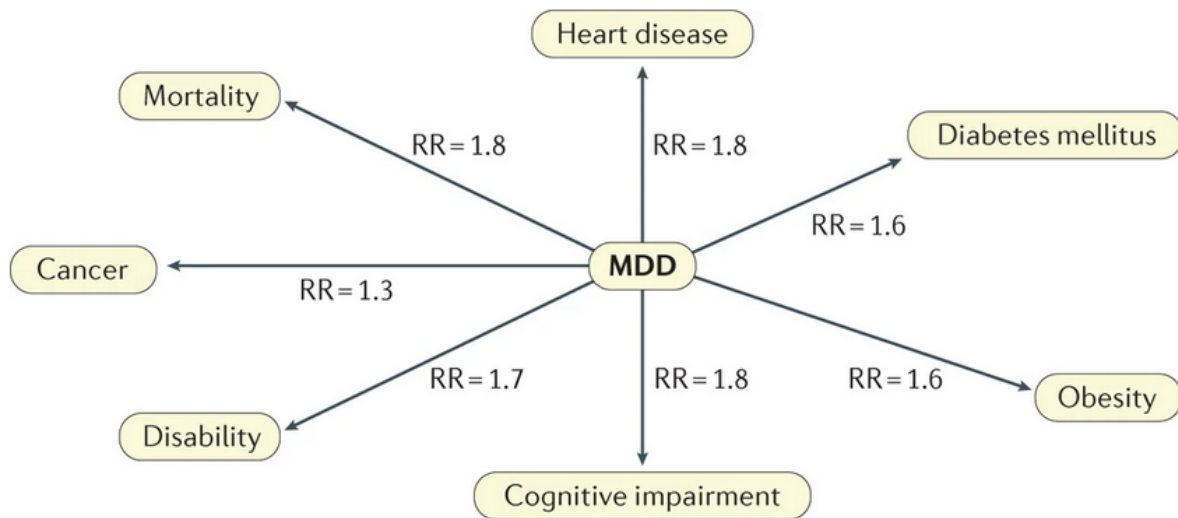
depressive episodes, without any occurrence of manic or hypomanic episode; whereas the presence of at least one manic or hypomanic episodes define bipolar disorders (type I or II, respectively), most of the time in alternation with depressive episodes (Figure 3, Vieta et al., 2018). The identification of previous manic or hypomanic episodes in depressed patients can be arduous because of recall biases (Swann et al., 2005). In addition, the onset of bipolar disorder can be through a depressive episode, leading to misdiagnosis between unipolar and bipolar depression, even though particular characteristics can suggest bipolarity (Swann et al., 2005). Behavioral difficulties starting during or before adolescence, family history of bipolar disorders in first-degree relatives, or abnormal activation during antidepressant therapy are examples of signs pointing towards bipolar disorders.

### ***1.1.2 Epidemiology: the burden of mood disorders***

#### **Depressive disorders**

Depression is the leading cause of disability worldwide, with a global total over 50 million Years Lived with Disability (YLD), accounting for 7.5% of all YLD (World Health Organization, 2017). More than 264 million people currently live with depression globally. The point prevalence is estimated around 4-5%, while the lifetime prevalence reaches 20-30% (Kruijshaar et al., 2005; Moffitt et al., 2010; Vandeleur et al., 2017), with females more affected than males. Co-morbidity is high, with depressive disorders associated to unfavorable physical health outcomes such as diabetes mellitus, heart disease, stroke,

hypertension, obesity, cancer, cognitive impairment and Alzheimer's disease (Figure 4, Otte et al., 2016).



**Figure 4. The somatic consequences of major depressive disorder (MDD).** From Otte et al. (2016). Evidence from meta-analyses of longitudinal studies has revealed that the relative risk (RR) of various diseases is increased in patients with MDD compared with patients who do not have MDD (Penninx et al., 2013).

## Bipolar disorders

Bipolar disorders affect more than 1% of the world population (Merikangas et al., 2011). Lifetime prevalence is estimated at 2-3% for the broad spectrum of BD, equally affecting males and females. They represent the 17<sup>th</sup> leading cause of global burden of disease, with considerable impact on the quality of life (Vigo et al., 2016). Importantly, bipolar disorders constitute the highest suicide rate factor among affective disorders, with about one-third to one-half of BD patients attempting suicide at least once in their lifetime (Gonda et al., 2012). Like in depressive disorders, life expectancy is reduced by increased risk of developing physical conditions like diabetes mellitus and cardio-vascular diseases (Correll et al., 2017).

### 1.1.3 Therapeutic management

#### Depressive disorders

The management of depression presents two initial treatment options: psychotherapy and pharmacotherapy (Otte et al., 2016). Usually, mild depressive episodes can be treated with psychotherapy alone, while moderate or severe depressive episodes require pharmacotherapy, alone or in combination with psychotherapy. Psychotherapies paradigms vary in their

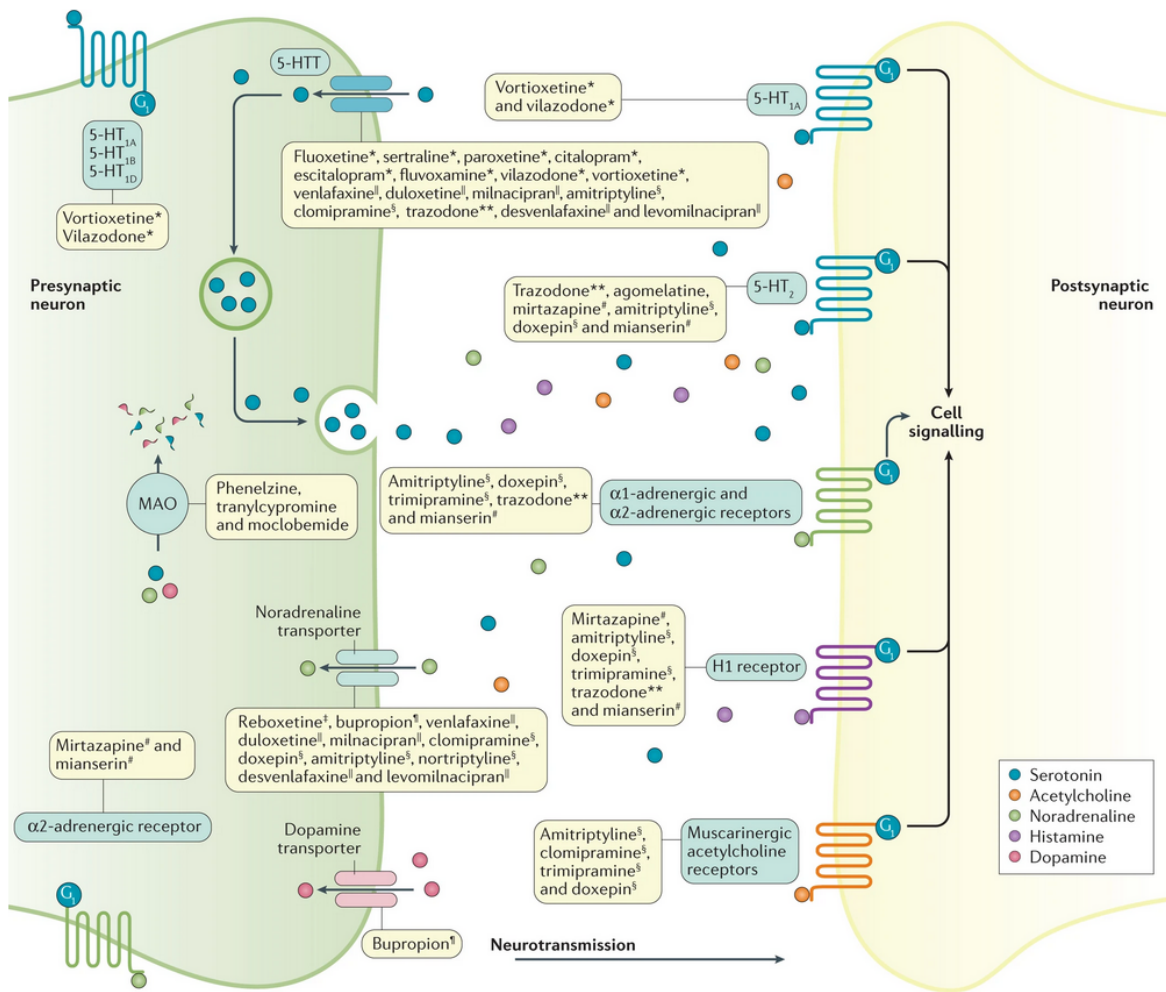
focus and methods, but have similar efficiency (Linde et al., 2015). The effects produced by psychotherapies are also equivalent to pharmacotherapy, although methodological issues like blinding prevent trusty comparisons (Amick et al., 2015). Despite clear efficiency of psychotherapy, barriers to access encompass time constraints, lack of available services and cost (Mohr et al., 2010, 2006).

Concerning pharmacotherapy, almost all of the available antidepressant drugs were discovered by serendipity between 1950 and 1960 (Figure 5, Berton and Nestler, 2006). The first tricyclic antidepressants (TCA) were mostly replaced by selective serotonin reuptake inhibitors (SSRI), noradrenaline reuptake inhibitors (NRI) and serotonin and noradrenalin reuptake inhibitors (SNRI), which share the same mechanism of action and efficacy, but with improved tolerance. Monoamine oxidase inhibitors (MAOI), which stop enzymatic breakdown of serotonin and noradrenaline can also be used, despite important side effects. Importantly, several weeks of treatment are necessary to observe their antidepressant effects. In addition, remission rates are as low as about 35% after the first treatment, increasing to 70% with up-to four different lines of treatment (Rush et al., 2006).

The choice of the antidepressant drug is mostly guided by the compromise between efficacy and tolerability, with new generation antidepressants including SSRI usually preferred as first-line treatments (Malhi et al., 2015). Some other clinical specifiers of the depressive episode (*e.g.* anxious distress, cognitive dysfunction, sleep disturbances, *etc.*) can also orientate towards particular pharmacological compounds (Kennedy et al., 2016).

Efforts to develop new antidepressant drugs are made towards non-monoamergic molecules, including neurokinin 1 antagonists, glutamatergic system modulators, anti-inflammatory agents, opioid tone modulators and opioid-K antagonists, hippocampal neurogenesis-stimulating treatments and antiglycocorticoid therapies (Otte et al., 2016). However, clinical trials for antidepressant drugs are extremely expensive because they involve chronic treatment of large number of patients (at least hundreds), with high risk of failure due to large placebo responses (Berton and Nestler, 2006). In 2019, intra-nasal ketamine, an antagonist of the NMDA receptor, was approved by the Food and Drugs Administration (FDA) in the treatment of resistant-depression, as defined by no response to one or more line of antidepressant therapy (Corrigan and Pickering, 2019). Classically, pharmacology-resistant depressions are treated by electroconvulsive therapy (ECT), eliciting a seizure under short anaesthesia. It is one of the most efficient antidepressant therapy, but availability and tolerability limit its use (Berton and Nestler, 2006). Other treatments





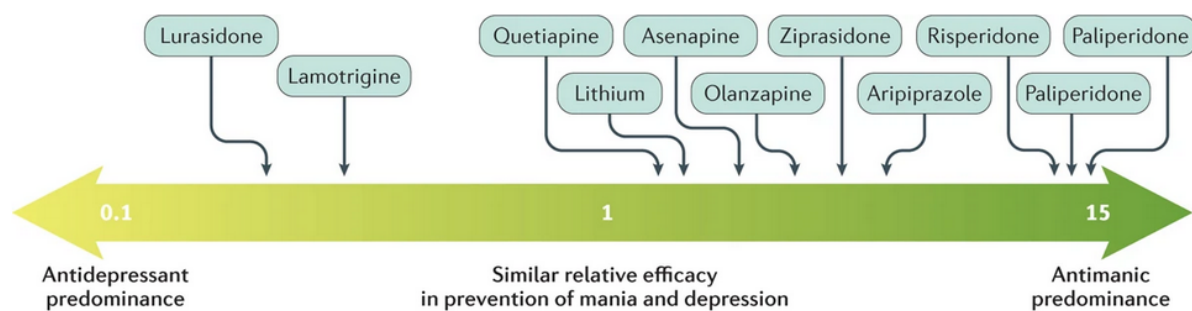
**Figure 5. The mechanism of action of antidepressant drugs.** From Otte et al. (2016). \* denotes selective serotonin reuptake inhibitors (SSRIs) (and \*\* denotes serotonin antagonist and reuptake inhibitor); ‡ denotes noradrenaline reuptake inhibitors (NRI); § denotes tricyclic antidepressants (TCAs); || denotes serotonin–noradrenaline reuptake inhibitors (SNRIs); ¶ denotes noradrenaline–dopamine reuptake inhibitors (NDRIs); # denotes  $\alpha$ 2-adrenergic receptor antagonists. 5-HTT: serotonin transporter; MAO: monoamine oxidase.

include for instance transcranial magnetic stimulation (TMS), deep brain stimulation (DBS) or vagus nerve stimulation (VNS).

## Bipolar disorders

Management of BD implies acute treatment of mania and depression episodes along with maintenance therapy for prevention of relapses (Vieta et al., 2018). Indeed, bipolar disorders are highly recurrent, even when they are correctly diagnosed and treated.

Lithium was the first anti-manic drug approved by the FDA and remains the mostly used. Its mechanism of action is mostly unknown. Other mood stabilizers medications include anticonvulsants like valproate and antipsychotics drugs like aripiprazole, prescribed alone or in combination (Vieta et al., 2018; Malhi et al., 2021; Goodwin et al., 2016). Anti-manic



**Figure 6. Polarity index of mood stabilizers.** From Vieta et al. (2018). “The polarity index of drugs used for the maintenance treatment of patients with bipolar disorders is the ratio of the number of patients needed to treat for prevention of depression to the number of patients needed to treat for prevention of mania on the basis of results of randomized placebo-controlled trials (Popovic et al., 2012). This index classifies therapies as those with an antimanic prophylactic effect and those with an antidepressant prophylactic effect. A polarity index of 1 reflects an equal efficacy in preventing manic and depressive episodes.”

drugs target the acute symptoms of mania, while any associated cognitive disturbance would be treated with antipsychotics, and any behavioral disturbance with antipsychotics and/or short-term benzodiazepines (Malhi et al., 2015). It is important to cease agents with mood-elevating properties such as antidepressants and psychostimulants. Therapeutic drugs for bipolar depression include second generation antipsychotics and mood stabilizers. Antidepressants are generally avoided in BD, or restricted to combination therapy with a mood stabilizer agent. Finally, continuation treatment for relapse prevention depends on the predominant polarity of mood episodes (Figure 6, Vieta et al., 2018).

Treatments for BD mostly target glutamate, serotonin and dopamine transmission, as well as intra-cellular calcium signaling and the inositol pathway (Kato, 2019). Antidepressant drugs can be added during depressive episode, but for short periods of time because they can induce mood switch towards mania. Suicidal risk must be evaluated, and lithium is preferred in this case (Smith and Cipriani, 2017). Most of these treatments have side effects, such as body weight gain (Fang et al., 2017).

Pharmacological resistance can happen during manic, mixed and depressive episodes, and requires to test different drugs or combinations. ECT is an alternative to medications that is efficient for each kind of mood episode (Malhi et al., 2021; Goodwin et al., 2016).

#### ***1.1.4 Hypotheses about the pathophysiology***

Despite tremendous progress in molecular, genetic and imaging fields of research, the understanding of the pathophysiology underlying mood disorders is still fragmented. Currently, no unifying mechanism can fully explain the diseases. We briefly present an

overview of leading research avenues for both depressive and bipolar disorders. Of note, the unresolved question about differences between unipolar and bipolar depression leads us to separately treat them, even though part of their pathophysiology could be shared (Smith and Craddock, 2011). Further details can be found for each part in cited reviews.

## **Depressive disorders**

**Genetics** Like most of psychiatric disorders, depression shares genetic and environmental causes. First-degree relatives of depressive patients display a 3-times increased risk of depression (Otte et al., 2016). Heritability is estimated around 35% (Geschwind and Flint, 2015). Large-scale genome wide association studies (GWAS) analyses suggest high polygenicity, with a large number of common variants at different *loci*, individually accounting for small effects on the disease. Importantly, the single-nucleotide polymorphisms (SNPs) identified in depression significantly overlap with those associated with other psychiatric disorders, such as schizophrenia, bipolar disorder and attention deficit hyperactivity disorder (ADHD) (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). Candidate gene studies have shown polymorphisms in genes implicated in the monoamine neurotransmitter systems, even though contradictory results exist (Shadrina et al., 2018). Variations in the hypothalamic-pituitary-adrenal (HPA) axis, neurogenesis, neuroplasticity, cytokines, circadian rhythm functions genes were reported but none of them were confirmed by meta-analyses. Whole-genome analyses did not replicate the candidate genes studies, and they were not themselves consistent from one to another (Bosker et al., 2011; Shadrina et al., 2018). Authors suggest a transition from an analysis of individual SNPs to sets of SNPs, and further to a polygenic risk score as it can be done for schizophrenia (Shadrina et al., 2018). Such GWAS studies require huge numbers of patients, and would benefit from more precise phenotype evaluation because of the significant heterogeneity among depression clinical presentations (Hyman, 2014).

It is worth mentioning the growing interest in epigenetics which can underlie the genes-environment interactions, and in particular the role of early life trauma in the depression occurrence, reviewed by Klengel and Binder (2015).

**Environmental stress** On the environmental side, different stressful events occurring either early in life or in adulthood are well-documented in the context of depression onset. They include childhood trauma among which physical and sexual abuse, psychological

neglect, exposure to domestic violence or early separation from parents following death or separation, and more recent stressors such as loss of employment, financial insecurity, chronic or life-threatening health problems, exposure to violence, separation and bereavement (Kessler, 1997; Otte et al., 2016). Possible biological mechanisms underlying such effects of environmental stress are reported below (see “HPA axis”).

**Monoamines** The monoaminergic hypothesis was the first pathophysiology proposed more than fifty years ago (Coppen, 1967; Barchas and Altemus, 1999). It was driven by the understanding of the mechanisms of antidepressant drugs action, that appear to increase serotonin (5HT) and noradrenalin (NA) levels in the brain, restoring potential deficiency of monoamines at functionally important cerebral sites. However, measures of 5HT and NA metabolites in the plasma, urine and cerebro-spinal fluid (CSF) of patients with depression, as well as post-mortem studies, demonstrated inconsistent results (Hasler, 2010; Otte et al., 2016). It seems that depression and antidepressant action could go through different neural mechanisms, since the action of antidepressants cannot accurately be described as reversing and normalizing the processes that are dysfunctional in the depressed brain (Willner et al., 2013). For instance, 5HT depletion induced by a tryptophan-free diet causes severe relapse in successfully treated depressed patients, whereas it has no major effect on mood of non-depressed people (Delgado et al., 1999; Ruhé et al., 2007). In addition, one could expect that drugs targeting monoamines would affect these neurotransmitter systems within hours after administration, whereas antidepressants usually show their therapeutic effect after several weeks of treatment. Thus, the monoamine hypothesis does not by itself explain the depression pathophysiology.

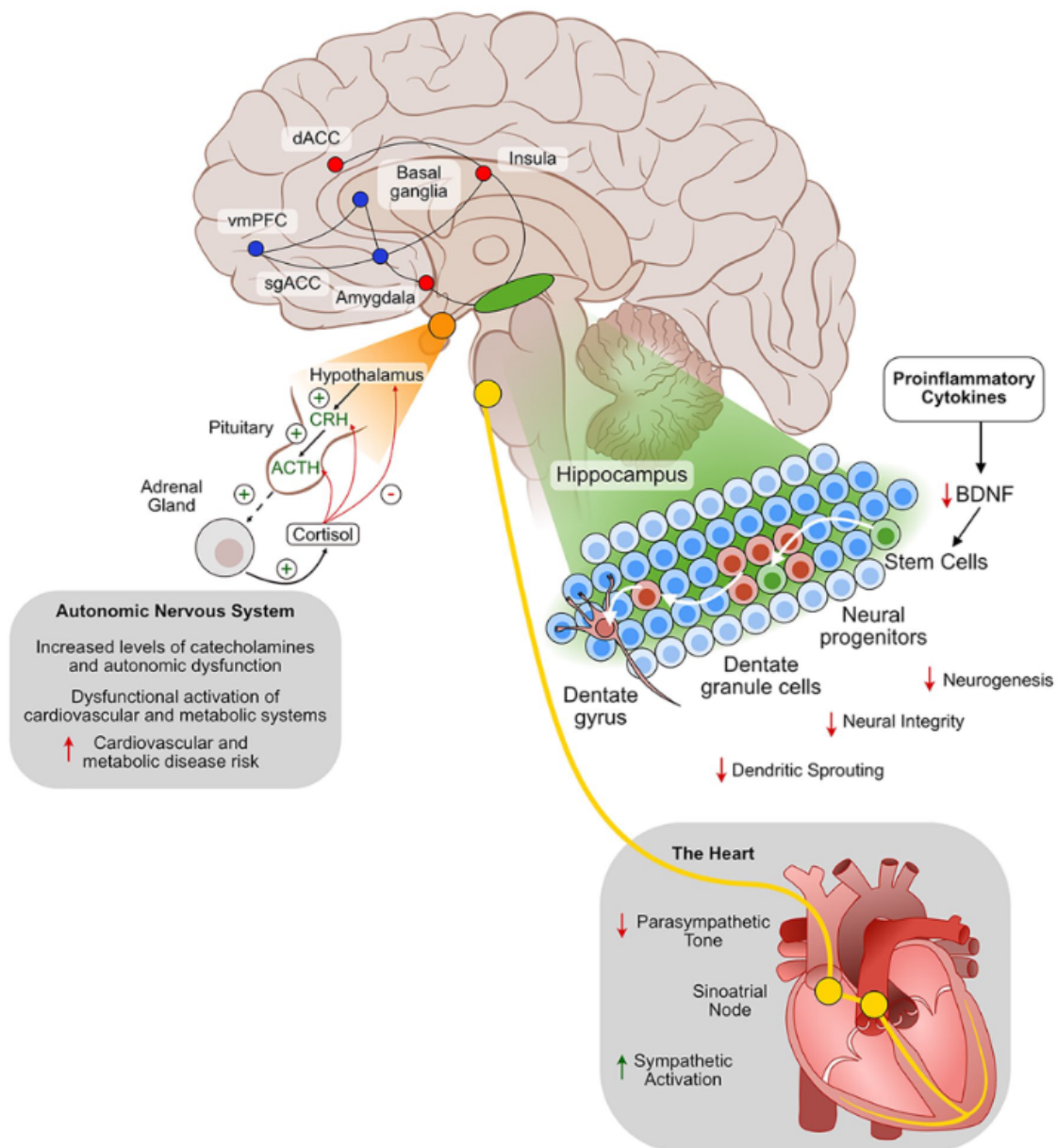
**HPA axis** According to the role of stress in the development of depression, a comprehensive pathophysiological model places the HPA axis in the center (Figure 7, Malhi et al., 2021). Indeed, depressive patients consistently display HPA axis hyperactivity, glucocorticoid resistance and increased inflammation (Otte et al., 2016). In animals, unpredictable chronic mild stress (UCMS) is a well-validated murine model of depression, based on the depressive-like phenotype of animals subjected to a varying schedule of minor stressors (Willner, 1997; Ménard et al., 2016). The cascade of events is explained as follows: emotional stressful stimuli are detected by the amygdala, which receives sensory inputs (Willner et al., 2013). The amygdala exerts excitatory control over the hypothalamus to stimulate the hypothalamic-pituitary-adrenal axis, the main physiological system involved

in response to stress. Cortisol (or corticosterone in rodents) acts in a positive feedback manner to further stimulate the amygdala. Conversely, the hippocampus exerts inhibitory control over the HPA axis, such that cortisol stimulation of the hippocampus acts in a negative feedback manner to inhibit the HPA axis. Chronic stress exposure leads to a loss of glucocorticoid receptors (GR) in hippocampal granule cells, with a consequent disinhibition of the HPA axis and a further increase in corticosteroid stimulation.

However, despite this well-characterized role of stress and HPA axis in the onset of depression, cortisol levels in patients do not necessarily decrease following treatment (McKay and Zakzanis, 2010). In addition, HPA antagonists appear not very efficient as antidepressants, and induce severe side effects (Otte et al., 2016; Willner et al., 2013). In summary, the dysregulation of the HPA axis in depression is unequivocal but has not improved therapeutic strategies.

**Inflammation** In close link to the HPA axis, the immune system constitutes an important part of the physiological stress-sensing system. A number of cytokines, these chemical inflammatory mediators primarily secreted by lymphoid cells in response to pathogens to regulate all other immune cells (*e.g.* lymphocytes, monocytes, neutrophils, basophils, eosinophils, natural killer cells) have been involved in the pathophysiology of depression (Medina-Rodriguez et al., 2018; Sharpley and Agnew, 2011). These include pro-inflammatory (interleukins IL-1 $\beta$ , IL-6, TNF $\alpha$ ) and anti-inflammatory (IL-4, IL-8, IL-10) cytokines whose levels are likely disturbed in depressive patients (Dowlati et al., 2010; Haapakoski et al., 2015). In addition, studies have shown that severe infections, as well as conditions inducing neuroinflammation such as traumatic brain injury and autoimmune diseases increase the risk of developing depression, just as cytokine treatments in the context of hepatitis virus infection or cancer for instance (Maes et al., 2011; Juengst et al., 2017; Benros et al., 2013; Myint et al., 2009). Peripheral cytokines can act directly on brain cells (including microglia, astrocytes and neurons) after transport through the blood-brain barrier, or indirectly through the vagus nerve. Animal studies also brought evidence supporting the role for peripheral immune dysfunction in depression, and have shown that these neuroimmunological mechanisms converge to alter neurotransmitter systems, neurogenesis and plasticity of the brain (Miller and Raison, 2016; Hodes et al., 2015).

In parallel, antidepressant therapies reduced cytokines concentrations in depressed patients



**Figure 7. The neurobiology of depression.** From Malhi et al. (2021). “This schematic shows some of the key nodes within neural networks thought to underpin emotional mentation. These brain regions and neural networks have individual functions and also serve collective functions and are impacted by their connections with each other and by influences and inputs from other parts of the body. Examples of these include connections with the neuroendocrine axis, in particular, the HPA axis that subserves responses to stress, and which in addition to being modulated by hormonal processes, is subject to autonomic nervous system control. Changes in the latter, and more specifically cardiac changes related to parasympathetic and sympathetic tone, can also modify inputs to the brain. A key region that is important to emotional processing and subject to many of these inputs, and itself provides outputs to many networks within the brain, is the hippocampus. The schematic shows the emergence of cells that begin as stem cells under the influence of brain-derived neurotrophic factor (BDNF). The generation of new cells and the many steps involved are all subject to influences such as those from proinflammatory cytokines that can diminish neurogenesis, neural integrity and reduce dendritic sprouting, thereby diminishing the functional capacity of the hippocampus. [...] In addition, neural networks involved in emotional regulation and processing of emotion play a significant role.” ACTH: adrenocorticotropic hormone, BDNF: brain-derived neurotrophic factor, CRH: corticotropin releasing hormone, dACC: dorsal anterior cingulate cortex, sgACC: subgenual anterior cingulate cortex, vmPFC: ventromedial prefrontal cortex.

in several studies (Miller et al., 2009). Nevertheless, anti-inflammatory drugs have inconsistent antidepressant properties, suggesting a more complex link between inflammation and depression, or a role for inflammation only in a subset of depressed patients (Köhler et al., 2016).

**Gut microbiota** This field of research extended more recently to studies on the role of microbiota and gut-brain axis in depression (see in Annexes: Siopi et al., 2020, 2019, and for a review: Yang et al., 2020). Arguments for the role of gut microbiota in depression arise from the observation that (i) depressed patients have disturbed gut microbiota composition, (ii) transplanting this fecal microbiota from depressed patients to germ-free rodents induces depressive-like behaviors, and finally (iii) specific probiotic bacteria have antidepressant effects (Sanada et al., 2020; Yang et al., 2020). Proposed mechanisms include the enteric and autonomic nervous system, metabolic and endocrine pathways as well as the immune system (Yang et al., 2020).

Interestingly, the monoamine oxidase inhibitors are antidepressants originally derived from the antitubercular agent isoniazid (Macedo et al., 2017). Since then, the antimicrobial properties of various antidepressant treatments have been highlighted, without knowing to what extent their antimicrobial effect relates to their antidepressant efficacy.

**Neuroplasticity** Increased peripheral levels of cortisol might induce depressive symptoms by disrupting molecular and cellular neuroplasticity in the brain. Indeed, prolonged corticosteroid stimulation leads to atrophy of apical dendrites and granular cell death in the hippocampus, along with impairments in hippocampal-dependant memory processes (Sapolsky, 2000; Bodnoff et al., 1995; de Quervain et al., 1998). Lower levels of the neurotrophin brain-derived neurotrophic factor (BDNF) have been observed in the depressed patients blood, and pharmacological as well as non-pharmacological antidepressant therapies normalize BDNF levels (Molendijk et al., 2014). In addition, the hippocampal neurogenesis, *i.e.* the growth and differentiation of new cells in the dentate gyrus of the hippocampus notably controlled by BDNF, is powerfully suppressed by stress (Egeland et al., 2015). Loss of neurogenesis has been reported in post-mortem samples from elderly depressed patients, as well as in various animal models of depression (Lucassen et al., 2010; Samuels and Hen, 2011).

Various antidepressant treatments such as classical monoamine targeting drugs, but also one single injection of ketamine or neurostimulation techniques (*e.g.* ECT, TMS, DBS)

induce robust adult neurogenesis in rodents. It appears that adult neurogenesis promotes resilience to stress by facilitating the corticosterone-mediated negative feedback on the HPA axis (Egeland et al., 2015). Efficient symptom relief has been suggested to undergo through this neuroplasticity effect (Egeland et al., 2015; Planchez et al., 2020). However, the role of neurogenesis in depression is debated. For instance, rodents do not demonstrate depressive-like behaviors upon adult neurogenesis reduction, unless a stressful context is added. In addition, part of the antidepressant effects are neurogenesis-independent (David et al., 2009).

**Brain structure** In relation with the neuroplasticity effects of stress, structural brain imaging studies have reported significant and reliable reduction of the hippocampal volume in depressed patients (Kempton et al., 2011; Schmaal et al., 2015). Moreover, antidepressant drug response correlates with the restoration of normal hippocampal volume (MacQueen et al., 2008). Other brain regions were found smaller in patients with depression such as the basal ganglia, thalamus, prefrontal, orbitofrontal, anterior and posterior cingulate cortices, insula and temporal lobes including the amygdala (Kempton et al., 2011; Schmaal et al., 2015; Spellman and Liston, 2020). Olfactory bulb volume reduction has also been proposed as a biomarker for depression (Rottstaedt et al., 2018). It is still not known whether these differences are due to neuronal loss, dendritic atrophy, loss of glial cells or other phenomena.

The impact of effective antidepressant therapy on the volume of these structure is inconsistent throughout the literature, except for the hippocampus as mentioned above, and the anterior and posterior cingulate cortices whose larger size predicting remission has been reported several times (Chi et al., 2015).

**Functional brain circuits** Changes in hippocampal structure, in addition to damage in other brain regions under prolonged exposure to high levels of glucocorticoids, lead to a global disruption of multiple brain networks.

The affective-salience circuit, including the dorsal cingulate, anterior insula, ventral striatum and amygdala, in addition to downstream targets like hypothalamus and brain stem structures, is involved in processing of and coping with affective information and guiding motivated behaviors (Willner et al., 2013; Krishnan and Nestler, 2010; Otte et al., 2016). Within this circuit, abnormal connectivity and activation of the amygdala is among the most robust reported alteration in depressed patients (Hamilton et al., 2012).



It is accompanied by hyperactivity of the dorsal anterior cingulate and anterior insula, and hypoactivity and connectivity of the ventral striatum, likely triggering depressive symptoms involving increased salience of negative information and self-directed thoughts, along with anhedonia (Hamilton et al., 2015; Pizzagalli, 2014; Satterthwaite et al., 2015). Dorsolateral prefrontal cortex activity is also decreased during various tasks, including during emotion regulation and executive control tasks (Spellman and Liston, 2020).

The default mode network, comprising the posterior cingulate cortex, precuneus, medial prefrontal cortex, rostral anterior cingulate cortex, inferior parietal cortex and temporal cortices normally activated during resting states with self-directed thoughts, is hyperconnected in depression and this positively correlates with rumination measures (Hamilton et al., 2015; Sheline et al., 2009; Dutta et al., 2014; Cooney et al., 2010).

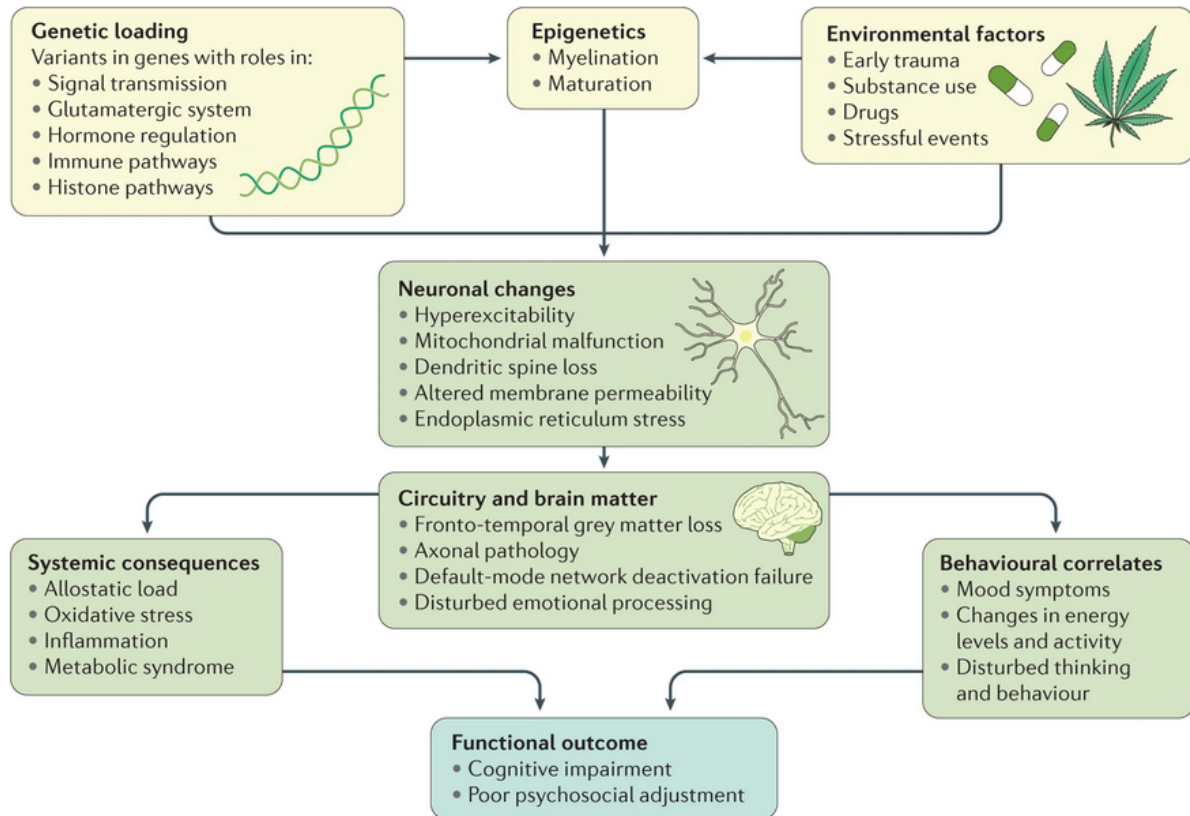
In parallel, the frontoparietal cognitive control circuit, which is engaged in many cognitive tasks, is found hypoconnected in depression, possibly underlying goal-directed attention deficits (Cole et al., 2013; Kaiser et al., 2015; Hamilton et al., 2012). Abnormal interactions between the frontoparietal control network and the default mode network emerge as a robust finding from meta-analyses (Broyd et al., 2009; Wang et al., 2012).

Such functional connectivity and activity modifications in these brain circuits is suggested to predict treatment response of at least some antidepressant interventions (for reviews, see Dunlop et al., 2019; Perlman et al., 2019). Anterior cingulate cortex hyperactivity is associated with positive antidepressant treatment outcome, as well as increased functional connectivity between frontal and limbic regions, decreased connectivity within the default mode network and maybe decreased amygdala function (Perlman et al., 2019). Specifically, not a single brain area could predict the response to any therapy or in any patients population (Dunlop et al., 2019). Instead, combined use of complementary biomarkers is hoped to help guiding treatment selection.

## **Bipolar disorders**

**Genetics** Heritability of BD is one of the highest for psychiatric disorders, estimated up to 85% (Goodwin and Jamison, 2007), although both environmental and genetic factors are involved. Siblings of a patient have a risk of developing the disease roughly 8-fold higher than general population (Craddock and Sklar, 2013). GWAS have uncovered several reproducible alleles associated, but with individual small effects. Several pathways, common with other mental illnesses such as schizophrenia, ADHD and autism spectrum

disorder are involved (Figure 8, Vieta et al., 2018; Lichtenstein et al., 2009). They include hormonal regulation, calcium channels, second messenger systems and glutamate transmission (Nurnberger 2014). Rare variants with high penetrance are also thought to be implicated, even though studies showed inconsistent results (Vieta 2018).



**Figure 8. Multifactorial model of bipolar disorders.** From Vieta et al. (2018). “Although bipolar disorders are amongst the most heritable psychiatric disorders, both genetic and environmental factors contribute to disease development. Gene–environment interactions might be mediated by epigenetic alterations. Genetic and environmental factors might contribute to the development of bipolar disorders through neuronal changes that modify brain circuitry. These changes systemically and behaviourally affect the body, leading to psychosocial and cognitive impairment.”

**Environmental and medical factors** Several perinatal risk factors have been identified, like cesarean section delivery, maternal influenza infection, maternal smoking during pregnancy and high paternal age (Vieta et al., 2018). Childhood adverse events and drug misuse during adolescence are also classically described risk factors (Aas et al., 2020; Parboosing et al., 2013).

Among the mania or hypomania inducers, antidepressant therapy without mood stabilizers, corticosteroids, androgens, and ECT have been highlighted (Goodwin and Jamison, 2007). Some medical conditions can increase the risk of bipolar disorders, such as multiple sclerosis, stroke, systemic lupus erythematosus and endocrine disorders involving cortisol hormone (Goodwin and Jamison, 2007). Other triggers of mood episodes include season change,

jet lag and increased light exposure (D’Mello and McNeil, 1995; Inder et al., 2016; Bauer et al., 2017).

**Monoamines** Historically, mood disorders are thought to originate from an imbalance of monoamine neurotransmitters, including serotonin, noradrenalin and dopamine (DA). The dopamine hypothesis of bipolar disorders is still on the table, with increasing use of antidopaminergics in therapy (Ashok et al., 2017). Reproducible findings highlight a hyperdopaminergia state underlying mania, induced by elevations in D2/3 receptor availability and hyperactive reward processing network. On the other side, it is speculated that increased striatal dopamine transporter (DAT) levels would reduce the dopaminergic tone and lead to depression, even though evidences are less robust.

Lithium as well as atypical antipsychotics are known to modify increase serotonergic and decrease dopaminergic transmission through  $5HT_{1A}$ ,  $5HT_2$  and  $D_2$  receptors (Yatham et al., 2005; Alda, 2015).

**HPA axis and stress** The role of stress in BD is less understood than in depression (Brietzke et al., 2012). Evidence of HPA axis alterations are more scarce in BD than in depressed patients (Vieta et al., 1999; Cervantes et al., 2001; Aubry, 2013; Brietzke et al., 2012). Stress has been postulated as a trigger for the first manic/hypomanic episode (Horesh et al., 2011; Horesh and Iancu, 2010), and stressful life events predict relapse in BD (Altman et al., 2006). Chronic stress exposure is associated with greater symptom severity and persistence, and higher number of episodes (Kim et al., 2007). This effect seems to be age dependent, with higher impact of early life stress and diminution of the stress effect when the person gets older (Daruy-Filho et al., 2011; Hillegers et al., 2004).

Lithium is thought to suppress the stress effects on the brain through neurotrophic effects (see below “Brain molecular and cellular alterations”, Alda, 2015).

**Circadian rhythm** Abnormal circadian rhythms are well-demonstrated during mood episodes but also during euthymic periods (Milhiet et al., 2011). Many circadian physiological functions such as hormone secretion (notably cortisol and melatonin), core temperature, appetite and sleep display altered rhythmicity in BD patients. Circadian clocks regulate a broad range of physiological processes related to mood, comprising monoamine and glutamatergic transmission, HPA axis function, metabolism and immune function (Ketchesin et al., 2020). Of note, circadian genes polymorphisms have been observed in bipolar

disorders (Milhiet et al., 2011).

Lithium is known to lengthen the free running cycle, probably through modulation of transcription factors and clock genes expression regulating the clock oscillations (Alda, 2015).

**Inflammation and immune system** Immuno-inflammatory dysfunction have been repeatedly observed in bipolar disorders, and could explain part of the comorbidity with cardiovascular diseases, diabetes mellitus, obesity and thyroid dysfunction (Hamdani et al., 2013). Pro-inflammatory cytokines could take a part in manic and depressive episodes, with increased levels of IL-6, IL-8, IL-4 and TNF $\alpha$  reported (Hamdani et al., 2013; Goldstein et al., 2009; Munkholm et al., 2013). C-Reactive Protein (CRP) is one of the acute phase proteins of systemic inflammation. Several studies associated bipolar disorders with elevated CRP (Hamdani et al., 2013; Dargél et al., 2017).

In the same line, few studies investigated the role of the microbiota and gut-brain axis in bipolar disorders (Liu and Zhu, 2018; Generoso et al., 2020), with results on gut microbiota composition modifications in BD patients (Evans et al., 2017). Probiotics are already tested as a therapeutic strategy (Reininghaus et al., 2020; Dickerson et al., 2018).

One effect of lithium therapy could go through a decrease in the production of pro-inflammatory cytokines (Sakrajda and Szczepankiewicz, 2021).

**Brain molecular and cellular alterations** Increasing evidence underlines the role of cellular pathways involving mitochondrial function, endoplasmic reticulum stress, neuroinflammation, oxidation and apoptosis in bipolar disorders (Figure 8, Vieta et al., 2018; Quiroz et al., 2008; Berk et al., 2011). Notably, mitochondrial deficits inducing the well-documented calcium dysregulation in BD could lead to disrupted cellular resilience and synaptic plasticity (Quiroz et al., 2008). Neuroplasticity, through neurotrophic factors such as BDNF, is decreased in bipolar patients and mood stabilizers have shown neuroprotective properties (Berk et al., 2011; Sarrazin et al., 2019).

Several mood stabilizers stimulate the release of BDNF (Kato, 2019). Lithium also increases the antiapoptotic b-cell lymphoma 2 factor (bcl-2), and decreases the proapoptotic factors tumor protein p53 and bcl-2 associated X protein (BAX). As antidepressants, lithium promotes neurogenesis in the hippocampus in animal models and increases hippocampal volumes in patients (Alda, 2015).

**Brain circuits** As a result of all the above mentioned biological systems alterations, specific brain circuits are disrupted, mostly those implicated in emotion and reward processing and emotion regulation (Phillips and Swartz, 2014). Neuroimaging findings reveal deficits in prefrontal cortex, especially in the ventro-lateral prefrontal and the orbitofrontal cortices inhibition onto the amygdala, associated with structural alterations of these regions along with the hippocampus in BD patients. In parallel, elevated activity in the ventral striatum, the ventro-lateral prefrontal and the orbitofrontal cortices is observed in bipolar disorders during reward processing.

Lithium appears to stabilize and inhibit excessive neuronal activity, mainly through calcium-related signaling pathways (Alda, 2015). However, few data are available on specific regulation of functional networks by lithium (Malhi and Outhred, 2016).

### **Trans-nosographic and dimension-based pathophysiology**

As reported above, most of the literature investigating mood disorders pathophysiology focuses separately on depression and bipolar disorders. However, these clinical entities suffer from heterogeneity in symptoms affecting different patients, with variable clinical presentations among one disease that suggest various pathophysiological mechanisms. At the same time, distinct mood disorders share the same diagnostic criteria defining the symptoms of mood episodes. The dimensional approach in psychiatry aims at identifying subgroups of patients homogeneous on their clinical presentation and biological alterations in a trans-nosographic way, meaning without separating them according to their diagnosis (Insel and Cuthbert, 2015; Cuthbert, 2014). Behavioral and cognitive dysfunctions are proposed to fall into different “domains” or “dimensions” such as sensory processing, motivation, arousal, social interactions, emotions, cognition, *etc.*

Increasing number of studies attempt to study specific biological mechanisms underlying particular dimensions alterations in a trans-diagnostic manner, for instance regarding apathy and anhedonia (Sharma et al., 2017; Spano et al., 2019; Guessoum et al., 2020; Gracia-García et al., 2021). However, this field of research, relatively recent, remains modest compared to the extensive literature focusing on pathophysiological mechanisms underlying specific DSM-defined disorders (Fusar-Poli et al., 2019). One current limiting factor may be the lack of consensual definitions of the dimensions or “trans-diagnostic constructs” involved, and widely-used, unified and translational tools to measure them.

### ***1.1.5 From mood to emotions***

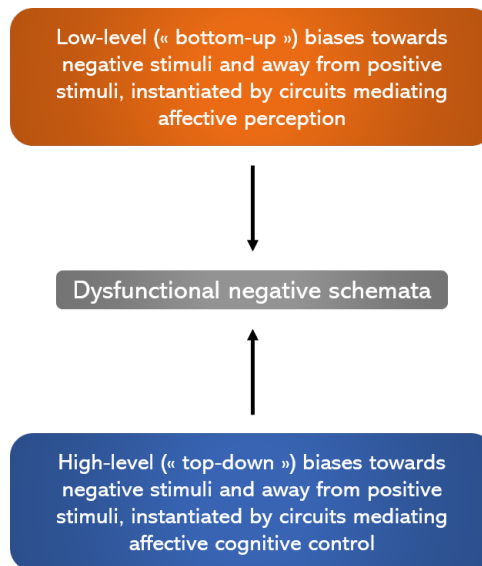
#### **The blurry boundaries of mood**

Mood is the main criterion for diagnosing mood episodes. Mood is defined as a temporary state of mind or feeling, a prevailing emotional tone or general attitude, according to the Collins dictionary. It has blurry time-boundaries. Such vague definition impedes possible exploration of its underlying neurobiology using functional imaging or preclinical tools.

In addition, current psychiatric classifications for mood disorders lead to significant heterogeneity among clinical presentations of patients yet sharing the same diagnosis (Olbert et al., 2014). In other words, patients with very different symptoms can be diagnosed with the same disease. Such clinical heterogeneity is another factor preventing efficient translational research on the pathophysiology of mood disorders.

#### **Emotional processing in mood disorders**

As mood is tightly linked to emotional responses, we think that a better understanding of mood disorders could arise from finer, more in-depth knowledge of emotional processes. From a clinical point of view, characterizing mood episodes with emotional responses is of clear interest for discriminating subgroups of patients pooled under the same diagnostic label by current classifications (Henry et al., 2007). It is well demonstrated that depressed patients attribute more negative valence to events or objects, this negative bias being an essential component of depressive episodes (Leppänen, 2006; Clark et al., 2009; Harmer et al., 2009; Roiser et al., 2012). For example, depressed patients display increased negative perception of ambiguous face emotions (Gur et al., 1992) and increased recall of negative *versus* positive self-referent words (Matt et al., 1992) compared to healthy controls. Large amount of data also supports the notion that depressed patients are hyposensitive to rewards and hypersensitive to punishments (for a review, see Robinson and Roiser, 2016). The cognitive theory of depression of Beck (1967) considered this negative bias tightly linked to dysfunctional pessimistic beliefs and expectations (“negative schemata”, Figure 9, Roiser et al., 2012) that colored the cognitive processing of external sensory stimuli and internal memories (“top-down” bias, Robinson and Roiser, 2016). However, more recent models include the possibility that such bias is “bottom-up”, meaning the perception of the inputs itself is negatively biased (Figure 9, Roiser et al., 2012; Robinson and Roiser, 2016). Roiser et al. (2012) closely relate their model with the monoamine hypothesis of

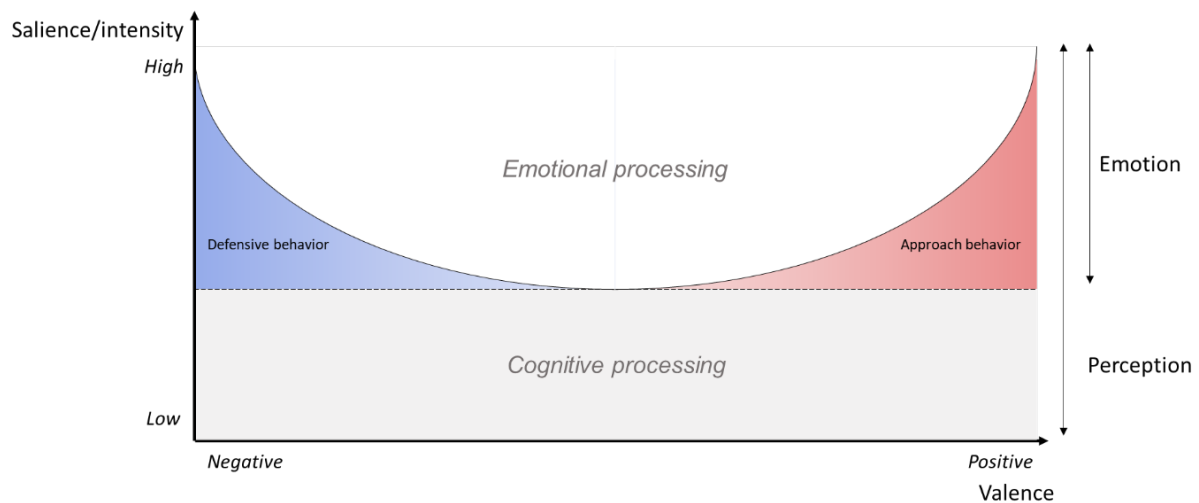


**Figure 9. The cognitive neuropsychological model of depression.** Adapted from Roiser et al. (2012). “Hot” cognition is represented in orange and “cold” cognition in blue.

depression, based on the fact that serotonergic antidepressants seem to act not on mood directly, but on the brain processing of affective stimuli (Harmer et al., 2009). Negative affective biases are suggested to play a causal role in the development, maintenance and treatment of depression (Roiser et al., 2012; Clark et al., 2009; Harmer et al., 2017).

Focusing on this “low-level affective processing”, we propose to reframe the current classification system based on mood onto an emotion-based model for mood disorders, extending the rationale to both depressive and manic states (see Bigot et al., 2020 and Henry et al., *accepted* in “Proposed hypothesis” section). Emotions are brief responses characterized by physiological arousal that are triggered by a stimulus to drive an adapted behavior (Schachter and Singer, 1962; Russell, 2003; Tye, 2018). They can be described by two quantifiable features: (i) the intensity of the response and (ii) the valence. Every mood state can be delineated according to the associated preferred emotional responses. Depressive states present decreased emotional intensity and negative valence bias, with loss of pleasantness (*i.e.* anhedonia). On the contrary, manic states display elevated emotional intensity associated with positive valence bias increasing the positive tone of emotions and so the rewarding properties of environmental stimuli, but also potentially decreasing the perception of danger. Moreover, this two-dimensional description of mood states allows a proper identification of mixed states. Indeed, increased intensity but with more or less positive or negative tone of the emotions determines whether the patient presents a depressive or a manic episode with mixed features.

## Sensory perception in mood disorders



**Figure 10. The link between sensory perception and emotion.** From Bigot et al. (2020). “When a perception is sufficiently salient, it triggers an emotion with a congruent valence (positive or negative). In turn, the emotion will trigger either a defensive (in blue) or an approach behavior (in pink). In animal models, it is possible to measure the valence attribution to various stimuli by measuring behavior and thus to infer the animal emotional state.”

We also think that these altered emotional responses can be reflected by modifications in the perception of sensory stimuli, following the same movement along the intensity and valence axes (Figure 10, Bigot et al., 2020). Thus, exploring sensory perception and more specifically valence assignment to environmental stimuli in patients affected by mood disorders can offer indirect insight onto the emotional biases they express (Figure 11, Parker, 2014; Parker et al., 2017).

Among all the sensory modalities explored with quantitative tools, several studies investigated the olfactory performances in unipolar depressed patients, with inconsistent results (for a review, see Naudin and Atanasova, 2014). Nevertheless, recent studies reported what they called “olfactory anhedonia” (*i.e.* pleasant odors are less pleasant) and “negative olfactory alliesthesia” (*i.e.* unpleasant odors are more unpleasant) (Naudin et al., 2012, 2014; Kohli et al., 2016; Kazour et al., 2020; Colle et al., 2020). Odor threshold detection impairments were also suggested, but with conflicting results (Naudin and Atanasova, 2014; Kohli et al., 2016; Kazour et al., 2020; Cole et al., 2013).

We reviewed olfactory exploration in bipolar patients (see below Henry et al., 2020). No detection or discrimination disruptions were identified, but a deficit in identification could be related to psychotic symptoms in BD patients, as it is described in schizophrenic patients (Moberg et al., 1999), or to a greater severity of the disease potentially indicating

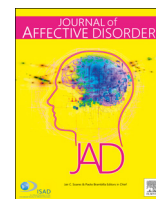


Sense	Hypomanic/Manic Episode	Depressive Episode
Smell	<p>"I'm more sensitive. Things smell better and worse, everything is a bit more intense."            "You can smell everything, you're alert, it's like, magical."            "I pay more attention to preferable smells, like flowers."            "A smell of a rose means more to me."</p>	<p>"Everything becomes dull."            "Things smell less vibrant. There is a lack of depth to whatever it is I'm smelling."            "Definitely repressed. I really have to center in order to smell something."            "All smells are less strong."            "I can smell things that other people can't smell."            "I can smell things that make me feel sick. Food smells."            "Everything is bland."</p>
Taste	<p>"Exaggerated, everything is more extreme. Spicy is spicier, sweet is sweeter."            "Things are more tasty, tastes are heightened. I taste more flavors in what I'm eating."            "Food tastes a lot better. I have gastrointestinal problems, but when I am high I eat more."            "Fruit tastes better, I'm more inclined to eat it."            "I will try more foods. I normally can't stand mushrooms but I enjoy them when I'm high."            "Everything is clearer. I don't need my glasses for reading."</p>	<p>"Things don't taste at all in some cases."            "I only eat not to die, but I can't taste anything."            "Reduced experience of tastes. I don't enjoy things as much as I normally would."            "I don't like anything. It all tastes rotten. Things just taste horrible."            "When I'm depressed, things aren't as bright, like there's a shade. Bright blues are dull."            "Sometimes I feel I don't see anything. I don't notice changes around me in my mind."            "I use my glasses a lot more, as things are out of focus."</p>
Vision	<p>"I might have just been imagining it, but I see vertical white lines."            "I feel like I can see auras or glows around people. I can see what they're thinking when they're saying something."            "I see everything. You see things more with your head. Seeing with your third eye."</p>	<p>"It becomes nothing. If I'm feeling depressed I can't feel a hand on my leg."            "My body goes numb, I lose all sensation, it's hard to discern things like temperature."            "Like my skin is crawling."            "I don't want to be touched. Being touched feels almost repulsive and I recoil from it."</p>
Touch	<p>"My skin feels more sensitive and more alive. Touch feels more real, especially humans or plants."            "Even my son tickling me is like someone stabbing me."            "Much more sensitive, sensual, sexual. Things are much more powerful. Heightened degree of sensuality."            "When I feel high, it's more a sense of being more sensitive to touch and your body feeling more connected to the universe."            "My sense of touch is the same but my interest in different textures is heightened."            "I'm much more tactile, I feel the need to touch things and have a tactile response, I especially notice it when shopping. I need to touch all the cushions."            "I'm a very kinesthetic person. I went into a lift with laser-etched surfaces, and it was the most wonderful thing."</p>	<p>"Minimal hearing. I don't notice the things I do when I'm up."            "I'm deaf as a head post."            "I'm more aware of things, especially yelling, chewing, etc."            "Unable to handle certain sounds, especially high-pitched, like a spoon against a bowl or the sound of people eating."</p>
Hearing	<p>"Everything is louder, more crisp."            "I zone in on something I wouldn't have otherwise noticed, like someone eating. It becomes a point of focus instead of being in the background."            "I guess that I hear things that other people struggle to hear or can't hear, like a beeping or telephone."            "I have tinnitus, but not so much when I'm high. This means I hear more."            "If listening to music, I can hear individual lines, not just the whole thing. Certain instruments stand out, my attention is caught."</p>	<p>"Minimal hearing. I don't notice the things I do when I'm up."            "I'm deaf as a head post."            "I'm more aware of things, especially yelling, chewing, etc."            "Unable to handle certain sounds, especially high-pitched, like a spoon against a bowl or the sound of people eating."</p>

**Figure 11. Altered sensory perception in bipolar disorders patients.** Adapted from Parker et al. (2017). Examples of participants qualitative responses describing sensory changes during hypomanic/manic and depressive episodes.

early signs of cognitive impairment. Concerning olfactory valence, one study reported decreased pleasant odors ratings (Kazour et al., 2020).

Therefore, the sensory perception modifications in mood disorders appears as an understudied field of research. The focus has been made instead on more cognitive "high-level biases", for instance with emotional face recognition (Leppänen, 2006). However, as a proper process occurring in these diseases, we think that investigating disturbed emotion-related perceptions could provide additional knowledge and understanding about mood disorders.



## Review article

## Can olfactory dysfunction be a marker of trait or states of bipolar disorders? A comprehensive review

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## ABSTRACT

**Background:** Olfactory deficits (OD) are reported as markers for a large spectrum of neuro-psychiatric disorders. Alterations can concern perception, identification, discrimination and assignment of odour's valence of olfaction process. We propose a comprehensive review to summarize which kind of OD were reported in bipolar disorders (BD) and in which phase of the disease, to know if they could be a marker of state or trait.

**Methods:** A Systematic Literature Review was conducted using PRISMA guidelines to include all studies assessing olfaction with objective measures in BD.

**Results:** 9 studies were identified. All of them have assessed odour identification and 3 reported deficits mainly in patients with psychotic features or elements of illness severity in comparison to healthy subjects. There is no difference in threshold of perception between BD patients and controls and it is not possible to conclude for discrimination because only one study has assessed this dimension in comparison to control. We cannot conclude for hedonic value of odours regarding these studies.

**Limitations:** These studies are very incomplete because only one has evaluated all the processes involved in olfaction process.

**Conclusions:** In light of this review, evidence is still missing to unveil potential disturbances of olfactory process as a marker of BD. These new avenues of research could help to clarify the links between OD and BD and provide information on the pathophysiology of the disorder according to the impaired dimension.

## 1. Introduction

Bipolar disorder (BD) is characterized by the recurrence of mood episodes, either depressive or manic alternating with periods of remission (American Psychiatric Association, 2013). Psychotic symptoms during acute phases lead to some confusion with schizophrenia diagnostic, while the recurrence of depressive episodes conducts to misdiagnosis with major depressive disorder (MDD). Unfortunately, we still do not have any biomarkers to help distinguish between all these pathologies.

Olfactory deficits are considered as markers for a large spectrum of neuro-psychiatric disorders. Actually, a number of studies report

olfactory dysfunctions in neurodegenerative pathologies such as Parkinson or Alzheimer's disease (Camargo et al., 2018; Jung et al., 2019; Sanjari Moghaddam et al., 2019). Concerning psychiatric conditions, schizophrenia is the most studied illness in the field and deficits in olfactory processes have been extensively reported (Chen et al., 2018; de Nijs et al., 2018; Kiparizoska and Ikuta, 2017; Urban-Kowalczyk et al., 2018; Zou et al., 2018). Studies about mood disorders are more inconsistent and it is not clear if olfactory dysfunctions are a marker of states or of traits in these disorders.

In humans, olfactory impairment has an impact on quality of life and is associated with frequent depressive symptoms (Gopinath et al., 2011; Katotomichelakis et al., 2014). Conversely, the attribution of the

*Abbreviations:* BD, bipolar disorder; CT, Controls; MDD, major depressive disorder; SZ, patients with schizophrenia; UPSIT, University of Pennsylvania Smell Identification Test

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positive hedonic values of pictures is improved in the presence of pleasant odours and decreased with unpleasant odours (Cook et al., 2015). In animals, an ablation of olfactory bulbs induces a depressive phenotype with a remodelling of limbic structures and in particular a reduction in the volume of the hippocampus (Morales-Medina et al., 2017; Yurttas et al., 2017). Chronic stress can induce depressive symptoms and alteration of olfaction which are associated with a reduction in neurogenesis and dendritic density in the hippocampus and the olfactory bulbs (Siopi et al., 2016). Reciprocally, the prolonged exposure of a rodent to a pleasant smell improves depressive behaviour induced by chronic stress (Xu et al., 2015). These elements emphasize the interest to explore the links between mood disorders and olfaction.

Olfactory sensory system performs a series of operations to process odour stimuli, including perception, discrimination and identification of odours as well as assignment of their hedonic valence. The level of perception is the threshold determined by the highest dilution of an odour that can be correctly identified. During test identification, the subject is required to choose from a list of proposals of several odours presented to her/him. The discrimination implies to identify which odour is different from a set of two identical odours. The assignment of hedonistic value consists in assessing the number of odours considered as pleasant or unpleasant in a set of smells.

The objective of this study is to provide a systematic review of studies evaluating olfactory functions in BD in order to have a comprehensive view of the four processes of smell (perception, discrimination, identification and valence) in the different phases of the disorder (remitted, depressive and manic phases). It can suggest whether certain olfactory alterations may be a marker of states or traits of BD. We will then discuss how these changes may differ from those found in unipolar disorders and schizophrenia and determine whether certain deficits are specific to each condition.

## 2. Methods

A Systematic Literature Review was conducted in April 2019. This systematic review was reported using PRISMA guidelines. To capture all the relevant studies, we searched the abstract, title, and keyword fields in the MEDLINE electronic database, to identify studies evaluating taste and olfaction in bipolar disorders. We used the following keywords for our first search: “olfaction” and (“bipolar disorder” or “psychosis”).

Searches were limited to English language and studies with human participants without any date restriction.

All studies describing research on olfactory function in BD were included. All studies describing research on BD patients (total or partial sample), and assessing any component of olfactory process were included. We kept only studies assessing olfaction with objective measures. We excluded studies evaluating olfaction in samples of patients with either affective or psychotic disorder, but not having any BD patients in the sample. Studies including first psychotic episodes without diagnostic details were excluded. Bibliography of reviews, meta-analysis and abstracts presented in congress not yet published and publications with less than five subjects were also excluded. Following removal of duplicates and citations from non-English, paper titles were scrutinized by MM and CH, those evidently outside the scope of the review were rejected (Fig. 1).

## 3. Results

We have identified 9 studies published as an original article assessing olfaction with objective measures and including BD patients. For each study, we have reported in Table 1: the number of BD patients and subjects in the comparative groups, relevant clinical characteristics, the mood status when subjects perform the olfactory test, the four processes of olfaction and the main results.

Four studies were performed during euthymic phases while mood

status was not reported for 4 studies. Only one study was conducted in depressed BD patients and none of them during manic episode. Two of the 9 studies included in the review do not compare BD with healthy control individuals (Krüger et al., 2006; Striebel et al., 1999). All studies have assessed the identification process (9/9) while the threshold of perception was reported in five studies (5/9), the assignment of valence in only three studies (3/9) and discrimination in only two studies (2/9) (Table 1).

Among the 7 studies comparing olfactory function in bipolar disorder with healthy controls, 4 studies report no deficit in odour identification. For the 3 studies reporting a deficit in BD patients, the study with the largest sample was limited to BD patients with a history of psychotic symptoms (Kamath et al., 2018). Lahera's study does not specify whether patients have a history of psychotic symptoms but the olfactory identification deficit is correlated with a deficit in verbal and non-verbal measures of social cognition. In the Cummings study, BD patients did not differ from patients with schizophrenia at the BPRS (Brief Psychiatric Rating Scale) score, indicating that they have the same level of psychotic symptoms.

No changes were found in the thresholds of odour perception with the exception of Krüger et al. (2006) which reports a better acuity in BD patients with a history of event-triggered episodes compared to those with spontaneous episodes. However, this result should be taken with caution since only seven patients were included in the first group and because there is no control group.

No differences were reported concerning discrimination of odours. Regarding valence, one study reported that euthymic BD patients rate odours as more pleasant compared to controls (Cumming et al., 2011), and another that BD patients with psychotic features rate pleasant odours as less pleasant in comparison to controls (Kamath et al., 2018).

Only one study assessed olfactory function during acute depressive episodes and did not report any differences.

## 4. Discussion

This review points out that only few studies assessed olfactory function in bipolar disorder. All studies assessed identification. Four of them found no alterations in odour identification. Only 3 of the studies reported deficits, mainly in patients with psychotic features or elements of illness severity such as cognitive impairment or high score on BPRS (Kamath et al., 2018; Striebel et al., 1999). It is not possible to conclude if assignment of valence could be considered as a state-marker BD since the thymic status of patients is not always reported.

Except for those by Kamath et al. (2018), studies have included generally a small number of patients. This study is also the only one that has assessed all the dimensions of olfaction, but unfortunately, the mood status is not documented. The University of Pennsylvania Smell Identification Test (UPSIT) is the most frequently used test and since it evaluates only identification, data is limited for the other types of odour processing (Cumming et al., 2011; Doty et al., 1984; Hardy et al., 2012; Hurwitz et al., 1988; Kamath et al., 2018; Lahera et al., 2016; McLean et al., 2004; Striebel et al., 1999). These results are consistent with the literature showing altered identification in patients with schizophrenia but not in patients with MDD (Amsterdam et al., 1987; Brewer et al., 2001; Coleman et al., 2002; Kopala et al., 1994; Lombion-Pouthier et al., 2006; Malaspina et al., 2002; Moberg et al., 1999; Pentzek et al., 2007). In BD patients, it should be clarified whether the identification deficits are related to a history of psychotic symptoms or to an overall higher severity of the disease or to a cognitive decline. Impaired identification in Parkinson's disease is correlated with mild cognitive impairment (Kotecha et al., 2018). In schizophrenia, patients with negative symptoms present more severe olfactory deficit and a study showed that an overall olfaction deficit is a predictor of mortality over 5-years (Pinto et al., 2014). The authors interpret these findings as follows: olfaction deficit is a biomarker of overall brain damage due to slow cellular regeneration or cumulative toxic exposure. In addition, we

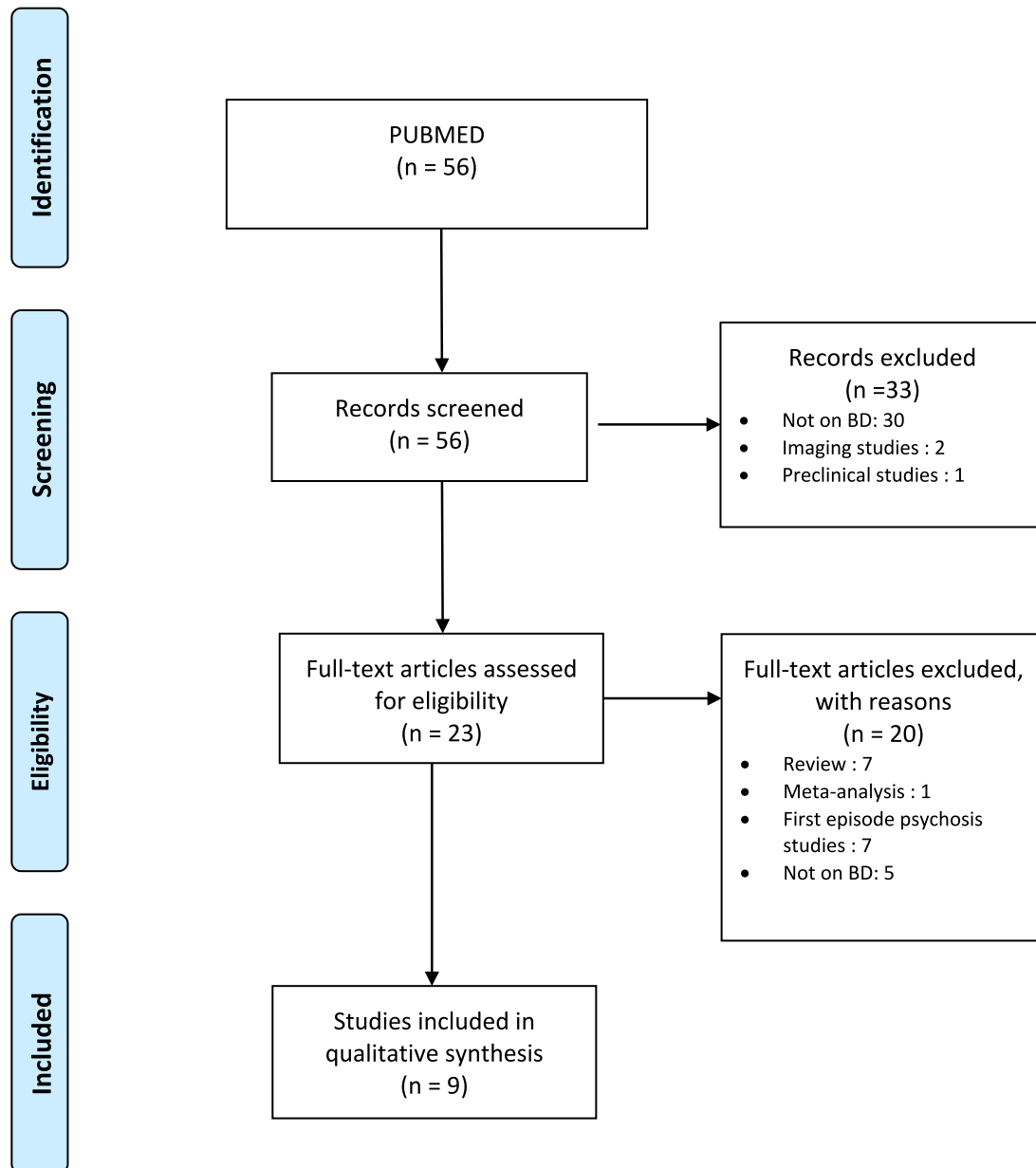


Fig. 1. Flowchart.

have previously demonstrated that plasticity and adult neurogenesis is determinant in olfaction process in mice (Gheusi and Lledo, 2014; Grelat et al., 2018). For BD, it would be interesting to know if deficits in odour identification could be a marker of neuro-progression of the disease and if changes in identification could represent early signs of cognitive impairment.

No difference in odour discrimination was found but only one study has compared BD patients and healthy subjects (Kamath et al., 2018). Further analysis, using odour mixtures is required to fully evaluate olfactory performance in BD patients.

With respect to the threshold of odour detection, studies found no difference between BD and control groups, except in the study by Krüger and colleagues which is not generalizable due to the small number of patients and because there is no control group. Conversely, in MDD patients, several studies have showed a reduced sensitivity to odours which seems to be associated with a reduced volume of the olfactory bulbs (Lombion-Pouthier et al., 2006; Negoias et al., 2010; Pause et al., 2001). Depressions during MDD are more homogeneous and are generally characterized by emotional hypo-reactivity meaning

that patients have an overall decrease in the intensity of experiences of emotions (Bylsma et al., 2008). It is possible that these blunt affects may extend to other stimuli, particularly to odours that are intimately linked to emotional states. However, bipolar depression is much more heterogeneous in terms of emotional reactivity with some forms of depression characterized by emotional hypo-reactivity and others by emotional hyper-reactivity during which patients describe feeling emotions with higher intensity than usual but with a majority of negative effects (Henry et al., 2010). It could be interesting to study if there is a link between the threshold of odour detection and the level of emotional reactivity. Emotion processing is one of the primary altered domains in mood disorders and encompasses the intensity of emotional responses as well as the attribution of valence. The tonality of emotional responses depends on the perception of our environment which is decoded by our senses, and the assignment of hedonic value to stimuli is a fundamental process that guides our emotional and behavioural responses. Perception biases are associated with mood states (M'bailara et al., 2009; Parker et al., 2017) but we cannot conclude on the hedonic value of odours regarding these studies. One study reported

**Table 1**  
Olfaction and bipolar disorders.

Authors	BD/ CT	Mood status of BD	Threshold	Discrimination	Identification	Valence
Cumming et al. (2011)	BD (n = 20)/ CT (n = 22)/SZ (n = 27)	Normothymic	No difference	No difference	Deficit (BD and SZ patients had no difference on the BPRS) No deficit Deficit (correlated to cognitive impairment) No deficit No deficit	BD rate odors as more pleasant
Hardy et al. (2012)	BD (n = 20)/ CT (n = 44)	Normothymic	No difference			
Lahera et al. (2013)	BD (n = 39)/ CT (n = 40)	Normothymic				
Hurwitz et al. (1988)	BD (N = 10 + 1 personality disorders)/ SZ (n = 18)/ CT (n = 10)	Unknown				
McLean et al. (2004)	Affective psychosis (n = 16 amongst them 12 BD)/ SZ (n = 22)/ CT (n = 21)	Unknown				
Kamath et al. (2018)	BD I (n = 43 among them 13 with psychotic features and 30 without)/ BD II (n = 48), MDD (n = 134), Anxiety disorders (n = 48)/ CT (n = 72)	Unknown	No difference	No difference	Deficit only in BD with psychotic features No deficit	Rate pleasant odour as less pleasant only in BD with psychotic features No difference
Swiecicki et al. (2009)	BD (n = 21)/ MDD (n = 20)/ CT (n = 30) NO HEALTHY CONTROL SUBJECTS	Depressed	No difference			
Striebel et al. (1999)	SZ resistant (n = 16)/Affective disorders with antipsychotic medication (n = 19 among them 18 BD and 1 MDD) / Affective disorders without antipsychotic medication (n = 20 among them 12 BD and 8 MDD) BD with (n = 7) and without (n = 9) history of event-triggered episodes	Unknown	No difference between groups		Deficit only in affective disorders with antipsychotic	
Krüger et al. (2006)		Normothymic	Lower in BD with an history of event-triggered episodes	No difference	No deficit in BD with an history of event-triggered episodes	

BD, patients with bipolar disorders; CT, healthy controls; SZ, patients with schizophrenia; MDD, patients with major depressive disorders  
The results are comparisons to the group of healthy control subjects for the first seven studies.

that euthymic patients perceive odours to be pleasant (Cumming et al., 2011) while another study found that BD patients with a history of psychotic symptoms found odours to be less pleasant (Kamath et al., 2018). Swiecicki's study found no difference in depressed patients with BD. However, several studies have reported that MDD patients perceived odours as less pleasant compared to control subjects during the acute phase and this feature disappears with episode recovery (Naudin et al., 2012). A better knowledge of mis-assignment of valence could give elements to a better understanding of pathophysiology and in particular the role of the amygdala. Indeed, a large amount of data in neuroscience have shown the key role of the different nuclei of amygdala in valence coding as a central trigger for valenced-behaviour (appetite or defensive) (Tye, 2018).

**5. Limitations**

Various elements can alter olfactory functions and then limit the interpretation of the results. All studies have excluded patients suffering from conditions that can compromised their sense of smell such as common cold, respiratory infections, chronic cocaine use or significant nasal injury. The main confounding factors concerning age, sex, smoking status were considered but the small number of patients in most studies does not allow to take into account those clinical characteristics.

**6. Conclusion**

In the current state of knowledge, it is not possible to answer clearly whether certain dimensions of olfaction can be markers of bipolar disorder because only few studies assessed olfactory function using objective tests. Moreover, these studies are very incomplete because only one has evaluated the four dimensions of olfaction.

In light of this review, evidence is still missing to unveil the importance of odour identification as a marker of the severity of the disease, the attribution of valences to odours according to the thymic state, as well as the acuity of perception as a function of the level of emotional reactivity. These new avenues of research could help to better clarify the links between olfactory deficits and BD and provide information on the pathophysiology of the disorder according to the impaired dimension.

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## 1.2 Animal models of mood disorders

In order to progress in the knowledge of the biological mechanisms underlying mood disorders and propose new therapeutic opportunities, we need valid animal models of the disease. Validity is usually assessed using three criteria: (i) construct validity means that triggering factors of the model should be identical to causal or risk factors of the disease, (ii) face validity stands for similar symptoms and biological patterns and (iii) predictive validity represents the fact that the model should respond to drugs used for treating the disease (McKinney and Bunney, 1969). Animal models of depression and of bipolar disorders, actually modelling mostly mania, are briefly presented in the following sections.

### 1.2.1 *Rodent models of depression*

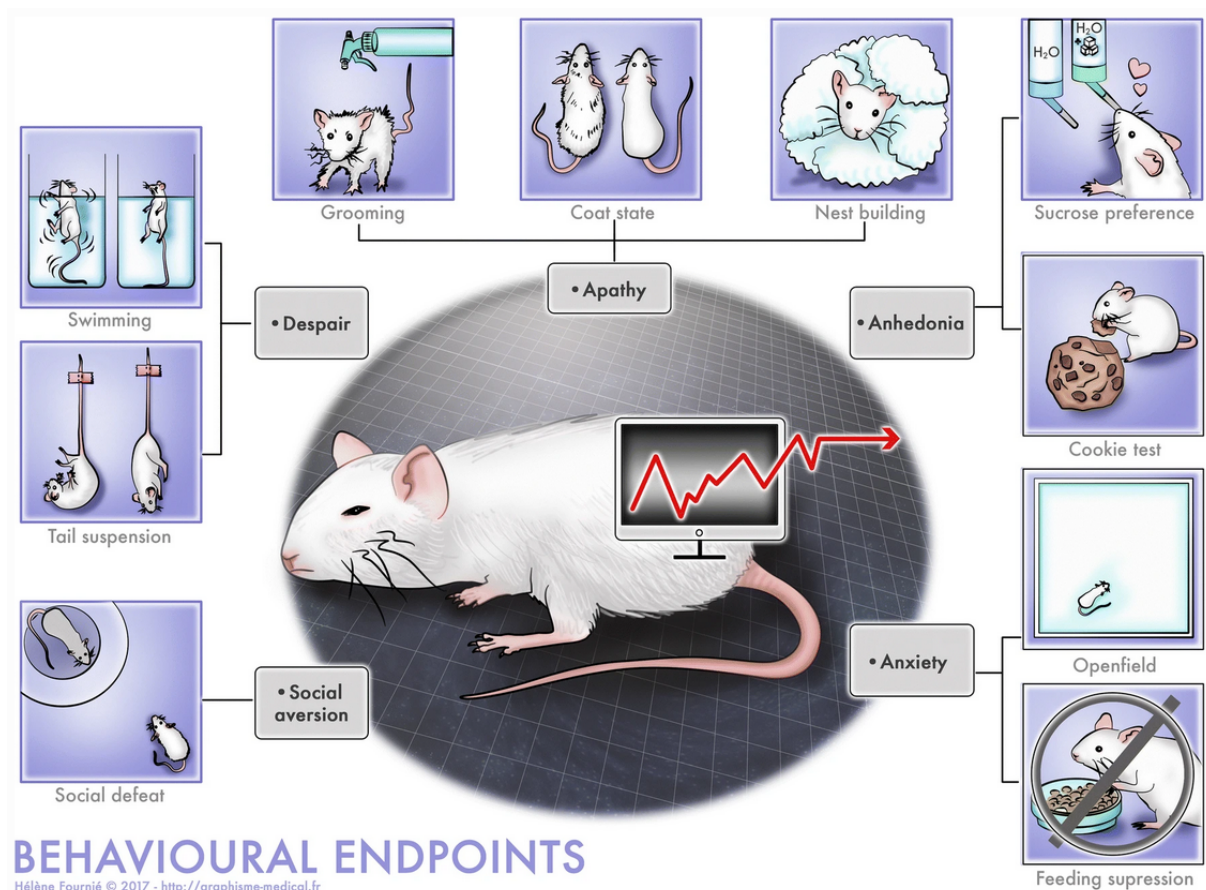
Rodent models for depression are mainly based on environmental, pharmacological and genetic manipulations. Other models include the bulbectomy and optogenetic or chemogenetic manipulations. To explore the face validity of these models, several behavioral approaches have been developed in rodents.

#### **Behavioral phenotyping**

Several symptoms of depressive state are translated into particular rodent phenotypes measured using behavioral tests. They include anhedonia, anxiety, social withdrawal but also sleep disturbance, changes in weight and appetite and psychomotor retardation that can be relatively easily assessed in animals (Figure 12, Planchez et al., 2019). Sadness or depressed mood is harder to translate. Indirect measures comprise slow gait (*i.e.* apathy, psychomotor retardation) and disengagement (*i.e.* despair behavior and social withdrawal), although not perfect. Obviously, others symptoms such as feelings of worthlessness, excessive guilt, recurrent thoughts of death, suicidal ideation or suicide attempts cannot be modeled in animals.

Anhedonia is mostly measured using the sucrose preference test, in which animals are given the choice between tap water and water containing sucrose or saccharose, and usually prefer the later. This preference is reduced in “anhedonic” animals. Other assays for anhedonia comprise sexual behaviors or intra-cranial self-stimulation using electrodes implanted in particular rewarding areas of the brain.





**Figure 12. Behavioral tests to measure anxiety- and depressive-like phenotypes in rodents.** From Planchez et al. (2019).

Most common tests for depressive-like behaviors actually measure despair-like behaviors, like the forced swim and the tail suspension tests. The animal is placed in an uncomfortable and inescapable situation (in water or suspended by its tail). At the start, active struggling behaviors can be observed. But at some point, with no escape possible, animals exhibit immobility bouts that rise with time. These protocols were labeled “depression tests” because single administration of antidepressant strikingly increased combativeness (Porsolt et al., 1977; Steru et al., 1985). As easily implemented, they became a gold standard for new potential antidepressants screening. However, as we discussed in the putative hypotheses for depression pathophysiology part, mechanisms underlying depression and antidepressant effects of drugs could undergo different pathways, without necessarily matching (see “Monoamines”). Consequently, the use of such tests has become controversial (Reardon, 2019).

Home cage locomotor activity can define psychomotor agitation or retardation, as well as locomotor activity in a novel environment like the open field.

Apathy, represented by a deficit in goal-directed behaviors, can be assessed through deficits

in nest building, self-grooming, maternal care, social interest and interest for novel objects (Cathomas et al., 2015). The coat state test, measuring the coat dirtiness in different parts of the body and the splash test, in which a sucrose solution is splashed over the back of the animal, normally inducing grooming behavior, can reveal a self-neglect-like phenotype. Various tests detect anxiety-like behaviors, sharing the same principle: placing the animal in an novel environment containing a more and a less stressful compartments, and assessing the exploration of both places (*e.g.* the open field, the elevated plus maze, the light and dark box tests). The novelty suppressed feeding test creates a conflict between the hunger of the food-deprived animal and its avoidance of the bright and opened space where a food pellet is located.

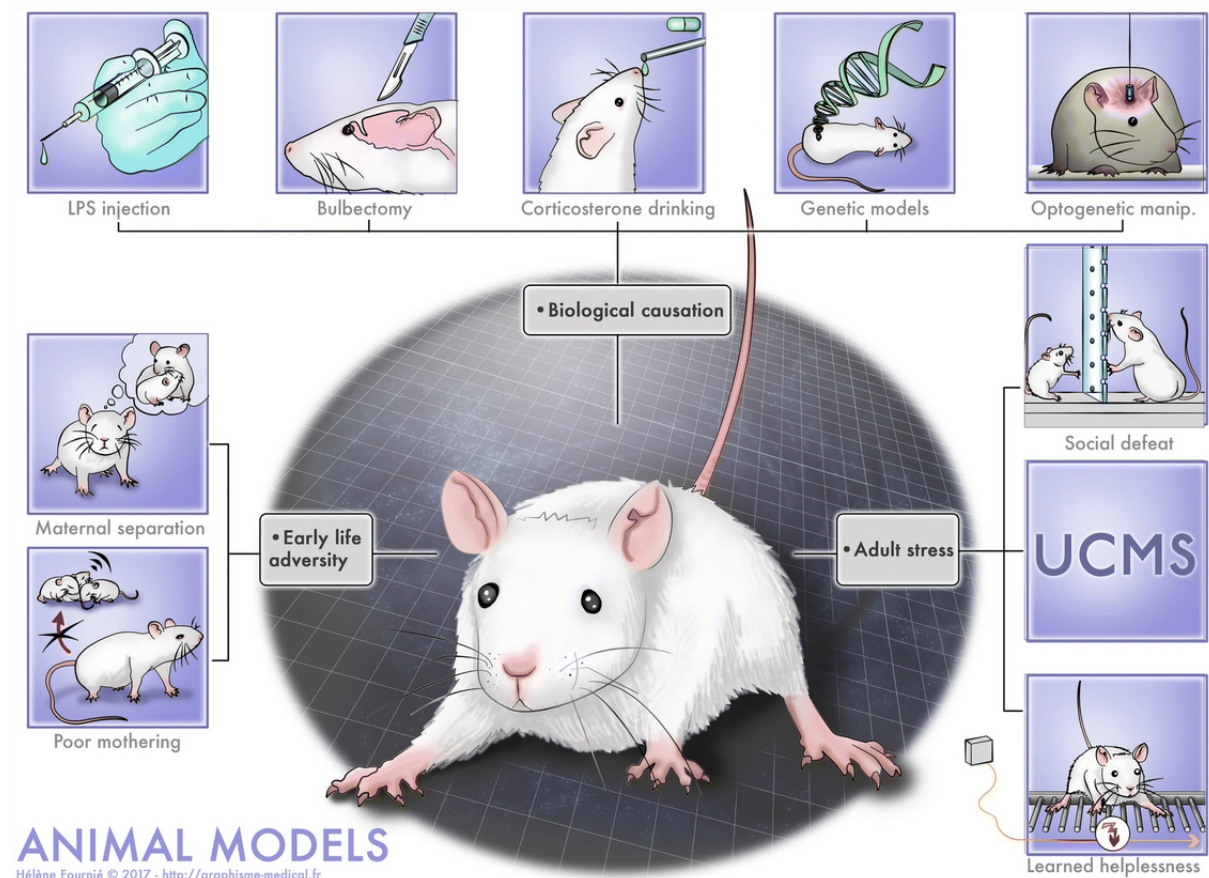
Cognitive impairment is another hallmark of depression, even if not specific. Novel object recognition and location (NOR/NOL) tests are widely used, based on the fact that rodents normally explore more unfamiliar stimuli. It exists a social equivalent of these tests, the social recognition test. The Morris water maze, in which rodents must find and remember the location of a platform hidden underneath the water, serves to check on spatial memory and learning. Finally, the fear conditioning, classically described as an emotional memory task, reveals deficits in associative learning. In this paradigm, a neutral stimulus which can be a sound or a specific environment is associated with an aversive stimulus, generally an electrical footshock. Following the conditioning, the sound or the environment alone triggers freezing fear response.

## **Classical models of depression**

**Environmental manipulation: the application of stressors** Early life adversity, through maternal separation for instance, is a powerful inducer of depressive-like and anxiety-like behaviors as well as deficits in learning and memory (Andersen, 2015). However, it does not consistently trigger anhedonic behaviors. Consistently with human findings, it decreases neurotrophins such as BDNF and increases corticosterone levels (Tractenberg et al., 2016). Other reported alterations comprise increased immediate early genes expression like cFos in the amygdala and paraventricular nucleus, suggesting hyperactivity of these areas. Similar findings were obtained with strains of rats that poorly lick/groom their offspring, or with maternal stress (Figure 13, Planchez et al., 2019). These paradigms parallel the effect of childhood abuse well-documented in depressed patients.

Stressors have also been applied during adulthood. An important feature is the uncon-

trollability and/or unpredictability of such stressors (Burger and Arkin, 1980). Learned helplessness, which consists in animals receiving inescapable foot shocks, and chronic social defeat, in which the test mouse is placed in the home cage of an aggressive resident mouse are two paradigms in which resilient and susceptible animals can be observed (Anisman and Merali, 2001; Kim et al., 2016b; Hammels et al., 2015). Susceptible animals later express anhedonia, despair-like behaviors and social withdrawal in case of social stress. Similar hypercortisolemia and decreased neutrophins have been demonstrated. Social defeat also produces prefrontal cortex dysregulation, in turn increasing amygdala activity (Hultman et al., 2016), and induces the release of pro-inflammatory cytokines (Reader et al., 2015). The chronic social defeat model responds to classical serotonergic antidepressant, as well as to acute ketamine administration (Planchez et al., 2019; Hammels et al., 2015).



**Figure 13. Animal models of depression.** From Planchez et al. (2019). “The models are mimicking different causes: early life adversity, biological causation, stress at adulthood.” LPS: lipopolysaccharide. UCMS: unpredictable chronic mild stress.

The unpredictable chronic mild stress protocol (UCMS) submits rodents to a wide variety of stressors on a chronic and unpredictable schedule, meaning different and randomly chosen stressors each day, at a different moment of the day, with a different duration. After several weeks, coat state deterioration, decreased grooming in the splash test and

anhedonia in the sucrose preference test are observed (Nollet et al., 2013; Willner, 2017). The phenotype is sensitive to antidepressants. This model is notably associated with dysregulation of the HPA axis, decreased hippocampal neurogenesis and BDNF levels among other plasticity markers and increased microglial activation (Hill et al., 2012; Planchez et al., 2019). Gut dysbiosis is another biomarker (Siopi et al., 2020).

One of the mild stressors used in the UCMS protocol is the restraint stress. Therefore, another model of depression involves to chronically restrain rodents for several hours a day, usually during one to three weeks (Wang et al., 2017). It leads to similar behavioral and biological alterations.

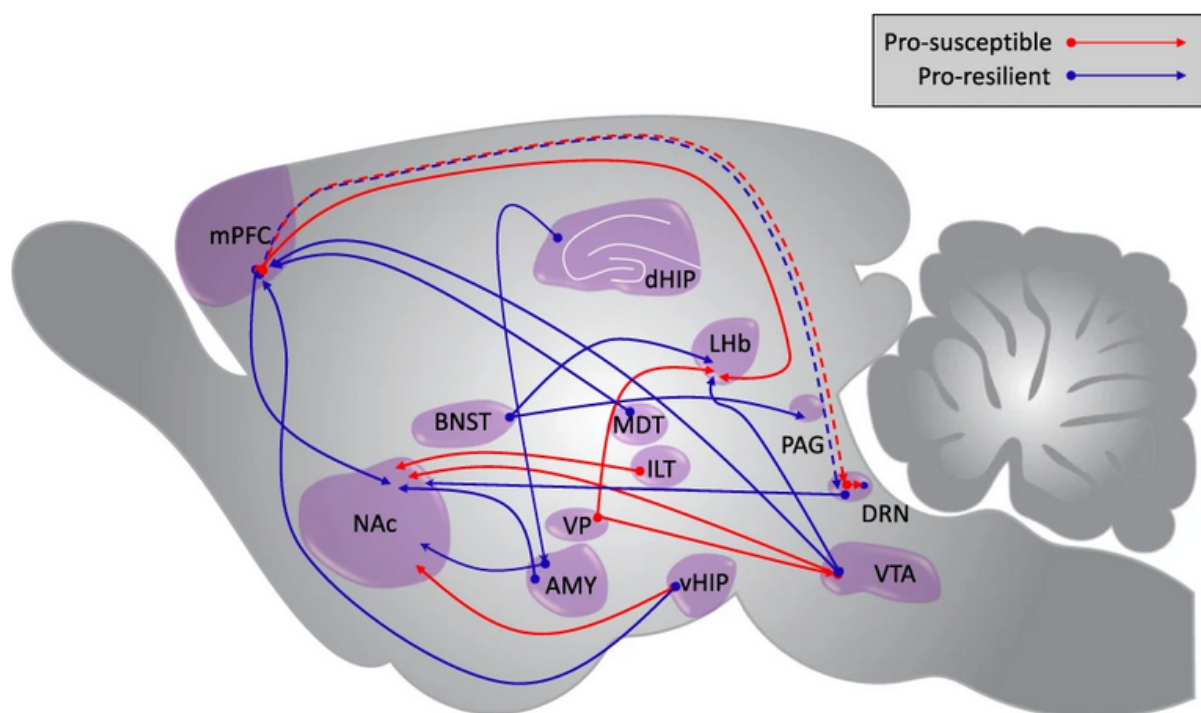
**Pharmacological drug administration** Chronic administration of corticosterone through drinking water or sub-cutaneous injections is thought to mimic chronic stress conducting to hypercortisolemia in depressed patients (Planchez et al., 2019; David et al., 2009). Chronic corticosterone elicits coat state deterioration, anxiety- and depressive-like behaviors in the open field, light and dark box and novelty suppressed feeding tests, splash test, tail suspension test and forced swim test. In addition, it dysregulates the HPA axis similarly than in depressed patients, and induces neuroinflammation, decreased hippocampal BDNF levels and changes in serotonergic neurotransmission.

A lipopolysaccharide (LPS) single injection mimicking bacterial endotoxin can be used to induce an inflammation-related model of depression (Planchez et al., 2019). Anhedonia and despair-like behaviors, along with increased brain pro-inflammatory cytokines and blood corticosterone, decreased BDNF levels in the hippocampus and changes in monoamine contents of corticolimbic structures are observed. Some of these effects are restored by antidepressant administration (Ohgi et al., 2013).

**Genetic models** As mentioned before, depression has an important genetic component, but with high polygenicity. Thus, genetic manipulation of one gene at a time in an animal can barely recapitulate the genetic ground of depression. Targets for genes mutations have been found in the monoamine system and HPA axis, but also in the endocannabinoid system or glutamatergic neurotransmission (Willner and Belzung, 2015). Genetic models also comprise bred lines selected according to particular features, such as increased emotionality and sensitivity to stress (Planchez et al., 2019).

**Bulbectomy** Bilateral surgical ablation of the olfactory bulbs in rodents creates, some weeks later, hyperactive behaviors when placed in a novel environment, transient cognitive deficits, disrupted sleep cycles, transient anhedonia and despair-like behaviors (Song and Leonard, 2005). These behavioral changes are thought to be linked with a major dysfunction of the cortical-hippocampal-amygdala circuit. Indeed, loss of olfaction alone, for instance peripherally induced, does not cause the same behavioral modifications. Inflammation and increased corticosterone levels have been reported, along with neurodegeneration of hippocampal neurons and disruption of several neurotransmitters transmission including serotonin, noradrenaline, glutamate, acetylcholine and Gamma amino butyric acid (GABA). Chronic antidepressant treatments alleviate the behavioral and most of the neurobiological alterations.

**Optogenetic and chemogenetic manipulations** In attempt to unveil the neuronal circuits involved in depressive-like behaviors in rodents, several activity manipulation



**Figure 14. Neuronal pathways implicated in depressive-like behavior by optogenetics or chemogenetics.** From Muir et al. (2019). Red arrows indicate that activation of this pathway leads to depressive-like behaviors, while blue arrows correspond to pro-resilient or antidepressant projections. Dashed lines represent conflicting findings. AMY: amygdala, BNST: bed nucleus of the stria terminalis, DRN: dorsal raphe nucleus, dHIP: dorsal hippocampus, ILT: intralaminar thalamus, MDT: medial dorsal thalamus, NAc: nucleus accumbens, PAG: periaqueductal gray, mPFC: medial prefrontal cortex, VP: ventral pallidum, VTA: ventral tegmental area (Walsh et al., 2014; Koo et al., 2016; Chaudhury et al., 2013; Friedman et al., 2014; Ramirez et al., 2013; Bagot et al., 2015; Vialou et al., 2014; Warden et al., 2012; Challis et al., 2013, 2014; Christoffel et al., 2015; Knowland et al., 2017; Johnson et al., 2016; Millan et al., 2017; You et al., 2016; Carreno et al., 2016).



through optogenetic and chemogenetic tools have been made. Optogenetics consists in expressing light-sensitive proteins in specific neurons of genetically engineered and freely moving rodents, timely activating or inhibiting those neurons (in the millisecond-scale) with the use of an optic fiber bringing light into the brain (Deisseroth, 2011). On the other side, designer receptors exclusively activated by designer drugs (DREADDs) enable to activate or inhibit specific neurons expressing modified G-protein-coupled receptors (GPCRs) upon the administration of specific drugs such as clozapine n-oxide (CNO) (Lee et al., 2014). Optogenetic and chemogenetic tools provide the opportunity to test causal relationships between circuits alterations and behavioral disturbances. The number of studies using such techniques for studying depressive-like behaviors increases exponentially (Figure 14, for reviews see Muir et al., 2019; Biselli et al., 2021; Fakhoury, 2021). They involve neuronal populations in the medial prefrontal cortex, the nucleus accumbens, the ventral tegmental area, the hippocampus, the raphe nuclei, the lateral habenula, the medial dorsal thalamus, the bed nuclei of the stria terminalis and the amygdala. Importantly, the effects of antidepressants are usually not assessed in these experiments.

### ***1.2.2 Rodent models of bipolar disorders are models of mania***

Animal models of bipolar disorders are almost exclusively models of mania because the cyclicity of the disease is barely reproducible in rodents.

#### **Behavioral phenotyping**

Behavioral phenotypes mimicking symptoms of mania encompass hyperactivity, decreased anxiety- and depressive-like behaviors, increased risk-taking behaviors and impulsivity, hyperhedonia, increased aggressivity and sexual activity and disrupted wake/sleep cycles, measured through various tests (Figure 15, Logan and McClung, 2016). Of note, hyperactivity is often considered as a gold standard parameter for describing mania models, paralleling one of the two main diagnosis criterion which is increased activity or energy (DSM-5, Cosgrove et al., 2016). It leaves mostly unsolved the question of the second criterion relative to mood modification, as in depression models.

#### **Classical models of mania**

As for depression models, rodent models of mania rely on pharmacological, genetic and environmental manipulations (Sharma et al., 2016). Psychostimulant like amphetamine

injection is the most commonly used model. Environmental models mainly correspond to sleep deprivation paradigm. Finally, genetic models have targeted the dopamine and the glutamate systems, intracellular signaling pathways as well as genes involved in the circadian rhythm. We reviewed the most common models of BD and provide after a summary table (see below Henry et al., 2018 and “Summary table of common mouse mania models”).

Behavioral endophenotype	Rodent paradigm
Reduced anxiety, increased novel exploration	Elevated plus maze; dark–light box; open field arena; social interaction test, novelty-suppressed feeding
Reduced depression	Forced swim test, tail suspension test, learned helplessness test
Impaired sensory processing	Paired-pulse inhibition (PPI)—sensorimotor gating*
Risk-taking behavior	Iowa Gambling Task (IGT)*
Impulsivity	IGT*
Impaired decision-making	IGT*
Psychostimulant-induced hyperactivity	Cocaine or amphetamine injection, repeated injections for locomotor sensitization
Hedonia	Sucrose preference; cocaine or amphetamine self-administration*; intracranial self-stimulation (ICSS) of medial forebrain bundle; two-bottle free-choice alcohol drinking; cocaine-conditioned place preference (CPP)
Hyperactivity	Open field arena; homecage monitors; wheel-running; behavioral pattern monitor (BPM)*
Increased sexual activity	Mounts, intermission, and ejaculation events
Increased goal-directed	BPM*
Repetitive movements	BPM*
Aggressive behavior	Resident–intruder test
Reduced or disrupted sleep	EEG; circadian activity; sleep–wake monitoring*
Disrupted circadian rhythms	Circadian wheel-running activity; temporal patterns of behavior

\* Demonstrated direct translational relevance to human bipolar disorder.

Figure 15. Modeling human bipolar mania in rodents. From Logan and McClung (2016).



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Communication

## Modèles animaux de troubles bipolaires : vers de nouvelles perspectives



*Animal models of bipolar disorders: Towards new perspectives*

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Relation dominant dominé

Trouble bipolaire

Trouble du sommeil

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### R É S U M É

De manière générale, il est difficile de modéliser les pathologies psychiatriques qui sont essentiellement humaines. Les troubles bipolaires rajoutent une difficulté supplémentaire du fait de la cyclicité des troubles. Comme nous le verrons, les modèles actuels de troubles bipolaires font référence à des modèles de manie. Les modèles classiques consistent à faire des manipulations pharmacologiques, comportementales ou par sélection de souche. Plus récemment, des manipulations génétiques ont permis d'étudier le rôle de gènes, potentiellement pertinents pour les troubles de l'humeur, dans la genèse de certains comportements. Cependant, tous ces modèles sont basés sur des phénotypes partiels et stables et ne permettent donc pas d'étudier les phénomènes de Switch de l'humeur. Une nouvelle technique, l'optogénétique, pourrait permettre de mieux explorer des phénotypes variables chez un même animal. En effet, cette technique permet d'activer ou d'inhiber de manière très spécifique des groupes de neurones, chez l'animal vivant et libre de mouvements, et donc d'étudier les modifications comportementales induites par ces manipulations. Nous présenterons quelques données récentes illustrant l'intérêt de cette technique pour l'étude des troubles de l'humeur.

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### A B S T R A C T

Animal models of psychiatric disorder are unlikely to be possible either conceptually or practically. Specifically for Bipolar Disorders (BD), the cyclic nature of the disease, characterized by a recurrence of manic and depressive states, creates an additional level of complexity. Moreover, animal models should ideally have construct (share the mechanism of the disease), face (mimic the symptoms) and predictive validity (share the same treatment response). To date, no such animal model for BD exists and current models mimic only mania. In this review, we first describe classical animal models of BD based on pharmacological, behavioral or strain selection manipulations and, more recently, on genetic modifications. However, all these models are based on a partial and stable phenotype of one polarity and do not allow the assessment of the neurobiology of the switch process. In this context, we discuss the interest of optogenetics, a technique that allows optical manipulation of a specific neuronal type and/or neuronal circuit in freely-moving animals, as a new tool to explore mood switches. We review recent studies using this relatively novel technique to identify potential brain circuits involved in mood swings. Recent work has shown that activation or inhibition of neurons projecting from the basolateral nucleus of the amygdala (BLA) to the ventral hippocampus induces an increase or respective decrease in anxiety and depression-like states. Moreover, it has been shown that two genetically distinct types of neurons in

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the BLA play a pivotal antagonistic role in valence attribution of positive and negative stimuli. These results give a glimpse into the interest to explore behavioral changes induced by activation or inhibition of specific neural circuits to better understand complex diseases. This approach is perfectly in line with the research advocated by the Rdoc program by working on preclinical models of isolated behaviors and domains involved in psychiatric disorders to go toward a nosology supported by physiopathology.

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## 1. Introduction

Les troubles bipolaires se caractérisent par la récurrence d'épisodes maniaques et dépressifs séparés par des périodes de normothymie de durée variable pendant lesquelles le retour à un état fonctionnel correct n'est pas forcément la règle [7]. Malgré des traitements efficaces, les rechutes sont fréquentes, le taux de suicide reste élevé, certains patients ne répondent que très partiellement aux traitements et présentent une évolution péjorative de leur maladie avec un retentissement majeur sur leur fonctionnement [17]. Il est donc nécessaire de développer de nouveaux traitements basés sur une meilleure connaissance de la physiopathologie.

Les modèles animaux sont un outil particulièrement pertinent mais complexe en psychiatrie. Il est en effet difficile de modéliser les pathologies mentales, essentiellement humaines, et certains symptômes touchant notamment le contenu de la pensée ne peuvent être étudiés chez l'animal. À ce jour, il existe des modèles animaux de manie et de dépression mais aucun ne combine les deux phénotypes chez un même animal. En effet, la nature dynamique et cyclique de la bipolarité représente une difficulté supplémentaire dans la modélisation de ces troubles. Pourtant, un modèle présentant les phénotypes des deux polarités permettrait de mieux comprendre les mécanismes de *switch* d'un état à l'autre, phénomène crucial de la physiopathologie des troubles bipolaires. À noter qu'à l'heure actuelle, lorsque l'on fait référence à des modèles de troubles bipolaires, il s'agit essentiellement de modèles de manie.

En principe, tout modèle animal reflétant une maladie devrait avoir des facteurs causaux identiques à ceux de la maladie (validité de construction), une symptomatologie superposable (validité apparente) et des réponses similaires aux traitements (validité prédictive). Une autre source de difficultés est donc notre ignorance des déterminismes précis des troubles bipolaires en dehors de l'interaction générale gènes/environnement. Ainsi, il n'existe aucun modèle remplissant tous les critères. Cependant, les modèles animaux restent indispensables pour mieux comprendre les mécanismes biologiques sous-tendant les comportements dans l'espoir de développer des traitements mieux ciblés pour les maladies mentales.

Après un bref rappel des modèles les plus classiques, cette revue a pour but de proposer l'optogénétique comme un outil pour créer de nouveaux modèles animaux des troubles bipolaires. Nous montrerons l'intérêt de cette technique dans la compréhension des circuits impliqués dans la régulation des émotions, et les perspectives qu'elle ouvre pour l'étude des phénomènes de *switch* d'un état émotionnel à un autre.

## 2. Modèles par manipulations pharmacologiques, comportementales ou par sélection de souche

Les modèles animaux classiques ont jusqu'ici soumis les animaux à des manipulations pharmacologiques ou environnementales qui déclenchent des symptômes proches de ceux que l'on veut étudier chez l'homme. Il est également possible de

sélectionner certaines souches ayant de façon innée des caractéristiques comportementales proches du phénotype recherché.

### 2.1. Manipulations pharmacologiques

L'injection de psychostimulants est le moyen le plus utilisé pour créer un état dit maniaque chez l'animal. En effet, des injections répétées de cocaïne ou d'amphétamine entraînent une hyperactivité locomotrice, une augmentation de l'agressivité, un déficit de l'inhibition de pré-impulsion [11]. L'administration de lithium, de valproate ou de lamotrigine réduit la plupart de ces effets [29].

Un des intérêts de ce modèle, largement sous-estimé, est l'inversion du phénotype vers un profil dépressif après arrêt des psychostimulants [1]. En effet, les états d'exaltation sont la plupart du temps suivis d'un état anhédonique et apathique chez les patients, souvent négligé car mis sur le compte des traitements par neuroleptiques. Pourtant, ces *switch* ont été décrits avant l'usage de psychotropes et sont observables chez les patients ayant un trouble bipolaire de type II dont les hypomanies ne sont pas traitées.

### 2.2. Manipulations comportementales

#### 2.2.1. Privation de sommeil

La privation de sommeil induit un état maniaque chez environ 30 % des patients bipolaires et potentialise les effets des traitements antidépresseurs. Chez les rongeurs, il est possible d'induire une privation de sommeil en les plaçant sur une petite plate-forme entourée d'eau d'où ils tombent dès qu'ils s'endorment. Pendant une courte période, les rongeurs soumis à cette privation de sommeil présentent une hyperactivité motrice, un comportement agressif ainsi qu'une hypersexualité [16], et cela indépendamment de l'état de stress que génère cette manipulation [2].

#### 2.2.2. Manipulation de la durée de la photo-période

Certains patients présentent des troubles à caractère saisonnier, généralement avec des épisodes dépressifs en hiver et d'exaltation au printemps. Une hypothèse explicative de ces variations saisonnières est la modification de la photopériode au cours de l'année. Pour étudier ce phénomène, il est possible de faire varier la durée d'exposition à la lumière des animaux. Ainsi, une augmentation de la période active (chez le rongeur, période nocturne) entraîne une réduction des comportements dits anxieux et dépressifs [10]. Ces modifications comportementales par modification de la durée de la période d'activité seraient liées au système dopaminergique.

### 2.3. Modèle dominant/soumis

La dominance se définit par la position sociale qu'a un individu au sein d'un groupe. Chez les animaux, il existe un continuum entre des profils dominants et soumis avec une majorité d'animaux au phénotype intermédiaire variant en fonction de leur environnement. La dominance aurait pour rôle le maintien de l'homéostasie du groupe. Il existe des similarités phénotypiques entre les

animaux soumis et les animaux présentant des symptômes dits dépressifs, à savoir un comportement défensif, une perte de poids, une altération du sommeil et une baisse de l'activité [24], et inversement pour ceux ayant un profil dominant plus proche d'un phénotype maniaque. Ces profils peuvent être étudiés en observant le comportement spontané des animaux évoluant en groupe ou bien forcés par des situations particulières. À titre d'exemple, si deux rats sont placés dans deux cages séparées par un couloir étroit où se trouve de la nourriture, les animaux ne pouvant se trouver en même temps dans le couloir, le rat le plus combatif aura accès à la nourriture et un rapport de dominance stable s'établira au bout de quelques jours. Utilisant ce modèle, Malatynska et Knapp [24] ont montré que le phénotype dominant était atténué par les traitements anti-maniaques tandis que le phénotype soumis était modifié par les antidépresseurs.

#### 2.4. Modèles par sélection de souche

Certaines souches de souris sont sélectionnées car elles présentent de façon innée un phénotype particulier. Ainsi, les souris Black Swiss présentent une appétence élevée pour le sucrose, développent une plus grande sensibilisation à l'amphétamine et sont agressives. Certains de ces comportements sont diminués par le lithium et le valproate. Une autre souche de souris, les souris Madison, présente une réduction de l'anxiété, une augmentation des comportements sexuels et une hyperactivité spontanée.

Ces modèles ont permis de confirmer les liens entre notamment certains traitements pharmacologiques ou des troubles du sommeil et les troubles de l'humeur et d'en étudier les mécanismes. Cependant, ils représentent des modèles très imparfaits de troubles bipolaires en ne se limitant qu'à un seul pôle.

### 3. Modèle par manipulation génétique

L'héritabilité des troubles bipolaires est estimée entre 60 et 80 % et repose à la fois sur des gènes spécifiques de la maladie et sur des gènes communs à d'autres pathologies psychiatriques [22]. Les modèles animaux par manipulation génétique permettent de répondre à plusieurs questions : quel est le rôle fonctionnel de variants génétiques associés à la maladie ? Expliquent-ils les anomalies du comportement ? Et quel est le mécanisme précis induisant ces modifications comportementales [5] ?

Ces modèles consistent à supprimer ou à augmenter l'expression d'un gène et à étudier les conséquences sur des comportements particuliers [5]. Le choix des gènes candidats repose sur la connaissance de leur implication dans des systèmes régulateurs connus pour être altérés dans le trouble, ou bien sur leur identification grâce à des études GWAS. Nous illustrerons l'intérêt de cette démarche par la présentation d'un modèle nous paraissant particulièrement pertinent, et nous renvoyons le lecteur à des revues récentes pour une liste exhaustive des modèles existants [4,5,19,23].

L'altération des rythmes circadiens et du cycle veille/sommeil fait partie des symptômes des épisodes thymiques, aussi bien dépressifs que maniaques, et une perturbation de ces rythmes chez les sujets vulnérables peut induire une rechute ou améliorer ponctuellement une symptomatologie dépressive. De plus, des psychothérapies focalisées sur une régulation des rythmes ont montré leur efficacité pour diminuer le taux de rechutes chez les patients bipolaires [14]. Plusieurs études ont montré une association entre troubles bipolaires et le gène CLOCK (Circadian Locomotor Output Cycles Kaput) [3]. Ce faisceau d'arguments en faveur d'une altération des rythmes circadiens dans le trouble bipolaire a conduit à manipuler des gènes liés à la régulation des rythmes circadiens pour induire un modèle de troubles bipolaires.

L'un des phénotypes maniaques le plus convaincant est induit par une délétion de l'exon 19 dans le gène CLOCK, ce qui empêche la transcription du gène [25]. Les souris Clock delta19 présentent une hyperactivité motrice et une augmentation des comportements exploratoires, une diminution du temps de sommeil, ainsi qu'une réduction des comportements dits anxieux et dépressifs [12,25]. Elles sont davantage sensibles à la récompense, étudiée par administration de cocaïne, consommation de sucrose et auto-stimulation. Enfin, l'administration chronique de lithium restaure une partie de ces modifications comportementales [28]. Au niveau cérébral, la mutation du gène entraîne une augmentation de l'activité des neurones dopaminergiques de l'aire tegmentale ventrale (VTA) qui pourrait rendre compte des symptômes dits maniaques [28].

Aussi sophistiqués soient-ils, ces modèles ne reproduisent que partiellement les symptômes maniaques et les phénotypes créés sont stables, et les deux pôles de l'humeur caractéristiques de la bipolarité ne peuvent être modélisés chez un même animal. Cela limite la validité apparente, et par conséquent l'apport de ces modèles dans la compréhension de la maladie et la recherche de nouvelles cibles thérapeutiques. Il est donc nécessaire d'utiliser de nouveaux outils permettant d'induire des phénotypes opposés chez un même animal.

### 4. Modèle de modulation des circuits neuronaux par l'optogénétique

En 2010 à Stanford, dans le laboratoire de Deisseroth, a été créée une nouvelle technique, l'optogénétique, qui permet l'étude des circuits cérébraux sous-tendant les comportements et des mécanismes cellulaires qui participent à leur régulation [15].

La technique de l'optogénétique repose sur l'insertion, grâce à un vecteur viral infectant les cellules de la zone où il est injecté, un gène codant pour une protéine photo-sensible, l'opsine. Le gène s'intègre dans le génome de la cellule hôte et produit des protéines qui pourront être activées grâce à la lumière apportée par une fibre optique implantée dans le cerveau. Le gène opsine peut être couplé à un promoteur pour transfecter spécifiquement certaines sous-populations de neurones (par exemple, les neurones glutamatergiques).

Les deux principales opsines sont la channel-rhodopsine 2 (ChR2), un canal sodique qui s'ouvre suite à la stimulation par de la lumière bleue, entraînant des potentiels d'action et donc une activation des neurones, et l'halarhodopsine (NpHR), canal chlore sensible à la lumière jaune, inhibant les potentiels d'action [9]. L'optogénétique permet ainsi de stimuler ou d'inhiber des circuits neuronaux précis et d'observer avec une très bonne résolution temporelle les modifications comportementales induites chez l'animal vivant et libre de ses mouvements [8]. De manière intéressante, l'animal étudié est son propre témoin, contrairement aux techniques de modifications génétiques de perte ou de gain de fonctions.

La conservation des circuits neuronaux sous-tendant les comportements communs entre l'homme et l'animal rend pertinentes les études chez l'animal, et complémentaires des études d'imagerie chez l'homme qui ne peuvent prétendre ni à cette résolution spatio-temporelle ni à la démonstration de liens de causalité. L'imagerie cérébrale chez les patients bipolaires a montré des modifications structurales, fonctionnelles et de connectivité qui concernent l'amygdale, l'hippocampe et le cortex préfrontal [18,26], certaines de ces anomalies étant prévenues ou restaurées par le lithium, traitement de référence des troubles bipolaires. Ces structures, et plus spécifiquement l'amygdale, ont un rôle majeur dans la régulation des émotions et de la motivation.

Il existe encore relativement peu d'études utilisant l'optogénétique pour explorer le rôle de l'amygdale dans les processus de switch. Jusqu'à présent, l'amygdale était surtout étudiée comme un élément clé générateur d'anxiété [21]. Ainsi, chez l'animal, de nombreuses études ont montré que l'hyperexcitabilité de l'amygdale et plus spécifiquement du noyau basolatéral (BLA) était fortement associée à des comportements dits anxieux, une hypervigilance et une incapacité à réguler les émotions [27]. L'amygdale est sous le contrôle inhibiteur de neurones GABAergiques venant de différentes structures corticales et sous-corticales et des perturbations de ce système inhibiteur entraînent une augmentation de l'anxiété [27].

Grâce notamment à l'optogénétique, des travaux très récents montrent que le BLA a un rôle central dans l'attribution de la valence des stimuli aussi bien positifs que négatifs et est associé à des phénotypes anxieux ou à l'apparition de comportements avec prise de risque. Ainsi, Felix-Ortiz et al. [13] ont montré que la stimulation optogénétique des neurones glutamatergiques projetant de la BLA vers l'hippocampe ventral engendrait un phénotype dit anxieux tandis que l'inhibition de ces mêmes neurones diminuait l'anxiété. En utilisant cette même technique, Stuber et al. [30] ont observé que la stimulation de neurones glutamatergiques projetant de la BLA vers le noyau accumbens (N Ac) entraînait des phénomènes d'auto-stimulation chez ces animaux, démontrant le rôle de ces neurones dans les phénomènes de récompense.

La BLA semble donc être une structure pivot dans la genèse d'un état émotionnel non seulement sur un versant anxio-dépressif mais également sur une polarité inverse. Dans une étude récente, Kim et al. [20] sont allés plus loin en montrant que les stimuli négatifs (choc électrique, odeur et goût aversifs) et positifs (présence d'une femelle, odeur et goût attractifs) activaient des sous-populations de neurones spécifiques au sein de la BLA, sur un axe antéropostérieur (antérieur pour les stimuli négatifs et postérieur pour les stimuli positifs). Ils ont également établi que ces neurones étaient déterminants pour attribuer une valence à un stimulus sensoriel et générer le comportement adéquat en réponse.

De plus, phénomène important, l'activation d'une de ces deux sous-populations de neurones antagonise l'autre. L'équilibre entre l'excitation et l'inhibition de ces deux populations de neurones est donc crucial dans la perception des stimuli et l'attribution de leur caractère hédonique ou aversif. Est-ce suffisant pour induire un état émotionnel vers un pôle « dépressif » ou « maniaque » ? Ou bien est-ce qu'un état émotionnel « dépressif » ou « maniaque » modifie l'équilibre de ce système et biaise la perception globale du monde environnant ? De plus, comme la BLA est directement reliée au système motivationnel via le N Ac, quelle est sa fonction pour augmenter ou diminuer la motivation, dimension essentielle à prendre en compte dans les troubles de l'humeur de l'animal en fonction de son état émotionnel ?

Ces résultats très récents obtenus grâce à l'optogénétique soulignent son intérêt pour étudier les oscillations entre des phénotypes « dépressifs » et « maniaques ». L'optogénétique permet ainsi de générer des phénotypes pouvant varier de façon dynamique d'un pôle à l'autre chez un même animal, offrant de grands espoirs dans la compréhension des mécanismes de switch. Les études parues jusqu'à présent utilisent des protocoles avec des stimulations ou inhibitions optogénétiques brèves, mais il est possible d'imaginer des modèles créés avec des manipulations optogénétiques plus chroniques.

Ce type de recherche est en parfaite adéquation avec les recherches prônées par le programme Rdoc. Ce programme stipule l'intérêt d'une approche dimensionnelle basée sur l'étude des systèmes sous-tendant les émotions, les cognitions, la motivation ou le comportement social en comprenant entre autres les mécanismes de régulation des valences et de l'attention/l'éveil [6].

## 5. Conclusion

Les modèles animaux dans leur ensemble sont intrinsèquement limités pour rendre compte de la complexité des pathologies mentales humaines. La dynamique temporelle caractéristique des troubles bipolaires est difficilement modélisable chez l'animal. L'optogénétique est une solution séduisante, puisqu'elle permet d'induire des phénotypes avec des caractéristiques opposées au cours du temps, chez un même animal, et donc de se rapprocher des fluctuations observées dans les troubles bipolaires.

On peut espérer que de telles manipulations de neuro-circuits ciblées, induisant des comportements mimant les principaux symptômes des troubles bipolaires, pourront permettre une meilleure compréhension de la physiopathologie de ces troubles. Toutefois, l'étude des circuits neuronaux par l'optogénétique ne sera pas suffisante, et il faudra combiner diverses approches pour évaluer l'impact de l'environnement ou de facteurs génétiques sur la physiologie et la communication de ces circuits afin de déterminer des facteurs étiologiques précis [15]. Bien qu'assez conservés d'une espèce à l'autre, les circuits neuronaux et les gènes impliqués dans la genèse des comportements ne sont pas forcément superposables chez l'animal et chez l'homme. Ces études pourront cependant stimuler la recherche translationnelle et aider à l'identification de nouvelles cibles thérapeutiques.

## Déclaration de liens d'intérêts

Les auteurs n'ont pas précisé leurs éventuels liens d'intérêts.

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Manipulation	Phenotype	Face validity	Predictive validity	Construct validity	Limitations
Psychostimulant administration	Hyperactivity Jumping Fighting Stereotypies	++	++	+++	Does not simulate hypomanic states Tolerance to psychostimulants Based on the assumption that only selective neurotransmitters(s) are involved in manic illness Nonspecific to mania
Sleep deprivation	Hyperactivity Insomnia Aggressive behavior Hypersexuality Stereotypies Cognitive deficits Circadian rhythm disruption	++	++	++	Significant stressor Nonspecific to mania
Dominant-submissive behavior paradigm	Hyperactivity Aggressive behavior	+	+		Laborious and time consuming Risk of injury to intruder animals Nonspecific to mania
Dopamine transporter (DAT) Knock-down	Hyperactivity Increased goal-directed behavior Repetitive locomotor patterns Impaired decision-making	++	++	+	Nonspecific to mania Limited data on construct validity
Circadian locomotor output cycle kaput (CLOCK) Mutant	Hyperactivity Risk-taking behaviors Increased impulsivity Impaired decision-making Disrupted circadian rhythm and sleep Low anxiety-like Low depression-like Rapid mood cycling Reward-seeking	++	++	++	Limited data on predictive validity Nonspecific for mania
Glutamate receptor 6 (GluR6) Knock-out	Hyperactivity Risk-taking behaviors Aggressive behavior Low anxiety-like	++	+	+	Limited data on predictive and construct validities
Black Swiss	Hyperactivity Hypersensitivity to amphetamine-induced hyperlocomotion Reward-seeking Risk-taking behaviors Low anxiety-like Low depression-like	++	++	+	Limited construct validity

**Summary table of common mouse mania models.** Adapted from Sharma et al. (2016); Logan and McClung (2016); Cosgrove et al. (2016).

### ***1.2.3 Towards new behavioral paradigms for animal models of mood disorders?***

#### **Classical rodent tests for depression are questioned**

Classical tests for depressive-like behaviors like the forced swim and the tail suspension tests are widely used. An analysis led in 2015 revealed that everyday on average was published one or more paper using the forced swim test (Molendijk and de Kloet, 2015). Yet, they are challenged (Reardon, 2019). Several reasons account for this debate. First, they are often used to measure depressive-like behaviors while they were not initially designed for that purpose, but instead to screen putative antidepressant drugs. As we mentioned earlier, mechanisms underlying the development of depression and the antidepressant effects could be different. Second, the effect of serotonergic drugs administration is immediate on rodents in these tests, whereas it takes several weeks to alleviate symptoms in depressed patients. Third, antidepressants with non-monoaminergic mechanisms of action do not always show their efficiency in these tests. Finally, they raise ethical questions about the intense stress inflicted to animals for data whose quality is questioned. Because of these issues, major drug companies such as Roche, Janssen and AbbVie recently dropped the forced swim test procedure. Several mental-health researchers advocate for more sophisticated tests.

#### **The affective bias test alternative**

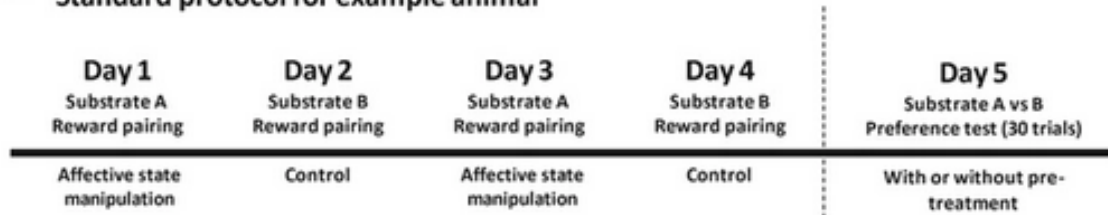
Following the cognitive theory of depression (Beck, 1967), Stuart et al. (2013) proposed an affective bias test in rodents. Affective biases in humans are defined by the way emotional states can influence higher cognitive processes, including attention, emotional processing, learning, memory and decision-making. Their rodent task aims at linking the effect of different psychotropic drugs, known to modulate mood in humans, with changes in associative learning performances in animals. During the affective bias test, animals learn to associate specific neutral cues with a reward. Two cues are learned either under control conditions or upon psychotropic administration attempting to change the emotional state during the learning. Then, the testing day measures the preference between the cue associated with control treatment *versus* the cue learned during a day of drug administration (Figure 16, Stuart et al., 2015).

A bench of pharmacological agents have been tested, with interesting results (Figure 17,



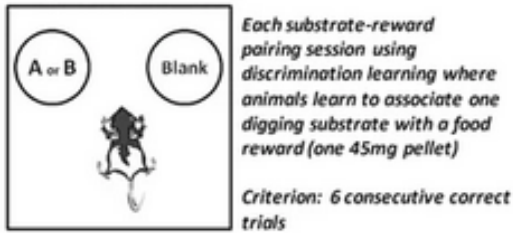
## Affective bias test (ABT) methods overview

### a Standard protocol for example animal



### b Experimental set-up

#### Substrate-reward pairing sessions (Day 1-4)



#### Preference Testing (Day 5)

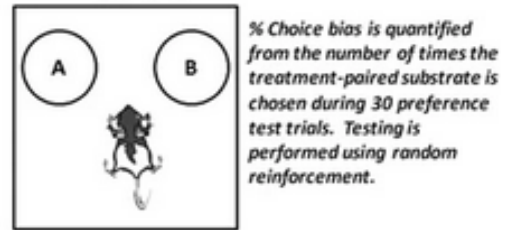


Figure 16. Affective bias test method overview. From Stuart et al. (2015).

Hales et al., 2014). A variant version of the test, the judgement bias task, evaluates the animals reaction to an ambiguous cue, mixing cues previously associated with positive and negative reinforcers (Harding et al., 2004; Hales et al., 2014). However, these tests present several drawbacks. They are not applicable to models that induce chronic alteration of the

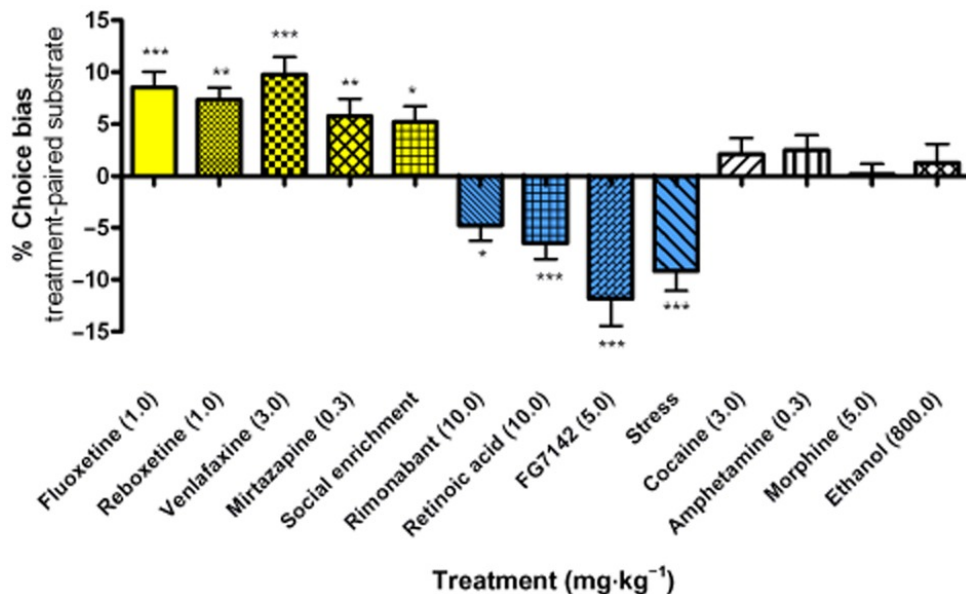
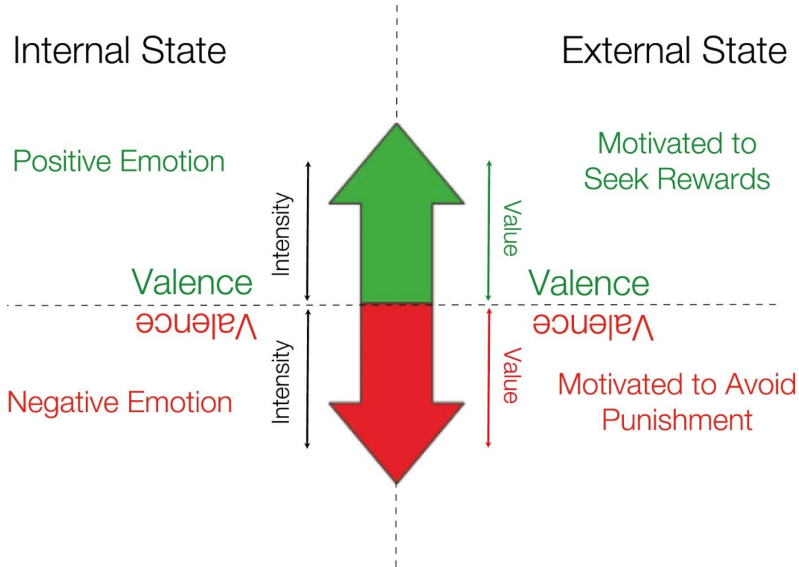


Figure 17. Pharmacological and environmental manipulations in the affective bias test. From Hales et al. (2014). “Yellow bars shows manipulations that caused a positive bias and blue bars indicate a negative bias. Drugs of abuse (white bars) had no effect in the test. Antidepressant drugs tested included typical and atypical drugs while pro-depressant treatments tested were the anxiogenic benzodiazepine inverse agonist, FG7142, cannabinoid CB1 receptor antagonist/inverse agonist, rimonabant and retinoic acid, the active ingredient of the anti-acne treatment, roaccutane. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, n = 16 animals per group. Data in this figure are taken from Stuart et al. (2013).”

behavior, or models using genetic triggering factors. In addition, they do not completely exclude the confounding effect of potential cognitive deficits, as well as alteration in sensory perception caused by the pharmacological compounds administered.

**Measuring emotional internal states by innate approach/avoidance response in animal models**

As we discussed earlier, mood cannot be directly measured in animals. However, emotional internal states in animals influence behavior, bringing an indirect way to measure them (Figure 18, Tye, 2018; Anderson and Adolphs, 2014). Motivated behaviors can be expressed in front of emotionally salient stimuli, innate or learned. They are thought to reflect emotional valence, defined as the subjective value assigned to sensory stimuli which determines subsequent behavior (Pignatelli and Beyeler, 2019). Positive valence leads to approach and consummatory behaviors (“motivation to seek rewards”) while negative valence leads to defensive and avoidance behaviors (“motivation to avoid punishment”).



**Figure 18. Linking emotional states to motivated behavioral outcomes.** From Tye (2018). “Green represents positive valence, while red represents negative valence. Valence refers to the ‘sign’ of the state, with positive representing rewarding states and negative representing aversive ones. Value tracks the worth of external stimuli on a continuum analogous to integers that can be positive or negative. Intensity indicates the absolute value of the strength of an internal state.”

According to our emotion-based model for mood disorders, we propose emotional responses measurement to describe mood states in rodents. As opposed to the affective and judgment bias tasks, we think that innate positive or negative stimuli need to be used, as it is done in humans.

The sucrose preference test already corresponds to such description, but consummatory



behaviors could somehow be dissociated from the assigned valence (*i.e.* the hedonic bias) of sucrose (Berridge et al., 2009). Not all depression models demonstrate differences in the sucrose preference test (Robinson, 2018), and in patients no obvious decreased sweet taste preference have been demonstrated (Dichter et al., 2010).

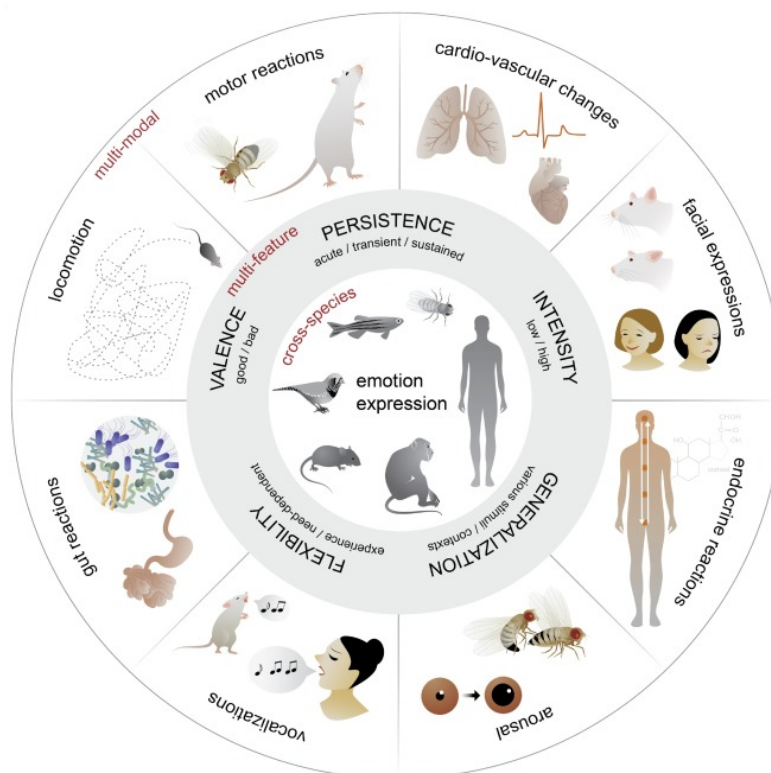
Another interesting approach is the female urine sniffing test (Malkesman et al., 2010; Zanos et al., 2016, 2017). It consists in measuring sniffing behavior of estrus female urine by male mice, in addition to ultrasonic vocalizations elicited. Both parameters were impaired in a learned helplessness model of depression (Malkesman et al., 2010). An additional compelling result from this study is the increase of female urine exploration by the GluR6-KO model of mania, suggesting this test could be used for describing mood episodes of both polarities.

We further extend the principle of this paradigm by proposing an olfactory preference test evaluating approach or avoidance responses to both appetitive (*e.g.* female urine) and aversive odors (*e.g.* predator odors). We suggest that approach or avoidance behaviors expressed by the animals in front of an odor, as assessed by the amount of time spent around the odor source, reflects the hedonic valence assigned to this odor. The olfactory preference test thus offers an indirect measure of the emotional state of the animal, in particular when disturbed compared to a control condition.

## 1.3 Circuits for emotional valence: focus on the amygdala

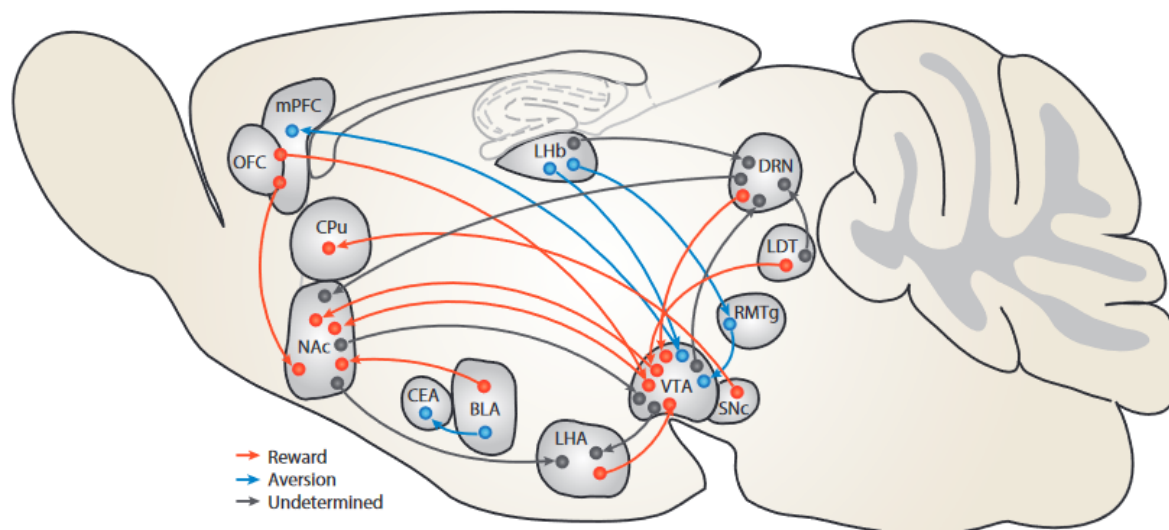
### 1.3.1 Brain circuits for emotional valence in rodents

There is a growing body of literature concerning the neuronal circuits involved in emotional processing and valence encoding. The neurobiological substrates for valence assignment are thought to be well conserved across evolution, as they serve the objective of recognizing appetitive and threatening stimuli, critical for survival. Emotions would then be a biological strategy for rapid integration and recording of significant stimulus of the environment, assigned with a particular motivation value, to trigger an appropriate behavioral response. Although emotions are difficult to identify and quantify, even in humans, various emotion expression can be considered as proxies for the emotional state of an animal (Figure 19, Zych and Gogolla, 2021; Nieh et al., 2013; Mauss and Robinson, 2009). For instance, fear triggers autonomic responses such as mydriasis (*i.e.* wide opening of the pupil), heavier breathing, redistribution of blood and release of hormones.



**Figure 19. Modes of emotion expression.** From Zych and Gogolla (2021). “Emotions are expressed through several modalities summarised in this schematic. Across species (inner circle) organismal response to a relevant stimulus can evoke changes in locomotion, motor behaviour, facial expressions, vocalisations, cardiovascular, gut and endocrine reactions and arousal (outer circle). Emotions, together with their expressions, have been proposed to encompass different features (middle circle).”

Facial expressions are other correlates of emotions (Anderson and Adolphs, 2014; Dolensek et al., 2020). Finally, we talk about motivated behaviors to encompass motor reactions resulting from emotions, notably in animals. Experimental testing of motivated behaviors comprises approach/reward-seeking or avoidance responses to innate and learned appetitive and aversive cues (*e.g.* conditioned or real-time place preference, pavlovian conditioning), but also intra-cranial self-stimulation, feeding, aggression and anxiety-like phenotypes (Namburi et al., 2016; Nieh et al., 2013).



**Figure 20. Neural circuits of reward and aversion.** From Hu (2016). “A simplified schematic summarizing the reward-mediating (red) and aversion-mediating (blue) neural pathways that have been verified by recent optogenetics-based behavioral studies. Prominent pathways that are implicated but unverified in reward and aversion are also delineated (gray).” BLA: basolateral amygdala, CEA: central amygdala, CPU: caudate putamen, DRN: dorsal raphe nucleus, LDT: laterodorsal tegmental nucleus, LHA: lateral hypothalamus, LHB: lateral habenula, mPFC: medial prefrontal cortex, NAc: nucleus accumbens, OFC: orbitofrontal cortex, RMTg: rostromedial tegmental nucleus, SNC: substantia nigra pars compacta, VTA: ventral tegmental area (Beier et al., 2015; Britt et al., 2012; Humphries and Prescott, 2010; Kirouac et al., 2004; Lammel et al., 2012; Lerner et al., 2015; Liu et al., 2014; Luo et al., 2015; McDevitt et al., 2014; Namburi et al., 2015, 2016; Nieh et al., 2015; Qi et al., 2014; Sesack and Grace, 2010; Stuber and Wise, 2016; Stuber et al., 2011).

Monitoring neuronal activity evoked by emotionally salient stimuli, as well as optogenetics and chemogenetics studies, which allow direct causal evaluation of the role of specific neurons in a particular behavior, reveal many areas of interest involved in emotional processing in the brain, belonging to distributed networks. They are mostly separated between circuits encoding reward, *i.e.* responding to positive valence stimuli and/or leading to approach and consummatory behaviors, and those encoding aversion, *i.e.* activated by negative valence stimuli and/or causing avoidance and defensive behaviors. Among them, we can cite the ventral tegmental area, the substantia nigra pars compacta, the rostromedial tegmental area, the nucleus accumbens, the lateral habenula, the hypothalamus, the raphe

nuclei, the locus coeruleus, the prefrontal and the orbitofrontal cortices, the hippocampus, the basolateral and the central nuclei of the amygdala and the bed nucleus of the stria terminalis (Figure 20, Hu, 2016; Nieh et al., 2013; Namburi et al., 2015).

We will primarily focus on the amygdala because of the well-demonstrated disturbances of this structure in mood disorders. An second argument stands on the recent literature unveiling the functioning of the basolateral nucleus of the amygdala in the encoding of valence in rodents.

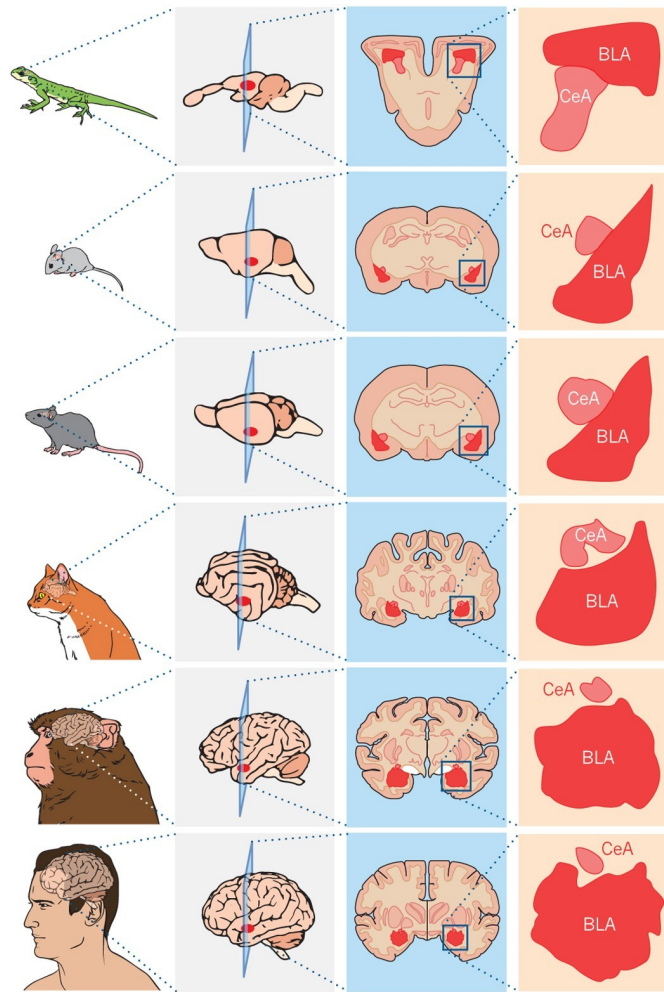
### ***1.3.2 The basolateral amygdala: a hub for valence encoding***

#### **Organization of the amygdala**

The amygdala consists of multiple interconnected nuclei located in the deeper region of the temporal lobe, distinguished according to several histological criteria including the density, configuration, shape and size of the cells, the trajectory of fibers and chemical signatures (LeDoux, 2007). The amygdala was first thought as two divisions, one more evolutionary primitive and more recent, associated with the olfactory system and the neocortex respectively. Nevertheless, it appears now that the amygdala does not represent a functional or structural unit, but rather different nuclei that belong to various systems of the brain. We will consider only the basolateral amygdala (BLA), which includes the lateral and the basal nucleus (BA, which is also called sometimes the basolateral nucleus). The other parts belong to the main and accessory olfactory system (medial amygdala, cortical amygdala, nucleus of the lateral olfactory tract and piriform-amygdalar transition area) and to the autonomic system (central nucleus or CeA) (Figure 21, Janak and Tye, 2015; Swanson and Petrovich, 1998).

Neurons among the BLA are mostly glutamatergic pyramidal projecting cells (Sah et al., 2003). The small remaining population is composed of local circuits GABAergic interneurons, expressing calbindin, calretinin, somatostatin or parvalbumin markers (Capogna, 2014). On the contrary, the CeA contains predominantly GABAergic medium spiny neurons (Sah et al., 2003).

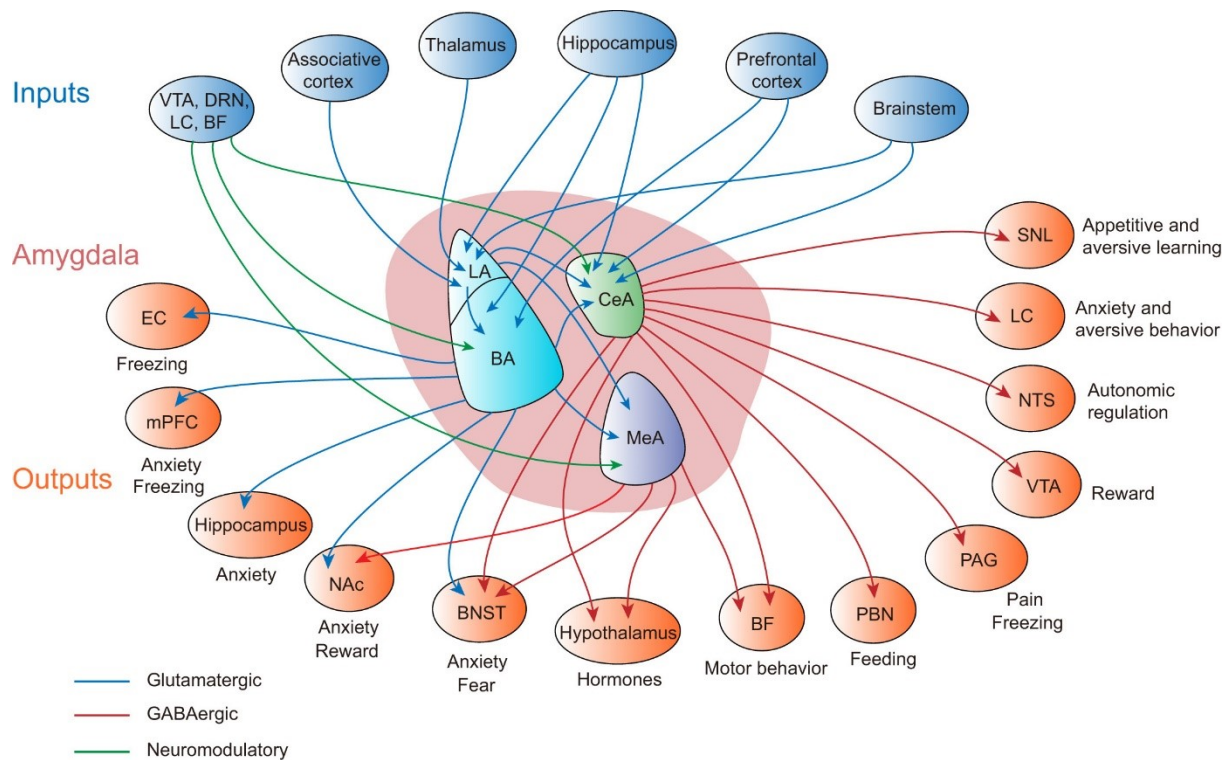
The BLA receives information originating from all sensory modalities via the thalamus or sensory cortex (Figure 22, Zhang et al., 2021; Sah et al., 2003). They mainly come from associative areas rather than primary sensory cortices, transmitting already processed information. These projections are glutamatergic, mostly ipsilateral. Polymodal sensory



**Figure 21. Evolution of the amygdala across species.** From Janak and Tye (2015). “Primary amygdalar nuclei and basic circuit connections and function are conserved across species. An enlarged image of the basolateral complex of the amygdala (BLA) and central nucleus of the amygdala (CeA) or analogues are shown next to a coronal section from the brains of a lizard, mouse, rat, cat, monkey and human.”

information also arrives from the prefrontal and perirhinal cortices and the hippocampus. Classically, sensory inputs first reach the LA, projecting itself to the BA, even if the BA also receives sensory cortical and thalamic afferents. Other inputs arise from the hypothalamus, involved in a variety of behaviors and physiological homeostasis such as feeding, mating, aggression and body temperature control, and from the monoaminergic and cholinergic systems that represent neuromodulatory inputs able to modify information processing within the BLA.

The main outputs of the BLA target the medial temporal lobe memory system including the hippocampus and perirhinal cortex, the ventral striatum (mainly the nucleus accumbens, NAc), the prefrontal cortex, the thalamus and inside the amygdaloid complex, the CeA (Figure 22, Zhang et al., 2021). The CeA forms a major output of the amygdala. It has reciprocal connections with the brain stem, more precisely the periaqueductal gray, the



**Figure 22. Input and output connections of the major amygdala subnuclei.** From Zhang et al. (2021). “Some of the major known functions of amygdala outputs are also indicated.” BA: basal amygdala, BF: basal forebrain, BNST: bed nucleus of the stria terminalis, CeA: central amygdala, DRN: dorsal raphe nucleus, EC: entorhinal cortex, GABAergic: gamma-aminobutyric acidergic, LA: lateral amygdala, LC: locus coeruleus, MeA: medial amygdala, mPFC: medial prefrontal cortex, NAc: nucleus accumbens, NTS: nucleus of the solitary tract, PAG: periaqueductal gray, PBN: parabrachial nucleus, SNL: lateral substantia nigra, VTA: ventral tegmental area.

parabrachial nucleus and the nucleus of the solitary tract, controlling autonomic responses. The CeA also targets the hypothalamus, the bed nucleus of the stria terminalis, and several monoaminergic and cholinergic structures, namely the noradrenergic locus coeruleus, the dopaminergic substantia nigra and ventral tegmental area, the serotonergic raphe and the cholinergic nucleus basalis.

### The role of basolateral amygdala neurons in positive and negative valence encoding

Fuster and Uyeda (1971) established that 37% of amygdala neurons selectively respond to motivationally significant stimuli, and their study has been followed by the demonstration that the firing rate of about half of the amygdala neurons selectively responsive to salient stimuli was modulated according to the affective significance of food or olfactory stimuli (Nishijo et al., 1988; Schoenbaum et al., 1999). Altogether, about one-fifth of BLA neurons could be encoding valence (Namburi et al., 2016).

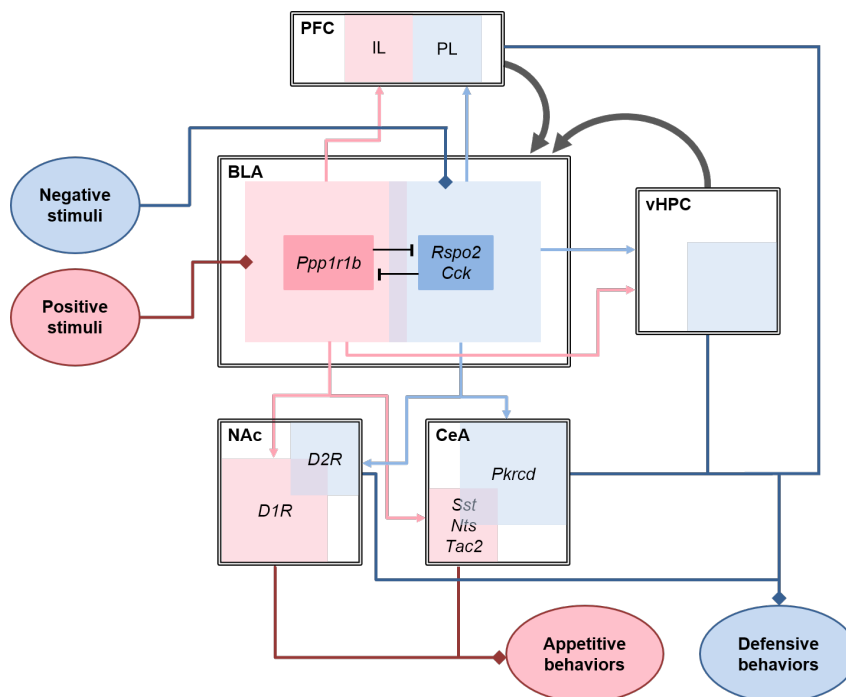
**Functional identification** Further examination of valence-specific activity responses of the BLA neurons to positive and negative stimuli uncovered two mostly non-overlapping, even if topographically intermingled neuronal populations (Zhang and Li, 2018; Gore et al., 2015; Redondo et al., 2014; Herry et al., 2008). Recent work also showed specific and stable BLA neuronal assemblies encoding engagement in spatial or social exploration, and non-exploratory defensive or self-centered states (Fustiñana et al., 2021; Gründemann et al., 2019). In addition, Corder et al. (2019) identified a distinct neural ensemble in the BLA representing diverse painful stimuli such as noxious heat, cold or pinprick, which mostly overlap, even though not completely, with neurons encoding non-somatosensory aversive stimuli like repulsive odor, bitter taste, loud tone, facial air puff. Less than one-third of neurons in this nociceptive assembly also responded to appetitive sucrose presentation or mild and light touch. Similar findings pinpoint the role of an ensemble of BLA neurons in histamine-response, inducing scratching behavior when injected intradermally, that are also active under administration of other itch or pain stimuli (Sanders et al., 2019). Such ensembles seem involved in the behavioral “affective” response to these aversive stimuli (Corder et al., 2019; Sanders et al., 2019).

**Topographical identification** The above mentioned studies described topographically intermingled neuronal populations. Contrary to these results, an antero-posterior gradient has been reported using the immediate early gene cFos expression under pleasant and unpleasant contextual, olfactory and gustatory stimuli (Kim et al., 2016a). This finding was not supported by further work by Beyeler et al. (2018), who rather proposed a dorso-ventral gradient. The technique for registering neuronal activity was however different: electrophysiological single-unit recordings in head-fixed mice trained to discriminate between reward- and aversive-predictive cues. In addition, Kim et al. (2016a) restricted their analysis to the basal nucleus, whereas Beyeler et al. (2018) included both the lateral and the basal nuclei.

**Genetic expression identification** Importantly, Kim et al. (2016a) also identified molecular markers, *Rspo2* and *Ppp1r1b* at the level of RNAs, for the preferentially encoding negative and positive neuronal populations, respectively. Such identification of *Rspo2* specifically labelling the “negative population” conjugated with the absence of *Ppp1r1b*, was further confirmed by other publications (Corder et al., 2019; Shen et al., 2019). *Cholecystokinin* (*CCK*) RNA expression is another marker which seems restricted



to neurons preferentially encoding negative valence and highly colocalizes with *Rspo2* (Shen et al., 2019). RNA sequencing or proteomics analyses of BLA neurons could provide additional insights about genetic markers discriminating both populations, further offering targets for pharmacological action (O’Leary et al., 2020; Pi et al., 2020; Kim et al., 2016a; Namburi et al., 2015). In that line, it is interesting to note that the protein Ppp1r1b is also known as DARPP-32 that plays a critical role in dopaminergic and glutamatergic signaling and is potentially implicated in schizophrenia and BD pathophysiology (Kunii et al., 2014).



**Figure 23. Anatomical connectivity and genetic identity of positive and negative value-coding BLA neurons and related structures.** From Bigot et al. (2020). Pink: positive pathways. Blue: negative pathways. Grey: regulatory pathways. BLA: basolateral amygdala, CeA: central amygdala, IL: infralimbic part of the PFC, NAc: nucleus accumbens, PFC: prefrontal cortex, PL: prelimbic part of the PFC, vHPC: ventral hippocampus, *D1R*: dopamine receptor type 1, *D2R*: dopamine receptor type 2, *Sst*: somatostatin, *Nts*: neurotensin, *Tac2*: tachykinin 2, *Pkrcd*: protein kinase C- $\delta$ , *Ppp1r1b*: protein phosphatase 1 regulatory subunit 1B+, *Rspo2*: R-spondin 2+, *Cck*: Cholecystokinin (Kim et al., 2016a, 2017; Beyeler et al., 2016, 2018; Namburi et al., 2015; Shen et al., 2019; Felix-Ortiz et al., 2013; Felix-Ortiz and Tye, 2014; McGarry and Carter, 2017; Sotres-Bayon and Quirk, 2010; Burgos-Robles et al., 2017; Senn et al., 2014).

**Anatomical identification** Besides potential topographical and genetic segregation of both populations, it has been proposed that projection targets could be different between neurons mostly encoding positive and negative valences and supporting approach or avoidance behaviors (Figure 23, Bigot et al., 2020; Tye et al., 2011; Stuber et al., 2011; Felix-Ortiz et al., 2013; Kim et al., 2013; Felix-Ortiz and Tye, 2014; Senn et al., 2014; Namburi et al., 2015; Beyeler et al., 2016; Kim et al., 2017; Shen et al., 2019; Pi et al.,



2020) (for reviews, see Janak and Tye, 2015; Namburi et al., 2016; O'Neill et al., 2018; Pignatelli and Beyeler, 2019).

To sum up this growing body of literature, the BLA-to-NAc projecting neurons are mainly implicated in positive valence encoding and approach behaviors, even though a subpopulation within this pathway seems on the contrary important for negative valence processing and defensive behaviors (Stuber et al., 2011; Namburi et al., 2015; Beyeler et al., 2016; Shen et al., 2019).

Among the BLA-to-CeA circuit, BLA-to-CeM (centromedial nucleus of the amygdala) neurons are assumed to be responsible for aversive valence processing, while BLA-to-CeL (centrolateral nucleus of the amygdala) neuronal activation has anxiolytic effects, supposedly through the subsequent inhibition of CeM by CeL GABAergic neurons (Tye et al., 2011; Namburi et al., 2015; Beyeler et al., 2016). However, contradictory results came from another study, in which CeM neurons elicited optogenetic self-activation, suggesting rewarding properties (Kim et al., 2017). More precise single-unit activity measurements in response to positive and negative valence stimuli should be performed in the subnuclei of the CeA to reconcile all these findings (Pignatelli and Beyeler, 2019).

The BLA projections to the ventral hippocampus (vHPC) were first suggested to drive defensive behaviors, but recent work showed that posterior BLA projecting neurons to the ventral hippocampus could also drive appetitive behaviors and have anxiolytic effects, while the anterior BLA-to-vHPC neurons activation is anxiogenic (Felix-Ortiz et al., 2013; Felix-Ortiz and Tye, 2014; Pi et al., 2020). Moreover, single-unit recordings of BLA-to-vHPC found that they respond to both positive and negative valence stimuli (Beyeler et al., 2016).

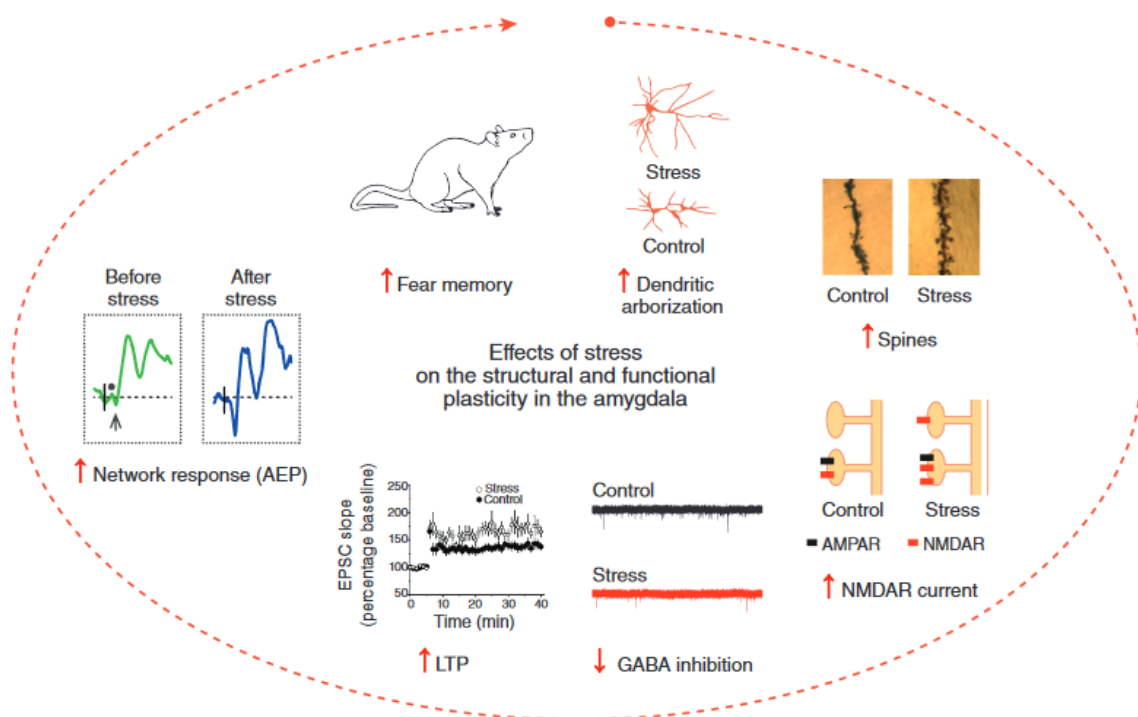
The BLA neurons projecting to the medial prefrontal cortex (mPFC), and more specifically to the prelimbic cortex (PL) respond more to aversive stimuli and trigger freezing during a fear conditioning task, whereas BLA-to-infralimbic (IL) cortex are more active during the extinction phase and would moderate freezing behavior (Senn et al., 2014; Felix-Ortiz et al., 2016; Burgos-Robles et al., 2017).

Finally, circuits such as BLA-to-anterior insula cortex (aIC) or to orbitofrontal cortex (OFC) could be important for reward associative learning, consolidation and extinction, even though their specific role in valence assignment is unknown (Lichtenberg et al., 2017; Gil-Lievana et al., 2020).

## BLA alterations under stress

Stress consists in “an actual or anticipated disruption of homeostasis or an anticipated threat to well-being” (Ulrich-Lai and Herman, 2009). Stressors recruit neural and neuroendocrine systems to produce physiological responses that aim at maintaining body integrity and restoring homeostasis. Therefore, appropriate responses to stress are critical for survival. The role of the amygdala in controlling physiological and behavioral responses to stress is well-documented (Ulrich-Lai and Herman, 2009; Zhang et al., 2021). Limbic structures including the amygdala are thought to tune the intensity of response depending on the stressor modality and intensity, also taking into account potential previous experiences (Ulrich-Lai and Herman, 2009).

The specific bidirectional relationship between the BLA and stress has been heavily investigated (Figure 24, Chattarji et al., 2015; Sharp, 2017). Depending on the type of stressor and its chronicity properties, amygdala neurons undergo structural and functional remodeling (Zhang et al., 2021). The main outcome is the hyperexcitability of BLA pyramidal neurons



**Figure 24. Stress effects on the amygdala.** From Chattarji et al. (2015). “Stress enhances fear by forming new synapses with greater capacity for LTP in the lateral amygdala. Chronic stress strengthens the structural basis of synaptic connectivity causing dendritic growth and spinogenesis. These newly formed dendritic spines have larger NMDAR-mediated synaptic currents as a result of the formation of NMDAR-only or silent synapses. Stress also lowers synaptic inhibition. This creates conditions that facilitate the induction of greater LTP in the amygdala. This, in turn, gives rise to stronger auditory-evoked potentials (AEPs) in awake, behaving animals. Together, these cellular and network level changes give rise to stronger fear memories.” These results can be further extended to anxiety- and depressive-like behaviors. LTP: Long-term potentiation.

resulting notably from disruption of local GABAergic circuitry under stress (Prager et al., 2016). Synaptic functional and structural plasticity under the control of neurotrophins, as well as expression of synaptic receptors and neuromodulatory mechanisms by a range of dopaminergic, noradrenergic, serotonergic, cholinergic, GABAergic and glutamatergic inputs (but also neuromodulators such as neuropeptide Y or endocannabinoids) either on GABAergic or glutamatergic BLA neurons are other factors regulating the excitability of principal neurons (Sharp, 2017; Prager et al., 2016; Boyle, 2013). Besides, genetic and epigenetic mechanisms can modulate the way the BLA responds to stress through mutations, DNA methylation or microRNAs for instance. Implicated genes comprise the ones for expression of cortico-releasing factor, glucocorticoid receptor, serotonin, BDNF but also belong to the endocannabinoid system (Zhang et al., 2021).

Globally, hyperactivity in the output of BLA neurons is thought to disturb the regulation of different brain regions such as the prefrontal cortex, the nucleus accumbens, the hippocampus and brain stem and thus impairs cognition, motivation, emotional and autonomic responses. While BLA neurons play a critical role in regulating adaptive stress response, facilitation of the BLA activity upon sufficiently intense and/or chronic stress is thought to lead to maladaptive stress reactions. Such pathological BLA hyperactivity has been associated with anxiety disorders, addiction, autism, Alzheimer's disease, ADHD, post-traumatic stress disorder (PTSD) and mood disorders (Sharp, 2017; Roozendaal et al., 2009).

## 1.4 Proposed hypothesis

Aforementioned evidence suggests that emotional biases play a fundamental role in the development, the maintenance and the resolution of mood episodes. Besides, the amygdala functional and structural disturbances in mood disorders are already well-established, and the preclinical knowledge increases about emotional valence processing in the basolateral amygdala.

In this context, we hypothesize that specific modifications in the BLA circuits during mood disorders lead to emotional biases. More precisely, considering that the BLA-to-NAc neurons are mainly involved in positive valence encoding and the BLA-to-CeA counterparts mostly encode negative valence, we think that imbalance of activity between those two populations could bias sensory and emotional valence assignment. Of note, “CeA” refers here and in the following parts to the CeM. We propose that the positive emotional bias encountered in mania states is underpinned by hyperactivity of the BLA-to-NAc pathway with concomitant hypoactivity of the BLA-to-CeA pathway. On the contrary, decreased BLA-to-NAc neurons activity along with elevated BLA-to-CeA neurons activity would lead to the negative emotional bias characterizing depressive states.

The publications below summarize the conceptual framework we draw on and the related evidences in the literature of the importance of emotional responses in mood disorders, to suggest a crucial role of specific amygdala circuits in the pathophysiology of these psychiatric illnesses.

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## Review article

## An emotional-response model of bipolar disorders integrating recent findings on amygdala circuits

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## ABSTRACT

Because of our classification system limitations for defining psychiatric disorders and understanding their pathophysiology, a new research area based on dimensions has emerged. It consists of exploring domains derived from fundamental behavioral components linked to neurobiological systems. Emotional processing is among the most affected dimensions in bipolar disorders (BD), but is excluded from the definition criteria. The purpose of this review is to synthesize the emotional responses disruption during the different phases of BD, using intensity and valence as the two key characteristics of emotions. We integrate those emotional disruptions into an original, emotion-based model contrasting with the current diagnostic frame built on mood. Emotional processing is underpinned by cortico-limbic circuits involving the amygdala. Recent publications showed the crucial role of the amygdala in emotional processes triggered by stimuli of negative, but also positive valence. We show how these neuroscience data can provide physiological basis for emotional disturbances observed in BD. We conclude with translational perspectives to improve the current knowledge about neural substrates underlying altered emotional responses characterizing BD.

## 1. Introduction

Lifetime prevalence of bipolar disorders (BD) is about 4.5 % in the general population with a high suicide rate and a huge impact on patients functioning (Grande et al., 2016; Merikangas et al., 2007). BD is defined by the recurrence of depressive, manic, and/or mixed episodes, in which manic and depressive symptoms are simultaneously present.

Our current classifications to define psychiatric disorders are based on clinical consensus relying on the observation of signs and symptoms but are questionable from both a clinical and research standpoint and therefore need to be refined (Bauer et al., 2018). We can note many shortcomings common for all psychiatric diagnoses. The clinical heterogeneity of syndromes results in patients with very different symptoms being diagnosed with the same disease. Moreover, these classifications do not provide access to the fundamental mechanisms underlying the syndromes, neither predict response to treatment. Concerning BD specifically, the altered mood is the main criterion to

define episodes, with sadness in depressive states and euphoria or irritability in mania (American Psychiatric Association, 2013). Nevertheless, the existence of mixed states associating manic and depressive symptoms challenges the relevance of this model of bipolarity based on mood (Koukopoulos et al., 2013). During mixed states, mood must be both exalted and sad at the same time or within a very short period. However, mood is a persistent and slow-moving feeling and then is not adequate to describe mixed states. This may explain the difficulties in diagnosing mixed states and its wide variations in estimating its prevalence (6–19.6%)(Shim et al., 2015). On the other hand, mood is not sensitive enough to detect sub-threshold or residuals symptoms that persist beyond clinically defined episodes. Then, they are not considered in current definitions despite their strong impact on functioning, while they are particularly difficult to treat (Serra et al., 2019; Dargél et al., 2018). Finally, the subjective assessment of mood is inappropriate for quantitative measurements and so for experimental research.

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To overcome these issues, a dimensional approach has been proposed. The principle is to explore not any more symptoms, but domains or dimensions that are constructs derived from fundamental behavioral components which can be linked to neurobiological systems (such as cognition, motivation, perception, etc.), and range from normal to pathological (Malhi et al., 2018; Cuthbert and Insel, 2013). One of the most impacted domains in BD is emotional processing whose disruptions underlie a large number of symptoms. Research in this area has long been neglected, as emotional processes remained poorly defined and difficult to measure in animal models. Currently, consensual and efficient definitions for research have made possible to carry out clinical and pre-clinical studies allowing us to determine the brain structures involved in emotional processes.

Emotions are brief responses characterized by a physiological arousal that are triggered by a stimulus to drive an adapted behavior (Schachter and Singer, 1962; Russell, 2003; Tye, 2018) and are characterized by two quantifiable features: (i) the intensity of the response and (ii) the valence. Emotional valence is the subjective value assigned to sensory stimuli which determines subsequent behavior. Positive valence leads to approach and consummatory behaviors while negative valence leads to defensive and avoidance behaviors (Pignatelli and Beyeler, 2019). Stimuli can be external via our senses or internal, and the perception of these stimuli will directly influence the behavioral response (Fig. 1). Conversely to mood, emotional response can be studied in real time during brain imaging studies in human and can be inferred in animal models by assessment of approach or avoidance behaviors (Beyeler, 2016a). Based on these two characteristics of emotion and fostered by the recent advent of sophisticated techniques, basic research is revisiting the role of amygdala circuits. Previously considered as the hub of fear, we now know that the amygdala is involved in valence assignment of both positive and negative salient stimuli, where potentially all kind of emotional responses could arise.

The purpose of this review is to synthesize the disruption of emotional responses during the different phases of BD and integrate them into a new model that is based on variations in the intensity and valence of emotional responses. We then propose an overview of recent neuroscience data that could provide a physiological basis for these disturbances. We conclude with translational research perspectives to improve current knowledge about the brain support of emotional responses that characterize BD.

## 2. Emotional disturbances in BD: from mood to emotional responses

### 2.1. Clinical data

As brief response following stimuli, emotional response can be assessed in day-to-day practice using self-questionnaires and ecological assessments or during laboratory experiments. We report here variations in emotional response during the different phases of the illness in adult BD patients.

Although considered in remitted phases, BD patients experience more frequent and intense emotions in response to environmental conditions relative to healthy subjects, which leads to mood instability (Henry et al., 2009). Emotional hyper-reactivity and mood instability have a detrimental impact on functioning, relapses, and suicide attempts (Strejilevich et al., 2013; Dargél et al., 2017). In a large cohort of BD patients clinically defined in a remitted phase by classical assessment, we showed that emotional response intensity is predictive of outcome (Dargél et al., 2018). Patients with higher emotional responses made more suicide attempts; had a higher level of C-reactive protein, a marker of inflammation; and had more physiological disturbances such as higher blood pressure and higher fasting glucose level. This pinpoints the relevance of assessing emotional responses to detect patients at risk of suicide, and at risk of developing cardiovascular diseases or cognitive decline linked to chronic inflammatory process, which are among the most important causes of the high mortality/morbidity of BD. Using ecological momentary assessment, specific profiles of emotional reactivity to daily events and mood instability were identified, which allowed us to distinguish BD I relative to patients with BD II, major depressive disorders (MDD), and anxiety (Lamers et al., 2018; Jepsen et al., 2019). Emotional responses could also represent an endophenotype, because subjects at risk for BD, defined by a score on a scale of hypomania, exhibited higher emotional reactivity (Kwapil et al., 2011; Gruber et al., 2008). Some authors suggest that excessive reactivity to positive events might be a core dimension of BD (Johnson, 2005; Gruber, 2011; Kærsgaard et al. (2018)). In this vein, we have reported that neutral pictures are assessed as more pleasant by BD patients relative to control, testifying that the misallocation of valence is in the direction of a positive bias (M'Bailara et al., 2009). However, the corpus of findings suggests that excessive emotional responses concern all kinds of stimuli, possibly depending on residual symptoms.

Compared to remitted phases, mood states are characterized by

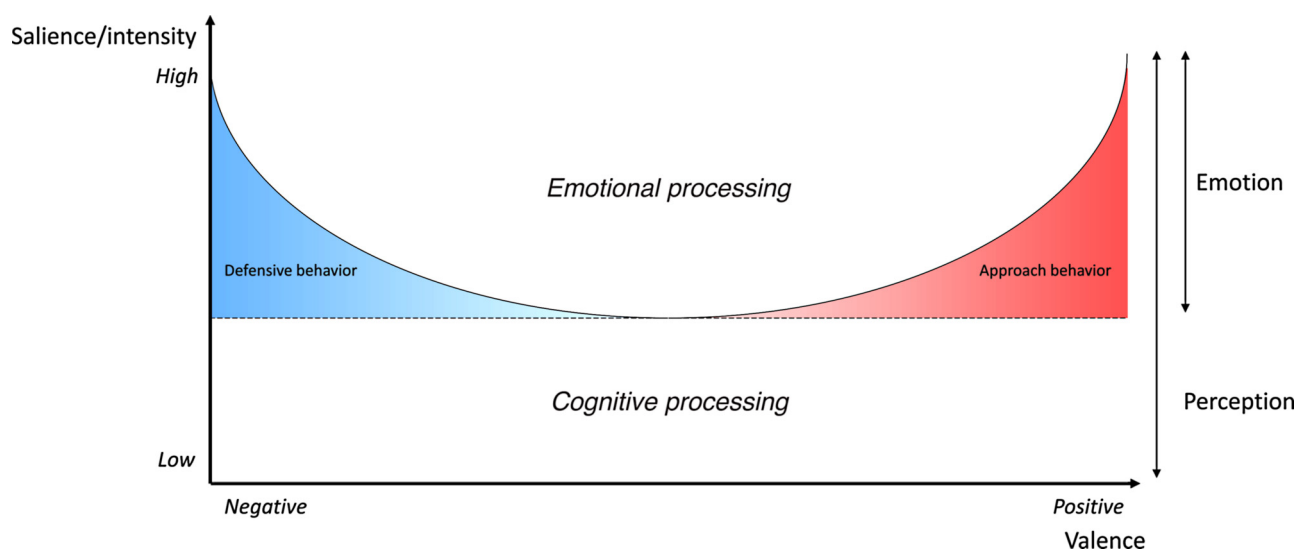


Fig. 1. When a perception is sufficiently salient, it triggers an emotion with a congruent valence (positive or negative). In turn, the emotion will trigger either a defensive (in blue) or an approach behavior (in pink). In animal models, it is possible to measure the valence attribution to various stimuli by measuring behavior and thus to infer the animal's emotional state.

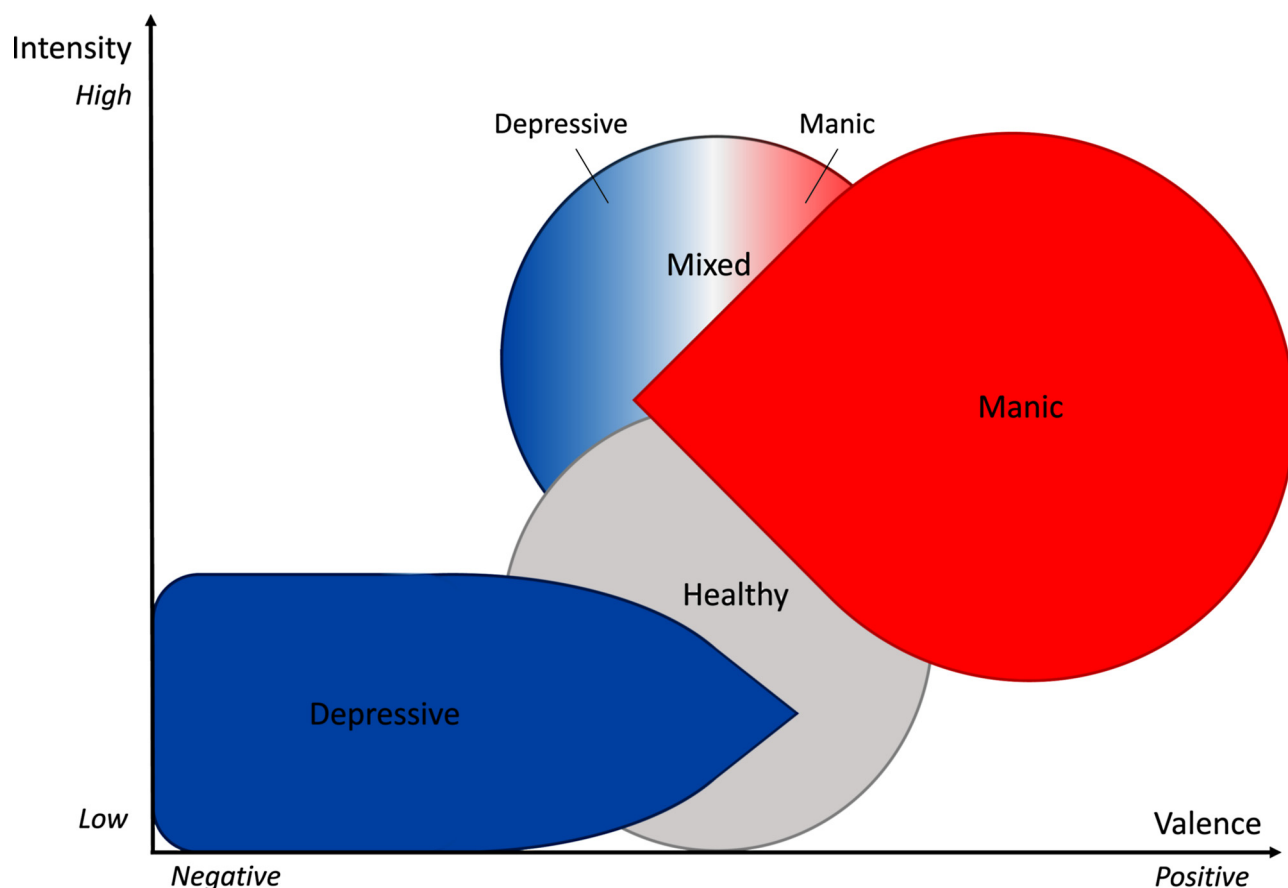


Fig. 2. In this emotional-response model, conversely to those of mood-model, mania and depression are not placed on the extremes of a unidirectional continuum, allowing us to integrate the broad spectrum of mixed and sub-syndromic states. During manic episodes (in red), there is a propensity to assign more positive valences with higher intensity. In mixed states (blue-white-red), the arousal is higher, but the attribution of valence is flexible. In depression (in blue), there is a decreased arousal and a greater weight assigned to negative valences, whereas during the remitted phase, as a function of the type of sub-syndromal symptoms, arousal and valence can slightly change in a chronic manner.

drastic changes in both intensity and valence of emotional responses. We showed that neutral, negative, and positive pictures are more arousing during both manic and mixed states in comparison to patients in remitted phases (M'Bailara et al., 2012). On the basis of the theoretical model of the behavioral approach system (BAS) and the behavioral inhibition system (BIS), bipolar mood states are supposed to result from either an increase or a decrease in the activity of these two systems (Meyer and Hofmann, 2005). Regarding the functioning of these two opposite systems, manic patients present increased reward awareness, whereas depressed patients avoid reward and exhibit higher sensitivity to punishment. In other words, during manic states, a positive emotional bias to reward cues drives a high level of goal-seeking behavior and magnifies and exacerbates sensory perception (Parker, 2014). This is accompanied by poorer perception of dangers leading to high risk-taking behaviors (i.e., lower attribution of negative value).

The intensity of emotional responses during depressive episodes is more complex, as bipolar depression is heterogeneous. In major depressive disorder (MDD), a meta-analysis including data collected by self-questionnaires, behavioral expressions, or physiological measurements has shown that there is a global emotional hypo-reactivity with a reduction of all emotional responses to both positive and negative stimuli, leading to a global insensitivity (Bylsma et al., 2008). In bipolar patients, because of the widespread clinical heterogeneity of depression, emotional responses are not homogeneous. We showed that depressed bipolar patients can be separated depending on the level of intensity of their emotional responses (Henry et al., 2007). One BD depression is characterized by emotional hypo-reactivity associated with overall behavioral inhibition, whereas the other is characterized

by emotional hyper-reactivity and mild activation that corresponds to depression with mixed features in the DSM-5 (American Psychiatric Association, 2013). That could explain why bipolar depressed patients as a whole are more reactive to emotional cues than healthy subjects, conversely to those with MDD (Stratta et al., 2014). Concerning valence assignment in depressed patients, there is a well-documented global negative bias (Leppänen, 2006). A human model suggest that the direct effect of successful antidepressant treatment is to modify negative biases in emotional processing and is an early marker of good response to monoaminergic antidepressants (Harmer et al., 2017).

From a clinical point of view, characterizing mood episodes with emotional responses is of clear interest for discriminating subgroups of patients pooled within the same diagnosis by current classifications (Henry et al., 2007). This strategy could help to understand various responses to pharmacological treatments for bipolar depressions and propose more personalized treatment. Currently, recommendations propose treatments with very different and sometimes opposed mechanisms of action, such as antipsychotics with an antidopaminergic action, molecules acting on other monoamines such as serotonin, but also dopaminergic agonists. For remitted patients, it could allow us to develop preventive strategies. Dialectical behavioral therapy would be particularly appropriate for patients with chronic emotional hyper-reactivity, whereas those with a hypo-reactivity may better benefit from interventions focused on behavioral activation (Dimidjian et al., 2011; Eisner et al., 2017). Regarding our results on patients during the remitted period, measuring emotional reactivity opens a new avenue to understanding the links between inflammatory processes and BD. Importantly, disruption in emotional responses is a transnosographic



dimension, which can also affect patients with personality disorders, attention deficit hyperactivity disorders, or substance users (Henry et al., 2001; Patel et al., 2015; Richard-Lepouriel et al., 2016). Therefore, assessment of emotional responses can also help to better understand links between these highly comorbid conditions or reduce misdiagnosis between them.

## 2.2. A new model based on emotional response in bipolar disorders

To guide research perspectives, we propose to deconstruct the mood-based model of BD and replace it with an emotional response-based model that can serve as a conceptual framework. Using the two-dimensional theory of emotion and the main results in clinical reports on emotional responses in BD, we propose here a model to characterize the variations of emotional responses during BD mood states using their two main features, intensity and valence (Fig. 2). The mood-based model is constructed from a unidimensional scale ranging from euphoria to sadness, circumscribing BD to two poles that does not allow us for a correct integration of mixed states and sub-threshold symptoms. Our model, by including two parameters, enables us to better account for the complexity of the emotional processing. During manic episodes, there is a positive emotional bias leading to assign more positive valences with higher intensity. Thus, neutral stimuli become positive, negative stimuli lose their negative value (e.g. a danger becoming less scary), and positive stimuli have a stronger rewarding value. In mixed states, the arousal is higher, but the attribution of valence will determine whether it is a depressive or manic episode with mixed features. In depression, there is a decreased arousal and a negative emotional bias, whereas during the remitted phase, as a function of the type of sub-syndromal symptoms, arousal and valence can slightly change in a chronic way.

This model can explain many of the symptoms such as frenetic pleasure seeking, risk-taking behaviors, emotional lability and disproportionate emotional responses in manic patients. On the contrary, loss of emotional responses associated with a negative bias may explain the apathy and anhedonia of depressed patients.

Obviously, mood disorders cannot be defined just by emotional responses and it is necessary to add other relevant dimensions that refer to different sets of symptoms. Recently, we highlighted the major interest in better studying motor activity (Scott et al., 2017). Other authors, using Kraepelin's model to explain mixed states, proposed a scheme with three dimensions: emotions, cognition and motor activity (Malhi et al., 2018). However, in this model, emotions are considered only for their valence. We believe that integrating intensity of emotion is essential to placing all domain on a continuum from inhibition to activation (Henry et al., 2010), as proposed in our emotional response model.

An important point to disentangle is whether those emotional disturbances are due to an alteration in emotional processing, involving brain limbic structures, or an alteration of the perception and sensory processing of the stimuli, relying on modifications in sensory areas. In a large sample of BD patients in remitted period, we performed a cluster analysis using five dimensions which are emotional reactivity, sensory perception, psychomotor activity, motivation and cognition that lead to discriminate four clusters. In all the clusters, emotional reactivity and sensory perception co-varied in the same direction (Dargél et al., 2019). Indeed, with a behavioral evaluation, it is very difficult to disentangle the sensory from the emotional contribution to the intensity and valence attribution to a particular stimulus. To completely distinguish both effects, a parallel evaluation of sensory system functioning is required, assessing several aspects of sensory perception including detection, identification and discrimination of stimuli, among other parameters. Alterations in sensory cortices activity, structure and connectivity have been recently reported (Shaffer et al., 2018; Thomas et al., 2019), but further research on this question is necessary to demonstrate the role of sensory processing in the pathophysiology of BD,

independently of the altered emotional processing. Concerning this emotional processing, limbic areas including the amygdala, have been consistently observed as disrupted in BD patients compared to healthy subjects (Chen et al., 2011).

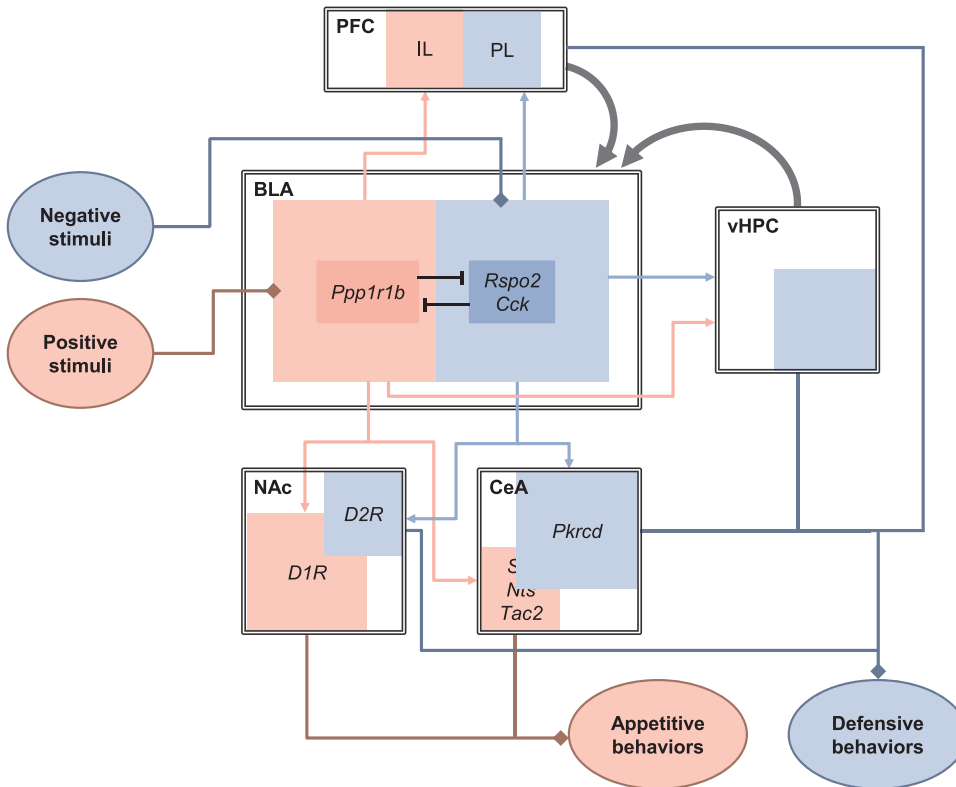
## 3. The amygdala: a key structure for emotional processing involved in the pathophysiological model of bipolar disorders

The amygdala has for a long time been identified as a key structure for emotional response. However, during the last decades, because of the tremendous work highlighting its role in fear mainly based on the very robust fear-conditioning model, it resulted in the amygdala being considered as the hub of fear (Rogan et al., 1997; Rodrigues et al., 2004; LeDoux, 2017). These pre-clinical data have been reinforced by data in patients with post-traumatic stress disorders, social phobia, and specific phobias for whom functional neuroimaging showed an over-activation of the amygdala triggered by negative emotional stimuli in comparison to control subjects (Rauch et al., 2006; Etkin and Wager, 2007). Moreover, fear conditioning in healthy subjects reproduced the same pattern of activation. Altogether, these data focused attention on the role of amygdala as a key component in fear and anxiety disorders.

However, structural and functional abnormalities of the amygdala are also found in other psychiatric disorders, in particular in BD. The current consensual model of BD assumes that a dysfunction in pre-frontal cortex regulation of the amygdala is the main pathophysiological cause of the disease. Hence, one of the most reproducible results using fMRI studies is an amygdala over-activation when facing emotional tasks in bipolar patients compared to controls (Strakowski et al., 2012; Phillips and Swartz (2014)). Some authors propose a state-dependent amygdala activation, with an over-activation during mania and hypo-activation during depression (Altshuler et al., 2005; Vizuetta et al., 2012). However, a meta-analysis of fMRI found an over-activation of limbic structures, most consistently in the amygdala and hippocampus, triggered by emotional stimuli in BD adult patients whatever the phases (i.e., remitted, manic, or depressive) in comparison to control subjects (Chen et al., 2011). Some authors have also shown that different patterns of amygdala activation may distinguish unipolar depressed patients from bipolar depressed patients (Almeida and Phillips, 2013). Moreover, it is worth mentioning that lithium intake, the gold standard treatment of BD, has been associated with modifications on amygdala in patients treated with lithium (Hartberg et al., 2015; Strakowski et al., 2016). Given the major role of amygdala in emotional processing, structural and functional changes in this area could underlie emotional disturbances both in depressive and manic episodes and explain the lithium effect.

### 3.1. New robust data from basic science: the amygdala, a hub for positive and negative valence encoding

The development of optogenetics over the past decade has provided novel opportunities to explore the role of neural circuits in behavior, particularly regarding amygdala functioning (Tye and Deisseroth, 2012). Optogenetics relies on the combined use of genetic and optical methods to control spatially and temporally specific neuronal activity. Neurons are first genetically engineered to express a given opsin, proteins sensitive to light, allowing control of their activity by light pulses. Under illumination, these neurons are transiently activated or inhibited, depending on the nature of the opsin that is chosen. The temporal properties of the optogenetic tool allow reshaping the style of the experimental design, and the activity of specific neurons can be controlled in free-moving animals, allowing to assess their behavior. Another way to link specific cell population activity to behavioral outputs relies on chemogenetic technology, which combines the use of chemogenetically engineered proteins (designer receptors exclusively activated by designer drugs, or DREADDs) with chemical reagents to activate or inhibit specific neurons (Roth, 2016). Using the



**Fig. 3.** Anatomical connectivity and genetic identity of positive and negative value-coding BLA neurons and related structures. The BLA is an important structure for valence processing, as demonstrated through recent pre-clinical studies. Distinct neuronal subpopulations encode preferentially either positive or negative values and antagonize the activity of the opposing neurons (Kim et al., 2016). Other studies also found that positive and negative valence-encoding BLA neurons are intermingled throughout BLA (Beyeler et al., 2018) and valence encoding in the BLA is mediated via activity of neural populations defined by their projections (Namburi et al., 2015; Beyeler et al., 2016b). The positive-encoding neurons respond to positively valenced sensory stimuli (in pink). These neurons project mainly to the NAc (Kim et al., 2016; Beyeler et al., 2016b, 2018), but also to the CeA (Kim et al., 2016, 2017), vHPC (Beyeler et al., 2016b) and the IL part of the PFC (Kim et al., 2016; Senn et al., 2014). Negative stimuli activate the negative-encoding neurons, which a subgroup expresses Rspo2 and Cck RNA (in blue; Kim et al., 2016; Shen et al., 2019), and are connected to the CeA (Kim et al., 2016; Beyeler et al., 2016b), NAc (Beyeler et al., 2016b; Shen et al., 2019), vHPC (Beyeler et al., 2016b) and the PL part of the PFC (Kim et al., 2016; Burgos-Robles et al., 2017).

Artificial activation of the BLA-to-NAc

pathway mainly lead to appetitive behaviors (Stuber et al., 2011; Namburi et al., 2015), even if specific inhibition or activation the negative subpopulation of the BLA projecting to the NAc bidirectionally regulate defensive behaviors (Shen et al., 2019). In the same way, global activation of the BLA-to-CeA neurons generates defensive behaviors, although activating BLA neurons that project to specific neuronal types of the CeA (Sst +, Nts +, Tac2 +) induces opposite behaviors (Kim et al., 2017). In contrary, inhibition of BLA-to-CeA projections impairs defensive behaviors and enhance appetitive ones (Namburi et al., 2015).

Enhancing BLA-to-vHPC neuronal activity triggers defensive behaviors (Felix-Ortiz et al., 2013; Felix-Ortiz and Tye, 2014), as well as BLA to PFC (Burgos-Robles et al., 2017). Finally, reciprocal connections from the PFC and vHPC would regulate BLA activity, as suggested by McGarry and Carter, 2017 and Sotres-Bayon and Quirk, 2010.

Pink: positive pathways. Blue: negative pathways. Grey: regulatory pathways. BLA: basolateral amygdala. CeA: central amygdala. IL: infralimbic part of the PFC. NAc: nucleus accumbens. PFC: prefrontal cortex. PL: prelimbic part of the PFC. vHPC: ventral hippocampus; D1R: dopamine receptor type 1; D2R: dopamine receptor type 2; Sst: somatostatin; Nts: neurotensin; Tac2: tachykinin 2; Pkrcd: protein kinase C-delta; Ppp1r1b: protein phosphatase 1 regulatory subunit 1B +; DARPP-32: Dopamine and c-AMP-regulated phosphoprotein; Rspo2: R-spondin 2 +; Cck: Cholecystokinin.

above-mentioned techniques, the role of the amygdala and its multiple nuclei has been studied, deconstructing the complex whole-amygdala circuits involved in various behavioral responses (Janak and Tye, 2015; Tye, 2018; O'Neill et al., 2018). The amygdala consists of multiple interconnected nuclei located in the deeper region of the temporal lobe. Its structure and functions have been well-conserved across evolution, and findings from mice provide a good approximation of amygdala functioning in humans (Janak and Tye, 2015). The two main structures of the amygdala include the basolateral (BLA) and the central nucleus (CeA) of the amygdala (Fig. 3). The BLA receives information originating from all sensory modalities via the thalamus or sensory cortex. The main outputs of the BLA are the ventral striatum (mainly the nucleus accumbens, NAc) and the CeA. The BLA is also reciprocally connected to the ventral hippocampus (vHPC) and the medial prefrontal cortex (mPFC) (Janak and Tye, 2015).

### 3.1.1. Molecular-defined valence populations: Rspo2 & Ppp1r1b

Using immunohistochemistry for the immediate early gene cFos, it was recently shown that two genetically distinct, spatially segregated populations of neurons within the BLA are differentially activated by positive or negative stimuli. Specifically, aversive stimuli such as foot shocks preferentially activates neurons expressing Rspo2 (R-spondin2), which correspond to magnocellular pyramidal neurons, whereas exposure to a reward (female mouse presentation to the tested male), preferentially activates the neurons expressing Ppp1r1b (protein

phosphatase 1 regulatory inhibitor subunit 1B, also known as DARPP-32), which correspond to parvocellular pyramidal neurons (Kim et al., 2016). The differential activation of these neurons was confirmed by other valence-specific stimuli, namely pleasant or unpleasant odors and gustatory stimuli. To confirm the role of these two neuronal populations in valence-specific behaviors, these authors used optogenetics to alternatively inhibit these two populations during fear-conditioning and reward-motivated learning. Inhibition of Rspo2-expressing neurons reduced the freezing in response to foot shocks, whereas inhibition of Ppp1r1b-expressing neurons decreased the reward-conditioning performance. The reverse was true when activating the same populations. Interestingly, activation of one population of neurons antagonized the activity of the other one (Kim et al., 2016). Another recent study using single cell calcium imaging showed that BLA neurons encoding negative affective valence of pain largely overlap with those encoding for other sensory-aversive cues (Corder et al., 2019). It is interesting to note that the protein Ppp1r1b is also known as DARPP-32 that plays a critical role in dopaminergic and glutamatergic signaling and is potentially implicated in schizophrenia and BD pathophysiology (Kunii et al., 2014).

### 3.1.2. Valence-defined topography: antero-posterior or dorso-ventral gradient?

There is no consensus regarding the spatial organization of the positive and negative valence encoding neurons. Kim et al. (2016)

showed, still using *ex vivo* cFos immunohistolabelling, preferential activation of neurons located in the anterior part of the BLA upon presentation of aversive contextual, olfactory and gustatory stimuli, whereas the neurons belonging to the BLA posterior part preferentially responded to pleasant stimuli of these same sensory modalities. In a recent study, more than 1000 neurons were recorded *in vivo* during a Pavlovian task to map their spatial location in the BLA (Beyeler et al., 2018). The data support the notion that although positive and negative neurons are intermingled through the antero-posterior axis, a gradient of valence responses could be seen within a dorso-ventral axis.

### 3.1.3. Projection-defined valence populations: BLA-vHPC, BLA-CeA, BLA-NAc

The idea that positive and negative valence encoding neuronal projections reach distinct brain areas seems more consistent across studies (Fig. 3). Work from the laboratory of Kay Tye successively explored BLA projections to the vHPC, the CeA, and the NAc in anxiety- and valence-related behaviors. Felix-Ortiz et al., 2013, 2014 showed that optogenetic stimulation of glutamatergic neurons projections from the BLA to the vHPC induces anxiety-like behavior and reduces social interactions, whereas inhibition leads to anxiolytic-like states and increased such interactions.

BLA-to-CeA projecting neurons activation induces real time place aversion, whereas its inhibition impairs fear conditioning and enhances reward conditioning (Namburi et al., 2015). Either BLA-to-NAc neurons projections stimulation (Stuber et al., 2011) or cell bodies activation of these same neurons (Namburi et al., 2015) induces intracranial self-stimulation (ICSS), suggesting a role for this specific circuit in reward-seeking behavior. Conversely, optical inhibition of the same BLA-to NAc neurons fibers decreased response to sucrose reward (Stuber et al., 2011). Finally, other findings employed optogenetic-mediated “photo-tagging” in combination with large-scale *in vivo* electrophysiological recordings to reveal the specific neural code of BLA neurons that synapse in the NAc, CeA or vHPC, in response to the presentation of cues associated with either rewarding or aversive outcomes. They demonstrate that during the retrieval of positive or negative associative memories, the valence encoding in the BLA is at least partially mediated via divergent activity of anatomically defined neural populations (Beyeler et al., 2016b).

Finally, recent articles demonstrated that chronic stress in mice specifically enhances synaptic connections and signal transmission from the BLA to the vHPC (Zhang et al., 2019a, b).

### 3.1.4. Projection- and molecular-defined valence population: BLA-to-NAc CCK + / -

At a molecular level, Shen et al., 2019 showed that cholecystokinin (CCK) gene expression could be a marker to subdivide the BLA-to-NAc subpopulation into positive (CCK-) and negative (CCK+) valence encoding neurons (Fig. 3). These two types of neurons co-express respectively Ppp1r1b and Rspo2, consistently with their role in positive and negative valence encoding. Furthermore, CCK + and CCK- neurons are respectively connected to D1R- or D2R-expressing neurons, mediating either appetitive or defensive behavior. Therefore, the BLA appears able to transmit information about the positive or negative valence of a stimulus to structures involved in motivational processes.

### 3.1.5. The amygdala, a central area for valence coding

Altogether, these studies suggest that specific populations of neurons within the BLA are key components to encode valence as they exhibit neural coding bias, meaning they respond preferentially either to positive or negative valence stimulus (Pignatelli and Beyeler, 2019). They attribute valence for different kinds of sensory stimuli and directly impact emotional responses and behavior. In other words, the amygdala circuit orchestrates behaviors by encoding and transmitting information about the external and internal environment. Thus, amygdala has a central position in the circuits controlling emotional processing to

trigger rapid and adapted behaviors.

Importantly, which algorithmic models are used by the amygdala to process valence is still debated (Tye, 2018). Is the predominant model based on the specificities of neurons, specific connectivity, or connectivity that can code for both types of valences but modulated according to the context, or does the modulation of these systems depend on specific receptors that can amplify or reverse a signal? An important issue is to know the impact of psychotropic medications on this valence-coding system. What is certain is that BLA circuits are a dynamic system likely to be adjusted over short or long periods of time depending on the context or the internal state.

### 3.2. Convergent data in human healthy subjects

Preclinical studies are congruent with data from human brain imaging showing an activation of the amygdala in response to both positive and negative stimuli. For instance, a meta-analysis revealed that negative as well as positive stimuli were likely to stimulate the amygdala (Costafreda et al., 2008). First studies in humans suggested that the amygdala is highly activated by negative stimuli and more specifically during fear response (Davis and Whalen, 2001). This may explain why the field primarily focused on the prominent role of this structure in threatening situations. These early studies were probably biased as it is hard to match the arousing properties of stimuli with negative and positive valence. Moreover, in some studies only the response to the fearful stimuli were analyzed in patients with amygdala lesions (Adolphs et al., 1994). However, when positive stimuli are arousing enough, such as humor or erotic pictures, activation of the amygdala is as important as for negative stimuli. In agreement, Pichon et al. (2015) show a larger activation of the amygdala in response to positive emotional movies, compared with negative ones. In the meta-analysis by Costafreda et al. (2008), it was also reported that all kinds of sensory stimuli can switch on the amygdala, but gustatory and olfactory stimuli have the strongest effect. Moreover, it was recently shown that the human amygdala responds to both pleasant and unpleasant odorant stimuli, covering the complete spectrum of valences (Jin et al., 2015). Conversely, autobiographic recalls are not efficient at activating the amygdala (Costafreda et al., 2008). Task instructions might have an impact, as attentional processing tends to decrease amygdala activity, maybe to ensure maintenance of performance of high-level cognitive function in the presence of disrupting emotional stimuli. The more complex the task is, the more it involves regulatory systems such as the pre-frontal cortex. In addition, some evidence pinpoints hemispheric specialization with a left-lateralization for stimuli involving language and a right-lateralization for masked stimuli (Costafreda et al., 2008).

A bias in the attribution of stimuli valence may explain emotional disturbances during depressive and manic states. An imbalance in the activation of neurons encoding positive and negative valence of the amygdala could represent a mechanism for perception modifications of the environment, changes in emotional responses and their associated inhibited or activated behavior in BD.

## 4. Suggested lines of research

To guide research perspectives, we propose to deconstruct the mood-based model of BD, characterized by a unidimensional scale ranging from sadness to euphoria, and replace it with an emotional response-based model that can serve as a conceptual framework. This new model we present here relies on the two main features of emotions, intensity and valence. It characterizes the variations of emotional responses during BD mood states using clinical data and allows us to better describe the complexity of emotional processing. Our model is based on a dimensional and translational approach, on a major domain which is emotional processing, as suggested by the RDoC programme to better understand the pathophysiology of mental illnesses (Insel, 2014).

Measuring emotional responses in connection with amygdala

activity seems to be crucial to better understand the pathophysiology of BD. In pre-clinical studies, animal models of depression and mania could help to assess the existence of perceptual biases and to understand their mechanisms.

Concerning clinical assessment, it would be worth developing research on variations in emotional responses in patients considering intensity and valence in a longitudinal way, potentially with digital monitoring, and evaluate them during the different mood episodes.

Until now, the strongest data on emotional bias existing in BD patients rely on facial emotional expression recognition. However, this remains a complex task that requires complex cognitive treatment, potentially activating the amygdala to a lower level. Studying the valence attribution to simpler stimuli could be a good way to understand how congruent emotions are generated.

About imaging experiments, it would be relevant to distinguish the activation of the different amygdala nuclei to see if human studies are consistent with animal data. Recent developments in high-resolution and high-field MRI allow for a reliable delineation and functional recording of amygdala sub-nuclei in humans, but it has not yet been applied to BD (Saygin et al., 2017). Finally, even if a systematic review synthesizes data on resting-state functional connectivity in BD patients during remission, we lack knowledge on functional connectivity between the amygdala and its projection areas involved in valence processing during acute episodes (Li et al., 2015; Man et al., 2019).

This research field provides new avenues to explore the pathophysiology of mood disorders (depression and BD) but also treatment responses. Lithium, which is the gold standard of BD treatment, modifies the amygdala structurally and functionally (Hartberg et al., 2015; Strakowski et al., 2016). However, some patients do not respond to lithium and others have side effects that require its discontinuation (Licht, 2012). On the other hand, a large proportion of depressed patients (30 %) do not respond to monoaminergic antidepressants (AD). Interestingly, it has been shown that AD may act to restore the balance between positive and negative emotional processing early in treatment, prior to mood improvement (Harmer et al., 2017). For example, 7 days of AD or even an acute dose were found to increase the perception of ambiguous faces as happy and the recall of positive self-referent words in both healthy volunteers and depressed patients compared to placebo (Harmer et al., 2009). This raises the question whether the restoration of emotional bias is a final pathway to all AD for the restoration of mood, and if early emotional bias restoration could predict it delayed response.

If the mechanisms explored are crucial in the pathophysiology of BD and its recovery, it will become essential to test new molecules on their ability to change valence attribution in very early phases (pre-clinical) of the development and phase I in human. The slow onset, unclear biological markers, and variable clinical efficacy even of approved psychiatric drugs makes the potential efficacy of candidate drugs difficult to measure and has led many pharmaceutical companies to withdraw from drug development. Biomarkers that capture how effective drugs modulate the brain's functional anatomy could prioritize candidate compounds for large clinical trials, thus improving the productivity and cost-effectiveness of drug development.

## 5. Conclusion

Recent literature, with extremely converging data, highlights the essential role of the amygdala in attributing the stimuli's valence, influencing emotional and behavioral responses. These data lead us to question the limits of current bipolar models in which the amygdala is involved as a whole, without considering its diversity and complexity. An imbalance in the activation of neurons preferentially encoding either positive or negative valence could have a great impact in the global perception of the environment, thus change dramatically emotional responses and their associated inhibited or activated behaviors.

We propose an original, emotion-based model to better describe the

different mood episodes that could be completed with assessment of other dimensions like sensory perception or motricity, to help classify BD patients within homogeneous groups, probably sharing the same pathophysiology. A better understanding of the fundamental dimensions of BD should allow us to propose more personalized treatments. For now, the genetically identified neurons in the rodent amygdala could be the starting point to develop specific drugs targeting the emotional dimension.

Exploring the mechanisms of altered emotional processing would be of great help to understand the global outcome of BD. Many patients have progressive disappearance of manic phases with more depressive episodes that become longer and more resistant to treatments, suggesting a progressive loss of neuronal activity encoding positive valence.

Emotional bias is a major component of BD and valence assignment can be assessed both in humans and animal models. Thus, it represents a tremendous opportunity to better understand the pathophysiology of BD and the response to treatment.

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## Declaration of Competing Interest

Authors have no conflict of interest for this review.

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Les troubles bipolaires : de l'humeur aux émotions.

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Résumé : Les troubles bipolaires sont caractérisés par une alternance de phases dépressives et d'exaltations. Il s'agit d'une pathologie fréquente, survenant chez l'adulte jeune, qui peut avoir un l'impact considérable sur le fonctionnement des patients du fait de la fréquence et de la sévérité des épisodes, des comorbidités psychiatriques et somatiques, des symptômes résiduels ou encore de l'altération des fonctions cognitives. Le risque majeur est le suicide. Son traitement repose sur la prescription de régulateurs de l'humeur et des prises en charge psychothérapeutiques. La pathophysiologie implique des interactions gènes-environnement et au niveau cérébral est sous-tendue par des anomalies du système cortico-limbique. A l'heure actuelle, les modèles animaux qui permettraient de mieux comprendre les mécanismes cellulaires et moléculaires sont imparfaits car ils n'explorent qu'une partie limitée des dimensions qui constituent les troubles. L'évaluation de l'humeur du fait de son caractère subjectif est le propre de l'Homme et n'est donc pas accessible à l'expérimentation animale. Nous souhaitons montrer l'intérêt d'étudier à la fois chez l'homme et chez l'animal les biais émotionnels, en évaluant les biais d'attribution de valence en réponse à des stimuli hédoniques, qui sont quantifiables chez l'animal par les comportements d'approche et d'évitement. Nous proposons ainsi un nouveau modèle des troubles bipolaires basé non plus sur l'humeur mais sur l'étude des réponses émotionnelles tenant compte de leur intensité et valence.

Mots clefs : troubles de l'humeur, biais émotionnels, système limbique

Abstract: Bipolar disorders are characterized by alternating depressive and exaltation phases. It is a frequent pathology, occurring in young adults, which can have a considerable impact on the patients' functioning due to the frequency and severity of the episodes, but also to psychiatric and somatic comorbidities, residual symptoms or altered cognitive functions. The major risk is suicide. Its treatment is based on the prescription of mood stabilizers and psychotherapeutic interventions. The pathophysiology relies on gene-environment interactions and at brain level is underpinned by abnormalities in the cortico-limbic system. Animal models that would allow a better understanding of cellular and molecular mechanisms are imperfect because they only explore a limited part of the dimensions that constitute the disorder. The evaluation of mood, because of its subjective character, is unique to humans and is therefore not accessible to animal experimentation. In a recent review, we have shown the interest of studying emotional biases in both humans and animals, quantifying the biases in attributing valence in response to emotional stimuli, for instance with approach and avoidance behaviors. We propose a new model of bipolar disorders no longer based on mood but on the study of emotional responses integrating their intensity and valence.

Keywords: mood disorders, emotional bias, limbic system

## Les troubles bipolaires en quelques chiffres

Le trouble bipolaire, ancienne psychose maniaco-dépressive, est une maladie fréquente avec une prévalence de 2 à 4 % en fonction de la sévérité des troubles [1]. Elle est caractérisée par la récurrence de phases de dépression, d'exaltation maniaque ou hypomane, ou encore d'états mixtes, qui associent des symptômes dépressifs et maniaques) [2]. La présence d'épisodes maniaques ou mixtes définit les troubles bipolaires de type I, alors que les troubles de type II sont caractérisés par des épisodes d'exaltation de moindre intensité, qualifiés d'hypomanie. Entre les épisodes, les patients peuvent avoir des phases de rémission plus ou moins longues et de plus ou moins bonne qualité [3]. Certains patients présentent des symptômes subsyndromiques ou des comorbidités qui impactent leur fonctionnement global [4,5]. Il s'agit d'une pathologie débutant la plupart du temps chez l'adulte jeune mais il se passera en moyenne dix ans entre le début des troubles et le diagnostic, qui ne débouche malheureusement pas toujours sur des prises en charge en accord avec les recommandations internationales [6]. En effet, la grande hétérogénéité des troubles et les nombreuses pathologies associées rendent parfois leur prise en charge complexe. Ceci explique en partie que l'espérance de vie des patients bipolaires est réduite de dix ans, du fait du taux élevé de suicide (15 % décèdent par suicide), mais également des comorbidités psychiatriques (addictions, troubles anxieux) ou somatiques (diabète, pathologie cardio-vasculaires...) [7,8]. Le traitement repose sur des thymorégulateurs au long cours et des prises en charge psychothérapeutiques. Malgré cela, les rechutes peuvent être fréquentes et impacter le devenir du patient.

Les défis concernant cette pathologie sévère et fréquente sont :

- Améliorer la précocité du diagnostic et les prises en charge
- Comprendre la physiopathologie afin de développer des traitements plus spécifiques

### Améliorer le diagnostic et les prises en charge

Il s'agit de troubles débutants généralement vers l'âge de 25 ans, bien qu'il existe des formes à début plus précoce à l'adolescence, voire chez l'enfant, et des formes plus tardives. A l'heure actuelle, le retard au diagnostic en moyenne de dix ans est causé par plusieurs facteurs. Les formes débutant par des épisodes dépressifs ou à polarité dépressive prédominante sont plus difficiles à diagnostiquer car la polarité exaltée est absente ou discrète [6]. Au-delà du diagnostic de la pathologie, il est important d'évaluer les caractéristiques cliniques ainsi que les comorbidités tant psychiatriques que somatiques pour orienter vers des prises en charge plus spécifiques et aider les patients à organiser leur parcours de soin [8–11].

Le traitement repose sur les régulateurs de l'humeur ou thymorégulateurs dont il existe trois classes, le lithium, les anticonvulsivants et les antipsychotiques qui sont prescrits seuls ou en association [12,13]. Lors de phase dépressive, des antidépresseurs peuvent être ajoutés mais généralement de manière ponctuelle. La prescription d'antidépresseurs au long cours favorise le risque de virage de l'humeur et la survenue de cycle rapide [12,13]. La polarité prédominante des épisodes et le risque suicidaire sont également à prendre en compte dans le choix du traitement pharmacologique, avec une préférence pour le lithium lorsque le risque suicidaire est élevé [14].

A l'heure actuelle, il n'existe pas de biomarqueur, le diagnostic reposant uniquement sur la clinique et l'anamnèse des troubles. En France, un réseau de centres de recours a été développé pour une aide au diagnostic et à la prise en charge [15]. Les nouvelles technologies pourraient devenir de nouveaux supports au diagnostic et à la prise en charge en enregistrant des données de manière continue [16]. Ainsi, de nombreuses applications sur smartphone ont vu le jour pour aider les patients à suivre leurs variations de l'humeur et à repérer précocement de nouvelles décompensations. Cependant, il est important que des études rigoureuses soient menées afin d'évaluer l'intérêt et la fiabilité de ce type d'outils avant d'envisager leur utilisation à grande échelle [17].

### État des connaissances sur la physiopathologie

Les connaissances actuelles reposent principalement sur la génétique et l'imagerie cérébrale. Nous évoquerons les données les plus marquantes dans ces différents domaines. Comme dans la plupart des pathologies psychiatriques, il existe des facteurs génétiques et environnementaux [18]. Un enfant d'un parent atteint de trouble bipolaire a un risque environ dix fois plus élevé de développer la maladie et l'héritabilité du trouble est estimée à plus de 80 %. La composante génétique repose sur des gènes multiples ayant chacun un effet modéré. Ainsi les études d'association pangénomique (GWAS), qui permettent d'identifier sans a priori (contrairement aux études gènes candidats) des variations génétiques fréquentes sur l'ensemble du génome associées à une maladie, ont identifié de multiples *loci* de susceptibilité dont la plupart ne sont pas spécifiques mais sont communs à diverses pathologies psychiatriques telles que la schizophrénie, le déficit attentionnel ou l'autisme [19]. De rares variants délétères pourraient jouer un rôle dans certains cas de troubles bipolaires, mais leur identité et leur contribution globale à la maladie restent floues. Les voies impliquées dans la prédisposition génétique aux troubles bipolaires comprennent la régulation hormonale, les canaux calciques, les seconds messagers, la signalisation du glutamate ainsi que le développement neuronal [20]. Certaines

circonstances environnementales, en modulant l'expression de ces gènes, favorisent l'émergence du trouble ou des formes plus sévères. Parmi ces facteurs environnementaux, les traumatismes psychologiques dans l'enfance, la prise de toxique ou des incidents obstétricaux sont reconnus comme ayant un impact sur le développement et/ou la sévérité de la maladie [21,22].

Il résulte de ces facteurs des modifications de certains circuits cérébraux touchant notamment la sphère émotionnelle, aussi bien en termes structural que fonctionnel. Ainsi, globalement, les systèmes de contrôles préfrontaux, notamment le cortex orbitofrontal et le cingulaire antérieur, présentent un déficit d'inhibition sur des structures plus profondes comme l'amygdale ou l'hippocampe [23]. Cependant, l'imagerie cérébrale ne nous livre qu'une connaissance très parcellaire de la physiopathologie, sans accès à l'échelon cellulaire et moléculaire. Pourtant, les avancées fantastiques des neurosciences en préclinique de ces deux dernières décennies, reposant notamment sur la possibilité de manipuler des neurones très ciblés par des modifications génétiques spécifiques, permettent d'explorer les mécanismes intimes des comportements et de leurs altérations chez des animaux libres de tout mouvement. Pour bénéficier de ces avancées, il est nécessaire d'avoir des modèles animaux de troubles de l'humeur valides, sur lesquels nous reviendrons dans le chapitre suivant.

### De l'humeur aux émotions

L'humeur est le critère principal de toutes les classifications pour définir les états thymiques survenant au cours des troubles bipolaires. Le Larousse définit l'humeur comme un état affectif qui s'installe généralement dans une certaine durée et ayant des variations entre tonalités agréable et désagréable. Ces états relativement mal définis et de durées variables sont peu compatibles avec les paradigmes de recherche clinique utilisant l'imagerie fonctionnelle. De plus, explorer l'humeur dans des modèles animaux est impossible car elle est propre à la nature humaine du fait de sa subjectivité. Ce constat nous a incité à réfléchir aux composants essentiels qui participent à générer cette humeur et qui pourraient servir de relais pour explorer les fonctions cérébrales sous-tendant ces variations affectives. De manière évidente, nous nous sommes tournés vers les émotions tant l'humeur est liée à la manière de ressentir les émotions. L'étude des émotions est facilitée par des critères permettant de les définir et donc de les caractériser. Les émotions sont des réponses brèves à des stimuli, suffisamment marquants pour déclencher des changements physiologiques destinés à réagir par un comportement adapté [24,25]. Elles sont caractérisées selon deux dimensions : l'intensité et la valence, c'est-à-dire la valeur hédonique attribuée aux stimuli [26]. Ces réponses émotionnelles universelles peuvent

être étudiées aussi bien chez l'homme que chez l'animal en évaluant chez ces derniers les comportements de fuite ou d'approche qu'elles déclenchent en fonction de leur caractère plaisant ou déplaisant.

A partir de données de la littérature et de nos travaux de recherche, nous avons proposé un nouveau modèle pour les troubles bipolaires, non plus défini par l'humeur mais par les émotions [27]. Selon ce modèle, toutes les phases thymiques peuvent être décrites selon leurs réponses émotionnelles associées. Ainsi, les états maniaques et hypomanes sont caractérisés par des réponses émotionnelles plus intenses qu'habituellement, associées à un biais émotionnel positif. A savoir, les stimuli neutres sont perçus comme plus plaisants, les stimuli positifs deviennent plus « récompensants » et orientent les comportements vers la recherche de plaisirs, tandis que les stimuli négatifs sont ressentis comme moins aversifs, entraînant une possible plus grande prise de risques. Au contraire, les dépressions sont sous-tendues par une abrasion globale de la capacité à éprouver les émotions. A cela s'ajoute un biais émotionnel négatif associant une moindre valeur hédonique aux stimuli plaisants, pouvant déboucher sur une anhédonie (impossibilité à éprouver du plaisir). Les stimuli négatifs, quant à eux, sont plus aversifs. Il semble que lorsqu'il existe une abrasion des affects, la capacité à ressentir des émotions négatives soit conservée plus longtemps. Dans une approche phylogénétique, on peut supposer qu'il s'agit d'un processus de préservation des espèces pour maintenir le plus longtemps possible les comportements de fuite face aux dangers. Notre modèle permet de distinguer des états dépressifs avec caractères de mixité qui se distinguent des dépressions classiques par une intensité émotionnelle augmentée. Cet élément pourrait être un facteur d'orientation du choix thérapeutique, les antipsychotiques étant particulièrement efficace pour atténuer l'intensité des émotions. Les états maniaques avec caractéristiques de mixité partagent cette même intensité des réponses émotionnelles mais la tonalité globale des affects est positive.

L'intérêt de ce modèle est multiple. D'une part, il permet de donner des définitions plus cohérentes des états mixtes, qui dans les classifications actuelles sont inopérantes. Cela conduit à de grandes variations dans l'estimation de la fréquence des états mixtes en fonction des évaluateurs [29]. D'autre part, il pourrait servir de base à une stratification des traitements. Les recommandations internationales sont à l'heure actuelle très décevantes pour le traitement pharmacologique des dépressions bipolaires, proposant sans distinction des molécules très variées avec parfois des mécanismes d'action opposés. Ainsi, au-delà des thymorégulateurs classiques tels que le lithium ou l'acide valproïque préconisés en première intention, il est possible de prescrire des antipsychotiques qui ont tous des propriétés antagonistes dopaminergiques, ou des antidépresseurs dont la plupart ont une action favorisant la libération

des monoamines, sérotoninergique, noradrénergique, voire ayant une action dopaminergique [30]. L'usage des molécules ayant des profils pharmacologiques aussi différents souligne l'hétérogénéité des tableaux cliniques des dépressions bipolaires et de leurs physiopathologies sous-jacentes. Enfin, ce modèle permet de développer des tests translationnels et donc d'étudier chez l'animal les mécanismes cellulaires et moléculaires impliqués dans les troubles bipolaires.

### Modèles animaux des troubles bipolaires

A l'heure actuelle, aucun modèle animal n'est capable de reproduire la cyclicité des troubles bipolaires, et tous reposent sur des modèles supposés de manie [31]. Ils consistent en des manipulations pharmacologiques, génétiques ou environnementales [32]. Les manipulations touchant notamment le système dopaminergique - les psychostimulants chez l'homme induisant des symptômes proches des états d'exaltation ou entraînant des décompensations chez les patients bipolaires - sont utilisés pour induire des modèles de manie. La caractéristique principale de ces modèles est d'induire une hyperactivité locomotrice. Utilisée comme un des critères majeurs de modèle de manie, cette caractéristique n'est pourtant pas pathognomonique des états maniaques, et se retrouve fréquemment dans les troubles avec déficit attentionnel et hyperactivité. La locomotion est ainsi évidemment loin d'être suffisante. Ceci nous a conduit à évaluer les biais émotionnels dans des modèles animaux de trouble de l'humeur (données non publiées). Chez l'homme, les biais émotionnels sont le plus souvent étudiés par des stimuli visuels avec des images à tonalités positives, négatives ou neutres ou bien par la reconnaissance d'expressions faciales ambivalentes [33], mais finalement ils concernent toutes les modalités sensorielles [34]. Ainsi, nous avons observé que des patients déprimés bipolaires percevaient les odeurs comme plus déplaisantes en comparaison à des sujets contrôles ou des patients bipolaires normothymique (données non publiées). Ces biais émotionnels olfactifs sont retrouvés également chez des patients unipolaires et semblent régresser avec l'amélioration de l'humeur. Nous avons donc mis au point un test chez la souris pour évaluer les biais d'attribution de valence à des odeurs, les stimuli visuels étant beaucoup plus difficiles à étudier chez les rongeurs. Ce test consiste à exposer des souris à des odeurs de valences naturellement positive et négative, et de mesurer le comportement d'approche ou d'évitement qui en résulte. Nous avons ainsi observé qu'il existe bien un biais émotionnel négatif dans un modèle de souris présentant un phénotype dépressif induit par administration chronique de corticostérone, les odeurs plaisantes étant perçues comme moins attractive et les odeurs négatives comme plus aversives (données non publiées).

## Intérêt de tester chez l'animal les biais émotionnels

Les biais émotionnels étant une composante essentielle des troubles de l'humeur au même titre que la motivation ou de l'activité locomotrice, il est important de pouvoir explorer les mécanismes qui les sous-tendent. Cela s'inscrit dans une approche dimensionnelle prônée par le RDoC programme qui propose d'étudier des dimensions correspondant à des fonctions cérébrales altérées dans les pathologies mentales, plutôt que d'étudier les syndromes dans leur globalité, démarche caduque du fait de leur hétérogénéité [35].

Concernant les biais émotionnels, des données récentes issues de travaux précliniques ont permis de disséquer les mécanismes de l'attribution des valences, indépendamment de la modalité sensorielle du stimulus (pour revue voir [27]). Les afférences sensorielles provenant du thalamus et des cortex sensoriels convergent toutes vers l'amygdale et plus spécifiquement vers son noyau basolatéral (BLA). L'amygdale semble donc jouer un rôle crucial pour déterminer la valeur hédonique d'un stimulus, afin de déclencher le comportement le plus adapté. Dans le passé, l'amygdale était considérée comme le centre de la peur, servant d'alerte pour décoder les situations de danger. En réalité, même si cette structure semble plus sensible pour coder les stimuli négatifs, elle répond également aux stimuli positifs [26]. Ces mécanismes dépendent de l'activation de deux types de neurones génétiquement distincts, dont les uns s'activent en présence de stimuli positifs et les autres avec les stimuli négatifs, l'activation d'une sous-population antagonisant en partie la seconde [36]. Ces deux populations de neurones sont entremêlées au sein de la BLA, bien que certains auteurs décrivent des gradients de densité spécifiques au sein de cette structure [36,37]. De plus, ces neurones ont des aires cérébrales de projection préférentielles distinctes. Ainsi, ceux codant pour les stimuli positifs vont en majorité vers le noyau accumbens (NAc) tandis que ceux décryptant les signaux négatifs projettent vers le noyau central de l'amygdale et l'hippocampe [37–39]. En comparant les animaux présentant un biais émotionnel négatif à des animaux contrôles, nous avons exploré les mécanismes qui le sous-tendent. Confirmant notre hypothèse, il existe bien une activation diminuée des neurones projetant de la BLA vers le NAc, et une augmentation de ceux allant vers le noyau central (données non publiées). Nous sommes en train d'évaluer le rôle causal de ces populations de neurones sur les biais émotionnels. L'amygdale est une structure très conservée dans l'évolution des espèces, ce qui laisse supposer que les données chez l'animal et les hypothèses étayées par les résultats précliniques vont pouvoir orienter des études cliniques [40].

Conclusion : Bien que décrits depuis l'Antiquité, les troubles bipolaires restent difficiles à diagnostiquer et à traiter. Une meilleure connaissance de leur physiopathologie pourrait aider à identifier des biomarqueurs et développer des traitements plus spécifiques. Malheureusement, la définition reposant sur le critère de l'humeur rend difficile l'utilisation d'approches de recherche translationnelle, et notamment des modèles animaux. Mis à part la mise en évidence d'un mécanisme majeur pouvant rendre compte des troubles de l'humeur, l'intérêt de l'étude des biais émotionnels tient dans la possibilité de tester de nouvelles molécules à visée antidépressive. En effet, des travaux chez l'homme ont montré que des antidépresseurs de type monoaminergiques nécessitent la restauration des biais émotionnels négatifs des patients déprimés pour avoir une amélioration de l'humeur [28]. Ces travaux doivent s'étendre à l'ensemble des molécules antidépressives ayant des mécanismes d'action très différents afin de savoir s'il existe une voie finale commune à tous les antidépresseurs qui passe par la restauration de ces biais émotionnels. Enfin, il est possible de tester en préclinique de potentiels effets dépressogènes de toute nouvelle molécule, par l'induction de biais émotionnels négatifs. A titre d'exemple, le rimodaban, molécule anorexigène, a dû être retiré du marché du fait de son imputabilité dans des gestes suicidaires. Testé plus tard chez l'animal, il apparaît qu'il induisait des comportements liés à un biais émotionnel négatif [41].



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## RESULTS

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### 2.1 Objectives and Methodology

As previously detailed, mood is a subjective symptom barely evaluable in animal. Disturbed emotional processing plays an important role in the causation and maintenance of mood changes associated with mood disorders. In particular, mood episodes can be described with modifications in intensity and valence of emotions. Investigating mood-congruent emotional biases in rodents, meaning positive bias in manic-like and negative bias in depressive-like states, could bring us insights on the neurobiological alterations underlying mood disorders. Indeed, the role of BLA neurons in valence processing starts to be unraveled, with different circuits preferentially encoding either positive or negative valence. Following our hypothesis stating that specific modifications in the BLA circuits during mood disorders could lead to emotional biases, our first goal was to identify corresponding hedonic biases in animal models of mania and depression. To do so, we chose two pharmacological models: (i) administration of GBR 12909, a dopamine transporter inhibitor, as a mouse model of mania and (ii) administration of corticosterone, the rodent stress hormone equivalent to cortisol in humans, as a mouse model of depression. Good face, construct and predictive validities were previously demonstrated in the literature for these models. In order to measure valence assignment biases corresponding to hedonic biases in mice, we set up an olfactory preference test. With this paradigm, spontaneous approach or avoidance behaviors in front of innate appetitive or aversive odors offer a proxy for the hedonic valence the animal assigned to these olfactory stimuli. Relying on innate instead

of learned valence allow to put aside potential cognitive deficits induced when modeling mania or depression.

The second objective was to study BLA circuits-specific functional alterations following the pharmacological manipulation of mood state. Considering that the BLA-to-NAc neurons are mainly involved in positive valence encoding and the BLA-to-CeA counterparts mostly encode negative valence, we labeled these two populations by injecting retrograde dyes in the NAc and the CeA. Immunohistological identification of neurons expressing the immediate-early gene cFos was used as a proxy for BLA neuronal activity in response to different appetitive and aversive odors.

Finally, to test the causal role of specific BLA circuits in the expression of hedonic bias by animal models of mood disorders, we employed a chemogenetical approach. By virally-inducing neuronal expression of DREADD (Designer Receptor Exclusively Activated by Designer Drugs), we were able to activate BLA-to-NAc and BLA-to-CeA neurons either in control mice or in models of mood disorders.

### **Objectives achievement in models of mania and depression**

As reported in the following study, we were unable to elicit a positive olfactory hedonic bias by injecting GBR 12909, while the hyperlocomotion behavior usually used as main criteria to define mania models was present. On the contrary, we highlighted a strong negative olfactory bias in GBR-treated mice, that was further confirmed in a gustatory preference test measuring consumption of both appetitive and aversive tastants. Since our first objective of describing a positive hedonic bias in a mouse model of mania was not reached, we did not go further in the investigation of BLA circuits modifications induced by GBR 12909 administration.

On the contrary, corticosterone-treated mice exhibited a negative olfactory hedonic bias as we predicted, along with the already described anxiety- and depressive-like phenotypes. Therefore, we could perform all the experiments described above to study the role of BLA circuits in the negative olfactory hedonic bias induced by chronic corticosterone administration.

## 2.2 Negative hedonic bias in a mouse model of mania (*submitted*) ..... 87

### *Summary*

Beyond mood, emotional biases differentiate bipolar states in humans. Mania is associated with positive biases, *i.e.* emotional stimuli become more rewarding and less aversive, and the opposite for depression. A mania model is therefore expected to exhibit positive hedonic bias. In the following study, we measured olfactory and gustatory hedonic responses to both positive and negative innate valence stimuli in a pharmacological mania model. Following injection of GBR 12909, we first observed the classical hyperactivity phenotype, along with low depressive-like but high anxiety-like behaviors. To our surprise, GBR 12909-treated mice exhibited strong negative hedonic biases, with lower appetitiveness of positive odor and taste stimuli, and higher aversiveness of negative odor and taste stimuli. Consequently, we suggest the GBR 12909 model of mania is not appropriate for studying emotional disturbances associated with mania states. Locomotor activity is no longer sufficient to measure validity of mania models. These results underline the interest of olfactory and gustatory preference tests as complementary translational assessment for emotional states biases observed in bipolar disorders.





# Negative hedonic bias in a mouse model of mania

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## Highlights

- Emotional bias is an essential feature of bipolar disorders in humans.
- Surprisingly, GBR 12909 mania model displayed a strong negative hedonic bias.
- Locomotor hyperactivity and combativeness are insufficient to define a mania model.

## **Abstract**

Bipolar disorders are defined by recurrences of depressive and manic episodes. The pathophysiology is still unknown, and translating clinical symptoms into behaviors explorable in animal models is challenging. Animal models of bipolar disorder do not exist because it is not possible to mimic cyclicity of the disease, and it is therefore necessary to study mania and depression models separately. Beyond mood, emotional biases differentiate bipolar states in humans. Mania is associated with positive biases, *e.g.* emotional stimuli become more rewarding and less aversive, and the opposite for depression. We propose to assess behavioral hedonic responses to innately appetitive and aversive olfactory and gustatory cues in mice as proxies for the assigned emotional valence. A mania model is therefore expected to exhibit positive hedonic bias. Using the GBR 12909 mania model, we observed the classical hyperactivity phenotype, along with low depressive-like but high anxiety-like behaviors. Contrary to our expectations, GBR 12909-treated mice exhibited strong negative hedonic biases. Consequently, we suggest the GBR 12909 model of mania is not appropriate for studying emotional disturbances associated with mania states. Locomotor activity is no longer sufficient to measure validity of mania models. We propose olfactory and gustatory preference tests as complementary assessment for emotional states biases.

## **Keywords**

bipolar disorders, mania, GBR 12909, olfaction, emotional state, animal models

## 1. Introduction

Bipolar disorders (BD) are defined by alternating phases of depression, mania or mixed exaltation, separated by periods of remission. Depressive and manic episodes are characterized by opposite symptoms (sadness/expansive mood, fatigue/increased energy, psychomotor retardation/agitation, *etc.*) and mixed states by a mixture of both depressive and manic symptoms. Due to subjectivity of diagnosis and reporting, these clinical criteria are difficult to transpose to animal models (Sharma et al., 2016). Such obstacles hinder the transfer of the immense progress in pre-clinical neuroscience made over the last two decades about neural mechanisms involved in major brain functions to the psychiatric field.

To overcome this issue, we need to translate the clinical criteria into domains or basic function alterations that are measurable both in humans and animals to be accessible for neurobiological multilevel analysis (Corlett and Schoenbaum, 2020; Cosgrove et al., 2016; Insel, 2014). Emotional processing is an essential domain affected in BD. However, this field of research has long been neglected due to the lack of clear definition of emotions that is suitable across species. The current consensus stipulates that emotions are brief physiological and behavioral responses to stimuli described by several dimensions, including valence (Anderson and Adolphs, 2014; Zych and Gogolla, 2021). Moreover, emotion expressions are thought as windows into the internal affective state of an individual across species from insect to humans (Zych and Gogolla, 2021). Based on this operational formalization, emotional processes can be explored both in clinical and pre-clinical studies. For instance, approach or avoidance behaviors are quantifiable motor readouts indicating the animal assigned valence of the presented stimuli and expressing an indirect measure of the triggered emotion (Tye, 2018; Zych and Gogolla, 2021).

We recently proposed an emotion-based model for bipolar disorders, contrasting with the current diagnostic frame built on mood (Bigot, 2020). According to our model developed from clinical data, many symptoms of manic and depressive states are linked to emotional biases, *e.g.* misattribution of the hedonic value or valence of stimuli. In manic patients, positive emotional biases lead to increased search of pleasure and risk-taking behaviors by decreasing the perception of danger. Conversely, depressive

states are characterized by emotional negative biases accounting for loss of pleasure. It is therefore crucial to study emotional biases in animal models of BD to better understand the pathophysiology of this key altered dimension. Importantly, thanks to recent techniques allowing the manipulation of specific neuronal networks, neuroscience has already made considerable progress in the knowledge of emotional valence neural processing (Namburi et al., 2016).

Animal models of BD are restricted to mania models because the cyclicity of the disease is almost impossible to reproduce (Cosgrove et al., 2016). These models have relied on pharmacological, genetic or environmental manipulations. Because a dysregulated dopaminergic system seems implicated in the etiopathology of the manic state, and because psychostimulants in humans induce manic symptoms, the injection of amphetamine is a common way to create a manic-like state in animals (Ashok et al., 2017; Logan and McClung, 2016; Young et al., 2011). However, this drug is also used for modelling other diseases such as schizophrenia, ADHD and drug abuse, and therefore is not specific for BD (Logan and McClung, 2016; Young et al., 2011). Consequently, amphetamine induces not only hyperlocomotion, but also addiction and hallucinations, generating several confounding factors. Another model has been developed based on a very specific dopamine reuptake inhibitor, GBR 12909 (Young et al., 2010). Human studies reported that polymorphisms of the gene coding for the dopamine transporter are associated with BD (Pinsonneault et al., 2011; Vaughan and Foster, 2013) and reduced striatal dopamine transporter levels have been observed in BD patients (Anand et al., 2011). Like classical psychostimulants, GBR 12909 induces significantly increased locomotor activity and a hyper-exploratory profile such as seen in BD patients (Young et al., 2010). Moreover, the effects on locomotion are reversed by lithium, valproate or aripiprazole (Bastos et al., 2018).

Therefore, based on its apparent correct face, construct and predictive validity criteria, we chose the GBR 12909 mania model to assess hedonic biases in response to olfactory and gustatory preference tests. Locomotion, anxiety and depressive-like behaviors were measured to complete the phenotype evaluation. We initially hypothesized GBR 12909 to trigger positive hedonic biases in response to innately attractive or aversive odor and taste cues.

## 2. Materials and methods

### 2.1. Animals

All animal care and experimental procedures followed national and European (2010/63/EU) guidelines and were approved by the French Ministry of Research (APAFiS: #16380-2018080217358599\_v1). C57BL/6N male mice (12–16 weeks old) purchased from Taconic Farms (Denmark) were used for all behavioral tests (n = 74). Mice were socially housed, 4-6 per cage, and maintained under standard housing conditions ( $23 \pm 1$  °C; humidity 40%) with a 14/10 h light/dark cycle (lights on from 6:30 AM to 9:30 PM) and food and water *ad libitum*, except for some behavioral experiments.

### 2.2. Drugs

GBR 12909 dihydrochloride (16 mg/kg, Sigma-Aldrich, Germany) was prepared in sterile saline solution for a final injection volume of 0.1 ml/10 g body weight, and dissolved after ~ 45 min heating at 40-60 °C. The animals received freshly made up solutions through intra-peritoneal injections between 0.5 and 1 h before behavioral tests.

### 2.3. Behavioral assessment

Before starting behavioral tests, mice were handled ~ 30 s at least twice a day for 3 days to habituate to the experimenter. All behavioral tests were conducted on separate days during the period of light (10 AM-7 PM).

2.3.1. Open-field. Animals were placed in Plexiglas containers (43 x 43 cm) and their behavior was recorded by a video camera during 20 min. A tracking system (Noldus Ethovision 3.0, Netherlands) was used to map the center and to measure the time spent in this zone and the total distance moved, expressed as percentages.

2.3.2. Elevated plus maze. The test was conducted using a plus-cross-shaped apparatus made of grey Plexiglas that was elevated 50 cm above the floor and comprised two open and two closed arms (30 x 7 cm) that extended from a central platform (7 x 7 cm). The Noldus Ethovision 3.0 tracking system was used to record behavior for 5 min. The probability of

entry in the open arms was calculated as the number of entries in the open arms divided by the total number of entries in the closed and open arms, and expressed as a percentage.

- 2.3.3. Tail suspension test. Mice were suspended at approximately one-third from the end of the tail, using regular tape, to a metal rod about 30 cm from the table, for 6 min. Upon viewing of the video recordings blindly to the treatment, the total time spent in an immobile posture was measured and expressed as a percentage.
- 2.3.4. Olfactory preference test. The olfactory preference test was adapted from Pérez-Gómez et al. (2015) and performed in a quiet and dimly lit room. Clean housing cages with regular bedding material were used as testing arenas, covered by transparent Plexiglas lids. A petri dish with a hooled cover was placed and adhered to one side of the arena for defining an odor zone. The Noldus Ethovision 3.0 system was used to track the position and locomotor activity of the mice for 15 min. During the first 4 days, only a Whatman paper filter (GE Healthcare Life Sciences, USA) was placed into the petri dish, to assess the baseline exploration. Then, 2 days were dedicated to each odor with the odorant placed on a paper filter in the following order: peanut oil (pure, 400  $\mu$ l), female urine (pure, 100  $\mu$ l), trimethylamine (Sigma-Aldrich, 6.75% in water, 400  $\mu$ l) and trimethylthiazole (Sigma-Aldrich, 5% in mineral oil, 400  $\mu$ l). The olfactory preference index was calculated as explained in Pérez-Gómez et al. (2015), as the difference between the time a given mouse spent in the odor zone when exposed to an odor and the average time of all saline-treated mice in this area during habituation (TH), divided by TH.
- 2.3.5. Olfactory detection test. Mice were trained using a custom-built computer controlled eight-channel olfactometer as previously described (Grelat et al., 2018, see also supplemental information). Odorants were diluted in odorless mineral oil (Sigma-Aldrich) for carvone+ (Sigma-Aldrich) or distilled water for 1-butanol (Sigma-Aldrich) to the desired concentration, and 10 mL of solution was used as the odorant source. The pairs of odorants used in the experiments were : carvone+ or 1-butanol vs their respective solvents. All mice were first trained to detect an odor at a high concentration without any treatment ( $10^{-2}$  v/v for Carvone+ and  $10^{-3}$  v/v for 1-butanol). After initial training, detection threshold

concentration were determined in mice injected with saline or GBR. Mice were given at least 200 trials in one session per day, one day out of two. The criterion performance was achieved when the mouse reached  $\geq 85\%$  of correct responses in at least one block of 20 trials. The concentration of the odor (S+ stimulus) was reduced 10-fold in the next session. If criterion performance was not achieved in 300 trials maximum, the preceding concentration was considered as the detection threshold.

2.3.6. Gustatory preference test. Mice were individually habituated to two 50 ml bottles filled with drinking water for 24 h. After habituation, the mice were given access to a two-bottle choice of water, or either sucrose solution (1%) or quinine solution (0.1 mM). Bottles containing water and tastant solution were weighed at several time points, 10 AM, 2 PM and 6 PM, for 48 h with the same solution, starting at 10 AM. The position of the bottles was changed (left to right, right to left) after each weight measurement to ensure that the mice did not develop a side preference. Animals were returned to their home cage for 24 h before starting the test with a new solution. Sucrose preference and quinine aversion were calculated as the percentage (amount of tastant solution consumed  $\times 100$  /total volume consumed of both tastant and water solution). Water and tastant solution bottles were prepared 24 h before their use and placed in empty cages to check leakage.

#### 2.4. Statistical analysis

Statistical analyses were performed with GraphPad Prism 9 software (USA). Normality was assessed using the Kolmogorov-Smirnov test. The Barlett test was then used to statistically compare the variances. Parametric or non-parametric tests were used accordingly : paired, unpaired or one sample Student, Mann-Whitney or Wilcoxon tests, One-way ANOVA or Kruskal Wallis tests with repeated measures when suitable, Two-way repeated measures ANOVA or Mixed-effect model when some values were missing. Following post-hoc analyses were applied with Holm-Sidak or Dunn corrections. All datasets were described using the mean; error bars in the figures represent standard error mean (SEM), except in Figure 3. Differences were considered significant for  $p < 0.05$ .

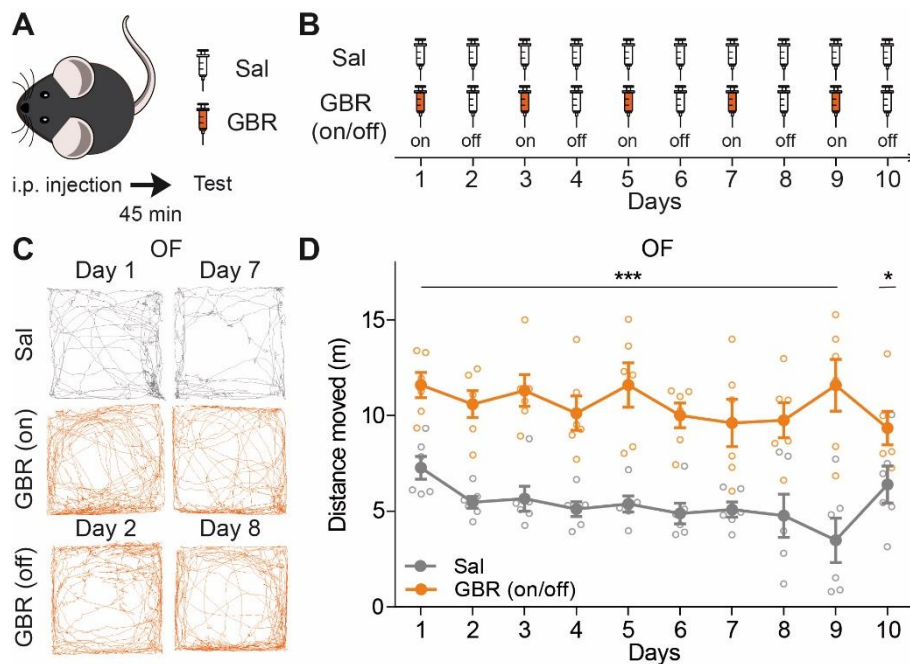
More detailed materials and methods can be found in supplemental information.



### 3. Results

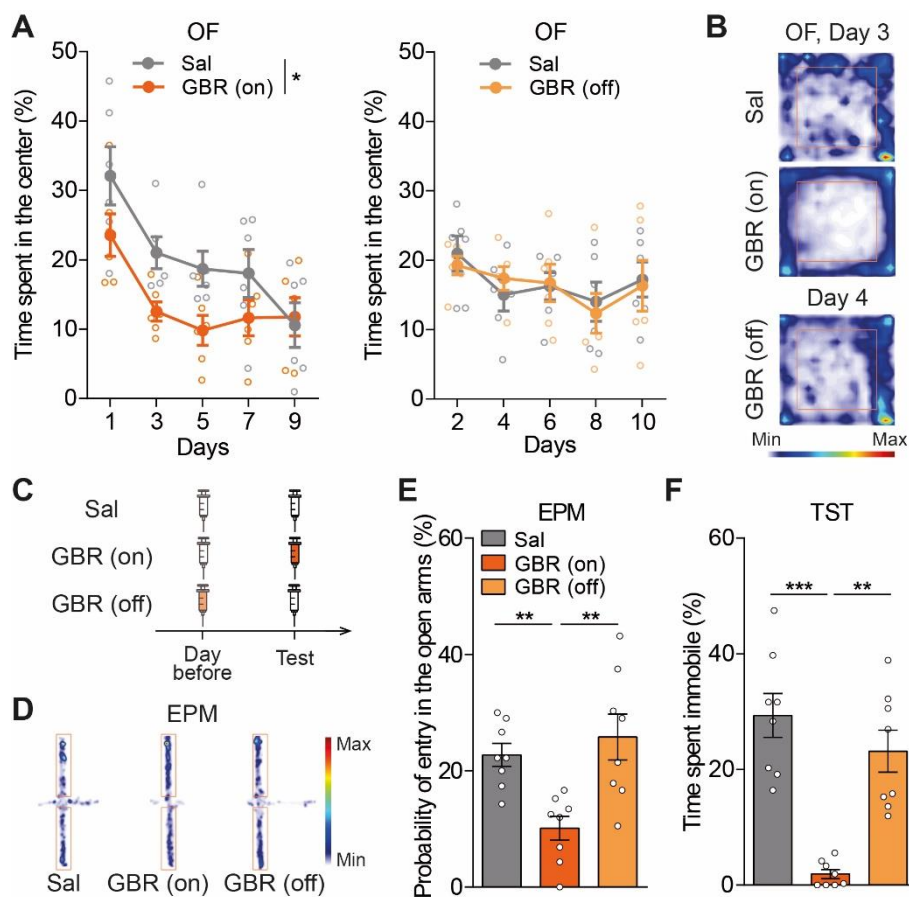
#### 3.1. GBR administration triggers hyperlocomotion, anxiety-like behaviors and combativeness

To assess hedonic biases, we needed a stable model of mania across multiple days. We injected GBR 12909 (GBR) every two days for 10 days (Figure 1A, B), alternating with saline (Sal) administration. Using the Open field test (OF), 45 min after either Sal or GBR administration (“on” days), GBR-injected mice showed a significant increase in locomotor activity relative to Sal-injected mice (Fig 1C, D). We observed that this regime of GBR administration maintained high levels of locomotion compared to Sal-treated mice during the whole 10 days of the experiment (Figure 1C, D). In particular, the typical hyperlocomotion observed for the “on” days was sustained to the “off” days. In contrast, daily GBR injections might have induced tolerance to the drug (Figure S1). Our results showed that injecting GBR every two days generates a robust and stable hyperlocomotion distinctive of mania models.



**Figure 1 : GBR administration induces strong hyperlocomotion.** A, Either GBR 12909 (GBR), 16 mg/kg, or saline (Sal) was injected intra-peritoneally (i.p.) 45 min before behavioral testing. B, GBR was administered every other day (“on”), alterned with saline (“off”) during 10 days to obtain a sub-chronic model of mania. C, Representative animal track for the 5 first minutes of the Open field test (OF). One same animal is showed for Sal group on days 1 and 7, and for the GBR group on days 1, 7 (“on”) and days 2 and 8 (“off”). D, OF revealed sustained hyperlocomotion in the GBR (on/off)-treated mice compared with the Sal-treated mice over 10 days (Two-way repeated measures ANOVA, Group :  $F_{(1, 10)} = 45.42$ ,  $***p < 0.001$ , Days :  $F_{(9, 90)} = 1.98$ ,  $p = 0.050$ , Interaction :  $F_{(9, 90)} = 1.91$ ,  $p = 0.060$ ,  $n = 6$ ). Data are shown as mean  $\pm$  SEM and individual data points.

Remarkably, GBR-treated mice exhibited lower center exploration in the OF than Sal-treated mice, indicating anxiogenic effects of the drug (Figure 2A left, 2B). This anxiety-like phenotype was present only on “on” days and not on “off” days (Figure 2A right, 2B). To confirm this observation, we measured the behavior in a classical anxiety paradigm, the Elevated Plus Maze test (EPM). We split the GBR-treated mice into two groups, GBR (on) injected on the day of test and the GBR (off) injected the day before (Figure 2C). Consistently with the OF, the GBR (on) mice showed a reduced probability of entry into the open arms relative to Sal and to GBR (off) mice (Figure 2D, E). This anxiety-like phenotype was not accompanied by a depressive-like state of the animals. Indeed, GBR administration 45 min before Tail Suspension Test (TST) almost completely suppressed immobility, whereas the administration the day before did not (Figure 2F). Therefore, GBR administration in mice triggered at short-term the major components of mania including hyperlocomotion and an increased combativeness phenotype, although these features co-existed with anxiety-like behaviors.

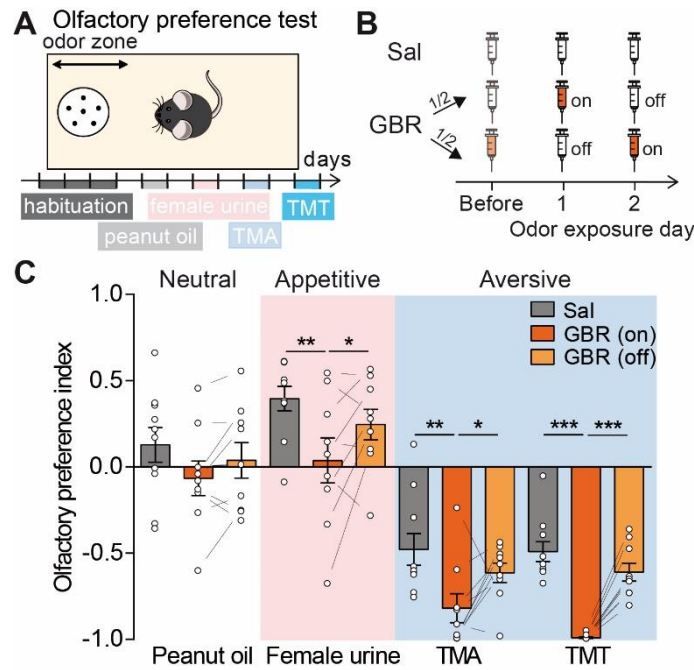


**Figure 2. Anxiety-like behaviors co-existed with anti-depressant-like phenotype in GBR-treated mice. A,** GBR-treated mice spent less time in the OF center compared to Sal-treated mice during the “on” days (left, Two-

way repeated measures ANOVA, Group :  $F_{(1, 10)} = 5.61$ ,  $*p = 0.039$ , Days :  $F_{(4, 40)} = 13.89$ ,  $p < 0.001$ , Interaction :  $F_{(4, 40)} = 1.54$ ,  $p = 0.209$ ,  $n = 6$ ), but not during the “off” days (right, Two-way repeated measures ANOVA, Group :  $F_{(1, 10)} = 0.01$ ,  $p = 0.911$ , Days :  $F_{(4, 40)} = 3.07$ ,  $p = 0.027$ , Interaction :  $F_{(4, 40)} = 0.38$ ,  $p = 0.821$ ,  $n = 6$ ). **B**, Representative heatmaps of the mean position in the OF of Sal and GBR (on) animals (day 3) and GBR (off) animal (day 4). The orange squares delimit the center of the arena. **C**, Scheme of the injection protocol to behaviorally test GBR (on) and (off) animals on the same day. **D**, Representative heatmaps of the mean position in the Elevated plus maze (EPM) of Sal and GBR-treated animals. The orange rectangles delimit the closed arms. **E**, GBR (on) mice showed a lower probability of entry in the open arms of the EPM, relative to Sal and GBR (off) mice (One-way ANOVA,  $F_{(2, 21)} = 8.78$ ,  $p = 0.002$  followed by Holm-Sidak post-hoc test,  $**p < 0.01$ ,  $n = 8$ ). **F**, GBR (on) mice spent less time immobile in the Tail suspension test (TST), compared to Sal and GBR (off) mice (Kruskal-Wallis test,  $H = 16.24$ ,  $p < 0.001$ , Dunn post-hoc analysis,  $**p < 0.01$ ,  $***p < 0.001$ ,  $n = 8$ ). Data are shown as mean  $\pm$  SEM and individual data points.

### 3.2. Strong negative olfactory hedonic bias in GBR-treated mice

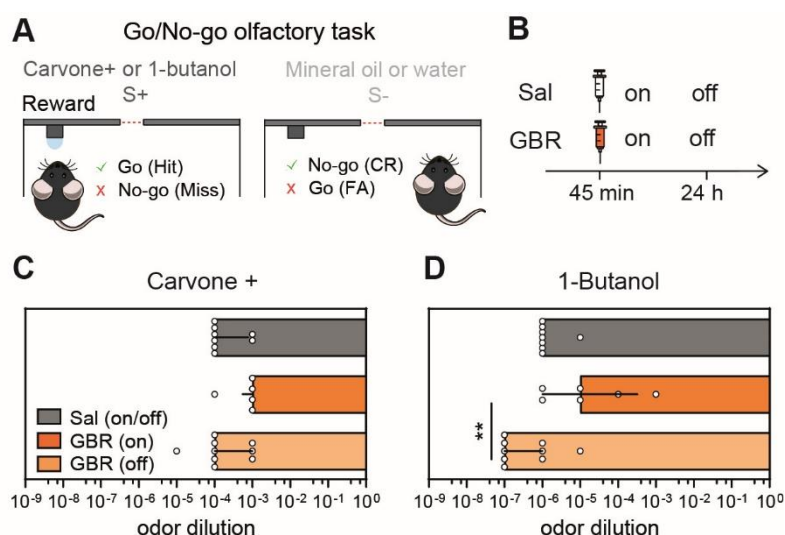
We next performed an olfactory preference test measuring innate behavioral hedonic responses to both appetitive and aversive odor stimuli as an indirect way to assess emotional states biases (Figure 3A, see Materials and Methods). GBR-treated mice were divided into two groups so that in each day of odor exposure both GBR (on) and GBR (off) mice were tested (Figure 3B). The exposure to peanut oil resulted in a preference index no different from zero in Sal-treated mice, indicating this stimulus was neutral in our experimental conditions (Figure 3C, Table S1). However, the stimulation with female urine induced an approach response in Sal-treated mice, as reflected by the positive preference index (Table S1). In contrast, TMA as well as TMT were aversive, as demonstrated by negative olfactory indexes (Table S1). Interestingly, GBR (on) mice exhibited a lower olfactory preference index than Sal mice for all non-neutral odors, causing decreased attractiveness to female urine and increased aversiveness to TMA and TMT (Figure 3C). On the “off” days, the index changed compared to the “on” days, becoming no statistically different from the Sal-treated mice, despite a trend for the group effect. Aversive odors usually decreases locomotor activity in control mice (Pérez-Gomez et al., 2015). In GBR-treated mice, it is worth noting that the aversiveness of TMT was sufficient to prevent the hyperlocomotion phenotype of GBR (on) but not of GBR (off) mice (Figure S2). In conclusion, the GBR (on) mice exhibited global negative hedonic bias in the olfactory preference test and, importantly, this effect was specific for odors triggering approach or avoidance (*e.g.* not neutral) as it was not present for peanut oil or during habituation (Figure S2).



**Figure 3 : The GBR mice model exhibit acute negative hedonic biases in olfactory preference test.** **A**, Scheme of the olfactory preference test protocol. The test occurred in a standard housing cage, with a covered petri dish installed on one side delimitating the odor zone. After four days of habituation without odorants, each odor was presented on two consecutive days inside the petri dish. Peanut oil and female urine (both pures) are used as innate neutral and attractive odors, respectively. TMA (trimethylamine, 6.75% in water) and TMT (trimethylthiazoline, 5% in mineral oil) are synthetic predator odors triggering innate aversion. **B**, Timeline of the injections protocol for the olfactory preference test. GBR-treated mice were divided into two groups, alternately injected each day with either Sal or GBR. **C**, The olfactory preference index, calculated with the time spent in the odor zone during odor presentation relative to the habituation is a proxy for the valence attributed to each odor by the mice. In this set of experiments, the peanut oil was neutral in Sal-treated mice (Student test *vs* a theoretical value 0,  $t = 1.265$ ,  $df = 9$ ,  $p = 0.238$ ,  $n = 10$ ; see also Table S1). GBR (on) mice exhibited a lower olfactory preference index than Sal mice for all non-neutral odors, meaning a decreased attractiveness of the female urine odor, and an increased aversiveness of the TMA and TMT, which was not present for GBR (off) (Sal *vs* GBR (on) : Two-way repeated measures ANOVA, Group :  $F_{(1, 17)} = 15.94$ ,  $p < 0.001$ , Odor :  $F_{(3, 51)} = 90.93$ ,  $p < 0.001$ , Interaction :  $F_{(3, 51)} = 1.53$ ,  $p = 0.217$  followed by Holm-Sidak post-hoc test,  $*p < 0.05$ ,  $***p < 0.001$ ,  $n = 9-10$  ; Sal *vs* GBR (off) : Two-way repeated measures ANOVA, Group :  $F_{(1, 17)} = 3.53$ ,  $p = 0.078$ , Odor :  $F_{(3, 51)} = 68.52$ ,  $p < 0.001$ , Interaction :  $F_{(3, 51)} = 0.06$ ,  $p = 0.981$ ,  $n = 9-10$ ). The “on” and “off” days were significantly different in GBR-treated mice upon presentation of female urine, TMA and TMT (GBR (on) *vs* GBR (off) : Two-way repeated measures ANOVA, On/off :  $F_{(1, 8)} = 14.49$ ,  $p = 0.005$ , Odor :  $F_{(3, 24)} = 52.64$ ,  $p < 0.001$ , Interaction :  $F_{(3, 24)} = 2.27$ ,  $p = 0.107$  followed by Holm-Sidak post-hoc test,  $*p < 0.05$ ,  $***p < 0.001$ ,  $n = 10$ ). Data are shown as mean  $\pm$  SEM and individual data points.

We next checked whether the observed hedonic bias could be explain by olfactory modifications following GBR administration. To do so, we evaluated odor detection threshold using a go/no-go operant conditioning paradigm. In this test, water-deprived mice were trained to discriminate between a pair of stimuli: a reinforced odor associated with a water reward (positive stimulus: S+; Figure 4A, left) and an unreinforced odor (negative stimulus: S-; Figure 4A, right). Mice were tested in two independent

tasks to recognize carvone+ or 1-butanol as the stimulus rewarded, and their solvent (mineral oil and water, respectively) as the non-rewarded stimulus. All mice were first trained to detect an odor at a high concentration without any treatment ( $10^{-2}$  v/v for carvone+ and  $10^{-3}$  v/v for 1-butanol), showing similar performances between groups (data not shown; Mixed-effect model, carvone+, Group :  $F_{(1.772, 14.18)} = 0.34$ ,  $p = 0.691$ ,  $n = 5-9$ ; 1-butanol, Group :  $F_{(1.429, 11.44)} = 0.17$ ,  $p = 0.778$   $n = 8-9$ ). Then, detection threshold concentrations were determined by presenting successively descending decimal concentrations of carvone+ and 1-butanol. Sal and GBR-injected mice were divided in two sub-groups, and the animals were tested 45 min (“on”) or 24 h (“off”) after injections (Figure 4B). We found that relative to Sal mice, neither GBR (on) nor GBR (off) groups had deficits in detection threshold for carvone+ (Figure 4C) or 1-butanol (Figure 4D). The only statistically significant difference was found for butanol detection threshold between GBR (on) and (off) using very low odor concentrations. In addition, the detection and movement times between groups remained unchanged, supporting the notion that odor investigation did not vary with GBR administration (Figure S3). Overall, our results showed that GBR administration does not alter odor detection threshold in a way that could influence the response to odors in our olfactory preference test.

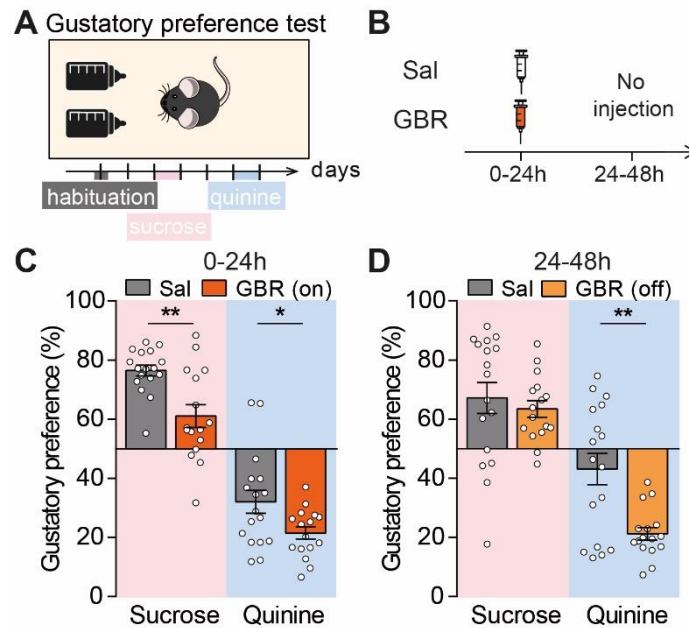


**Figure 4 : GBR administration does not alter odor detection threshold.** **A**, Schematic of the Go/no-go learning task. In response to S+, licking the water port triggered water delivery. This constitutes a correct response (Go, a hit), whereas not going to lick is considered an error (No-go, a miss). In response to S–, trained mice refrained from licking, thus constituting the correct response (No-go, correct rejection, CR), whereas going to lick constitutes an error (Go, false alarm, FA). **B**, Experimental timeline and injection protocol. All mice were first trained in a detection task without any treatment (carvone + ( $10^{-2}$ ) vs mineral oil; 1-butanol ( $10^{-3}$ ) vs water, data not shown). Performances were similar for all groups (Mixed-effect model, carvone+, Group :  $F_{(1.772, 14.18)} = 0.34$ ,  $p = 0.691$ ,  $n$

= 5-9; 1-butanol, Group :  $F_{(1.429, 11.44)} = 0.17$ ,  $p = 0.778$   $n = 8-9$ ). **C, D**, Detection threshold concentration were determined in mice injected with Sal or GBR every other day. Sal and GRB-injected mice were divided in two sub-groups, and the animals were tested 45 min (“on”) or 24 h (“off”) after injections. Sal (on) and (off) showed identical performances and were pooled together (Mann-Whitney test, carvone+,  $p = 0.429$ ,  $n = 4$ ; 1-butanol,  $p > 0.999$ ,  $n = 4$ ). Odorant concentration (v/v) are given as the dilutions used in the olfactometer. GBR administration does not alter odor detection threshold for both carvone + (C) and 1-butanol (D) with respect to the Sal group. A significant difference was found for 1-butanol detection threshold between GBR (on) and (off) (Kruskal-Wallis test, carvone+:  $p = 0.165$ ; 1-butanol:  $p = 0.008$ , followed by Dunn’s test :  $**p < 0.01$ ). Data are shown as median with interquartile range and individual data points.

### 3.3. Negative gustatory hedonic biases in GBR-treated mice

To go further, we evaluated if the olfactory negative hedonic bias observed in the GBR (on) mice could also be detected in another sensory modality by evaluating gustatory preference (Figure 5A, see Materials and Methods). Sucrose was used as an appetitive stimuli and quinine as the aversive one which were presented for 48 h along with water in a two-bottle choice test. Mice were injected with either Sal or GBR at the beginning of the 48 h (Figure 5B). Sucrose was indeed attractive for Sal-treated mice in both the 0-24h and the 24-48h periods (Figure 5C, D, Table S2), as reflected by gustatory preference superior to 50%. On the contrary, quinine was aversive in these mice, as they exhibited a gustatory preference lower than 50%, but only during the first 24 h (Table S2). Consistently with what we observed in the olfactory preference test, GBR (on) mice expressed lower appetite for sucrose and increased aversion for quinine than Sal mice during the 0-24h period (Figure 5C). Whereas quinine was no longer aversive during the last 24 h in Sal-treated mice, GBR-treated mice still avoided this tastant causing Sal and GBR (off) mice had different gustatory preference for quinine (Figure 5D). However, no differences were observed between the Sal and the GBR (off) mice in their preference for sucrose. We noticed that Sal-treated mice drank more than GBR-treated mice during all the gustatory preference tests, except during the 24-48h period of the quinine presentation (Figure S4). Altogether, the olfactory and gustatory preference tests brought to light the negative hedonic olfactory and gustatory biases provoked by the acute administration of GBR in mice.



**Figure 5. GBR-treated mice show negative hedonic biases in gustatory preference test.** **A**, Scheme of the gustatory preference test. The test occurred in a standard housing cage, with two bottles containing either water or the tastant solution. After one day of habituation with two water bottles, each tastant was presented *vs* water for 48 h. **B**, Timeline of the injections protocol for the gustatory preference test. Mice were injected at the beginning of the test. **C**, **D**, Sucrose is an appetitive tastant in Sal-treated mice both during the first and the last 24 h of test, whereas quinine is aversive for these mice only during the first 24 h (Student or Wilcoxon test *vs* a theoretical value 50, see also Table S2). GBR (on) mice showed a lower preference for both sucrose and quinine solution compared with Sal mice (Two-way repeated measures ANOVA, Group :  $F_{(1, 31)} = 17.09$ ,  $p < 0.001$ , Tastant :  $F_{(1, 31)} = 197.8$ ,  $p < 0.001$ , Interaction :  $F_{(1, 31)} = 0.65$ ,  $p = 0.426$ , Holm-Sidak post-hoc test, \* $p < 0.05$ , \*\* $p < 0.01$ ,  $n = 16-17$ ). Contrary, GBR (off) mice only exhibited a stronger aversion for quinine, relative to Sal mice for which quinine is not aversive, and no difference for sucrose preference (Two-way repeated measures ANOVA, Group :  $F_{(1, 31)} = 8.13$ ,  $p = 0.008$ , Tastant :  $F_{(1, 31)} = 70.69$ ,  $p < 0.001$ , Interaction :  $F_{(1, 31)} = 5.29$ ,  $p = 0.028$  followed by Holm-Sidak post-hoc analysis, \*\* $p < 0.01$ ,  $n = 16-17$ ). Data are shown as mean  $\pm$  SEM and individual data points.

## 4. Discussion

We used olfactory and gustatory hedonic responses in a pharmacological mania model to study emotional biases, an essential, yet overlooked dimension of mood state in humans. For that purpose, we evaluated behavioral responses to olfactory and gustatory stimuli with both positive and negative innate valences in rodents. To our surprise, we found, apart from the classical features of mania models (hyperlocomotion and anti-depressant-like phenotype), very strong negative hedonic biases in both olfactory and gustatory preference tests shortly following GBR administration. This effect disappeared at 24 h, suggesting a time- or dose-dependent effect.

### 4.1. GBR administration to trigger manic states in mice

Therefore, multi-dimensional behavioral assessment revealed that the GBR model, rather than mimicking mania, exhibits mixed-like features. Indeed, following acute GBR administration, characteristics of manic-like and depressive-like states co-existed. First, this model clearly showed increased locomotor activity, a parameter well-described in the literature and often considered as the gold standard of mania models (Bastos et al., 2018; Kelley and Lang, 1989; Queiroz et al., 2015; van Enkhuizen et al., 2013a; Young et al., 2010). Compared to previous studies mainly studying the locomotor modifications after one single injection, we observed a stable induction of locomotor hyperactivity by administrating GBR every other day, in contrast to daily injections that instead might have caused tolerance to the drug. Unlike these results, de Queiroz et al. (2018) did not observed any difference in locomotor activity between the 1<sup>st</sup> and 14<sup>th</sup> day of treatment with daily GBR injections. This difference could be due to the GBR doses (16 vs 10 mg/kg), test conditions (day vs night) and/or the mice strains (C57Bl/6NTac vs Swiss).

We found that GBR administration increases anxiety-like behavior, at least in the “on” condition, as it was previously described for amphetamine treatment (Lapin, 1993). These data go against several reports showing low-anxiety-like phenotypes in different genetic models of mania (Cosgrove et al., 2016). It is important to stress that anxiety-like measurements were not always clearly distinguished from risk-preference behaviors, sometimes measured by the Iowa Gambling test (van Enkhuizen et al.,



2013a). Nonetheless, anxiety is not a clinical criteria for mania diagnosis, and would rather be associated with mixed features.

We also measured strong increased combativeness effects on the TST after acute GBR administration. A similar effect was already reported in the literature using the forced swimming test (Hemby et al., 1997). Interestingly, this effect disappeared on the day after injection, whereas locomotor hyperactivity persisted.

#### 4.2. Negative hedonic biases in GBR model for both olfactory and gustatory stimuli

In contrast with its anti-depressive-like properties in the TST, acute GBR induced negative hedonic biases. Indeed, testing olfactory and gustatory preference uncovered a reduced attractivity to pleasant stimuli while unpleasant stimuli became more aversive. These hedonic biases observed with olfactory cues could not be explain by alteration of the odor detection threshold, since there was no significant difference between Sal and GBR (on) mice. All other parameters analyzed in our study support that olfactory function is not notably altered after GBR administration. Moreover, odor concentrations used in the olfactory preference test were extremely high. A previous work used a female urine sniffing test to measure reward-seeking behavior in the GluR6-KO model of mania (Malkesman et al., 2010). They observed increased female urine exploration in these mice compared to control mice, as we initially expected in the GBR model. However, Kamdar et al. (2007), showed that acute injections of GBR resulted in a dose-dependent reduction in sugar water intake, consistently with our results. When examining studies involving sucrose preference in mania models, it appears that only genetic models show increased sucrose consumption (Kirshenbaum et al., 2011; Leussis et al., 2013; Roybal et al., 2007; van Enkhuizen et al., 2013b). It would be interesting to evaluate hedonic biases in different kinds of mania models to decipher if the emotional dimension is altered in a positive way only in genetic models, and to state on the presence of mixed-like features in the other kinds of pharmacological or environmental models. Another study examined GBR-treated mice behavioral responses to damp cloth impregnated with domestic female cat fur, and concluded with increased risk-preference through increased exploration of this predator stimuli (de Queiroz et al., 2018). However, the olfactory stimulus used was extremely uncontrolled, and further experiments are necessary to clarify these discrepancies

with our results. Finally, our protocol only evaluated behavioral hedonic responses to well-defined innate positive and negative cues that do not require previous experience to avoid any confounding effects due to potential impaired cognitive processes in the GBR model (*e.g.* attention or memory). Indeed, another test developed by Robinson and collaborators (2018) to evaluate affective biases resulting from acute intake of pro-depressant or antidepressant drugs relies on modulation of associative emotional memory.

Contrary to other classes of dopamine transporter inhibitors also acting as dopamine agonists, *i.e.* the amphetamines and cocaine, the GBR dopaminergic activation is lower *in vivo*. Indeed, GBR 12909 binds to the dopamine transporter approximately 50 times more strongly than cocaine (Izenwasser et al., 1990), but simultaneously inhibits the release of dopamine (Singh, 2000). These combined effects only slightly elevate dopamine levels, giving GBR mild stimulant effects. This limited pro-dopaminergic action of GBR might result in the mixed phenotype observed in our study. A pro-dopaminergic drug would have been expected to have rewarding properties and therefore, to possibly induce a positive hedonic bias. The surprising negative hedonic bias induced by GBR could be linked to mild elevation of dopamine levels in different brain areas from amphetamine and cocaine, and potentially by resulting in activation of different dopamine receptors.

#### 4.3. Emotional states assessment: an essential dimension in animal models of mood disorders

Our results demonstrate the importance of multi-dimensional assessment of animal models. In the DSM-5 (American Psychiatric Association, 2013), the two main criteria to define a manic state are elevated and expansive mood with increased activity and energy. It is therefore particularly important to have translational evaluations of these two elements. While the second criterion has largely been retained to define mania models, based on the increased motor and exploratory activity of animals, the mood main criterion has to date not found an equivalent measure applicable to animals. Indeed, mood is a subjective symptom peculiar to human nature. However, as proposed in our emotional-based model for bipolar patients, emotional biases can be explored both in humans and animals as a proxy for mood (Bigot, 2020). Our data support the notion that GBR-treated mice exhibit a mixed-like state in which manic and depressive symptoms occur simultaneously, unlike classical mania models. We therefore propose that

emotional states bias assessment *via* hedonic responses is a reliable and essential method to model and characterize different BD states. The attribution of emotional valence is underpinned by mechanisms common to different sensory modalities and is well-conserved across species (Costafreda et al., 2008; Janak and Tye, 2015; Xia and Kheirbek, 2020). Studying hedonic biases in animal models of BD could unravel the underlying biological mechanisms involved in mood alteration.

Animal models are crucial to understand the physiopathology of a disease, but also for the development of targeted pharmacological treatments. Our results underscore the need to test in pre-clinical phases how potential anti-depressant drugs restore emotional biases. Indeed, human models propose that the direct effect of successful anti-depressant treatment may be to improve emotional negative biases (Harmer et al., 2017). GBR was first suggested as a new anti-depressant drug, based on classical pre-clinical depression tests (Hemby et al., 1997; Sjøgaard et al., 1990), although we uncovered the fact that it induces strong negative biases.

## **5. Conclusion**

To conclude, we propose to translate the mood criteria of BD into emotional biases to more closely link neurobiological findings in rodents with human studies. The occurrence of hedonic biases in animal models opens up great prospects for understanding of the underlying mechanisms of mood states, and most importantly provides an opportunity to discover new anti-depressant drugs capable of restoring them.

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## **Author contributions**

MB: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – Original Draft, Writing – Review and Editing; EV: Investigation, Formal analysis, Visualization, Writing – Review and Editing; PML: Writing – Review and Editing, Supervision, Funding acquisition; MA: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – Original Draft, Writing – Review and Editing, Supervision; CH: Conceptualization, Methodology, Writing – Original Draft, Writing – Review and Editing, Supervision, Funding acquisition.

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## Supplemental information

### Negative hedonic bias in a mouse model of mania

#### Supplemental Methods and Materials

##### Animals

All animal care and experimental procedures followed national and European (2010/63/EU) guidelines and were approved by the French Ministry of Research (APAFiS: #16380-2018080217358599\_v1). C57BL/6N male mice (12–16 weeks old) purchased from Taconic Farms (Denmark) were used for all behavioral tests (n = 74). Once they arrived in the animal facility, the mice were given at least one week to habituate. Mice were socially housed, 4-6 per cage, and maintained under standard housing conditions (23 ± 1 °C; humidity 40%) with a 14/10 h light/dark cycle (lights were on from 6:30 AM to 9:30 PM every day) with food and water *ad libitum*, except for some behavioral experiments. All behavioral tests were conducted during the period of light (10 AM-7 PM). We used the minimum number of animals as estimated from our previous knowledge in performing the same type of experiments.

##### Behavioral assessment

Olfactory preference test. The test was adapted from Pérez-Gómez et al. (2015). The test was performed in a quiet and dimly lit room (~ 40 lux), around 3 to 6 pm. Clean housing cages (17 x 32 cm) with regular bedding material were used as testing arenas, covered by transparent Plexiglas lids. Each testing arena received mice socially housed in the same cage. The first day, all the mice from the same cage were placed together to overcome neophobia, in each testing arena for habituation during 15-20 min. The second day, a petri dish (94 mm diameter) with a hooled cover was placed and adhered to one side of the arena. For 12 consecutive days, the behavior was recorded by a video camera for 15 min and the Noldus Ethovision 3.0 system (Netherlands) was used to track the position of the mice. The time spent in an odor zone, defined as a rectangle drawn to cover all the cage side around the petri dish and 2 cm from it (representing about one-third of the cage), and the locomotor activity were used as measures of olfactory valence. During the first 4 days, only a Whatman paper filter (GE Healthcare Life Sciences,



USA) was placed into the petri dish, to assess the baseline exploration. Then, 2 days were dedicated to each odor, placed on a paper filter, in the following order: peanut oil (pure, 400  $\mu$ l), female urine (pure, 100  $\mu$ l), trimethylamine (Sigma-Aldrich, Germany, 6.75% in water, 400  $\mu$ l) and trimethylthiazole (Sigma-Aldrich, 5% in mineral oil, 400  $\mu$ l). Peanut oil and female urine are classical appetitive odorants, whereas trimethylamine and trimethylthiazole are predator urine synthetic compounds, aversive at these concentrations (Li et al., 2013; Pérez-Gómez et al., 2015; Root et al., 2014). However, in our experimental conditions animals were not food deprived before the test and consequently peanut oil had a neutral value, not triggering attraction. Repeating the measurement of each odor twice allowed to decrease the inter-subject variability of this spontaneous behavior. The olfactory preference index was calculated as explained in Pérez-Gomez et al. (Pérez-Gómez et al., 2015), as the difference between the time a given mouse spent in odor zone when exposed to an odor and the average time of all saline-treated mice in this area when only paper filter was present (TH, during habituation), divided by TH.

Olfactory behavioral testing.

Mice were trained using a custom-built computer-controlled eight-channel olfactometer, as previously described (Grelat et al., 2018).

Pretraining Procedures. Partially water-deprived mice (at 80–85 % of their normal body weight) were trained using a go/no-go procedure. First, standard operant conditioning methods were used to train mice to insert their snouts into the odor-sampling port and to respond by retracting the head and licking the water port (located to the left of the odor port). The mice initiated each trial by breaking the light beam positioned across the odor port which led to the opening of an odor valve and a diversion valve directing all airflow away from the sampling tube. This resulted in the odorant vapor being combined with the main air stream and the diversion of the main air stream to an exhaust path. The diversion valve closed 1 s later, and the odor stimulus was presented to the odor sampling port. The stimulus valve closed 2 s later (maximum odor stimulus duration), terminating delivery of the odor. Reward delivery depended on the mouse licking the water delivery tube (maximum response criterion, 2 s). Trials in which the mouse did not keep its snout in the odor-sampling port for at least 0.1 s after odor onset were aborted and counted as short-sample trials. A 3  $\mu$ L water reward was delivered if the mouse satisfied the response

criterion. The mouse then had to retract its head (the beam was resealed) and wait at least 5 s (intertrial interval) before initiating a new trial. All mice underwent at least five pretraining sessions without odor stimuli. We considered that mice had learned the rules when they responded by introducing their snout in the odor port, waited 1,200 ms before leaving to lick the water port for at least 40 trials, and exhibited fewer than 50 short samples. A pseudorandom protocol was used during behavioral experiments to assign animals to the different olfactometers (six olfactometers in total). A given animal was never trained two consecutive days in the same device.

**Training Procedures.** The trial procedures were identical to those used in the initial pretraining sessions. Mice were trained to respond to the presence of an odor (S+ : positive stimulus) by licking the water port and to refrain from responding to the presence of odorless mineral oil or water (S- : negative stimulus). In each trial, a single stimulus (S+ or S-) was presented. If the response criterion was met in S+ trials, a droplet of water (3  $\mu$ L) was given as a reward, and the trial was scored as a hit; if not, the trial was scored as a miss. Failing to lick in a S- trial was scored as a correct rejection (CR); otherwise, the trial was scored as an false alarm (FA). S+ and S- trials were presented in a modified random order (each block contained equal numbers of S+ and S- stimulus trials, and neither stimulus was presented more than three times consecutively). The percentage of correct responses was determined for each block of 20 trials [(hits+CRs)/20 $\times$ 100]. Scores  $\geq$  85 % implied that mice had correctly learned to assign the reward value to the S+ and the non-reward value to the S-. The odor-sampling time (detection time) was the latency from odor onset to withdrawal of the nose from the odor port. The movement time was the latency from the withdrawal of the nose from the odor port to the first lick on the water port. Detection time and movement time were quantified on Hit trials for the first 5 blocks after reaching the criterion on the highest tested odor dilution.

The datasets that support the findings of this study are available on Mendeley Data website (<http://dx.doi.org/10.17632/7rhz66hwfw.1>).

## KEY RESOURCES TABLE

<b>REAGENT or RESOURCE</b>	<b>SOURCE</b>	<b>IDENTIFIER</b>
Chemicals, Peptides, and Recombinant Proteins		
GBR 12909	Sigma-Aldrich	D052
Carvone+	Sigma-Aldrich	22070
1-butanol	Sigma-Aldrich	87906
Mineral oil	Sigma-Aldrich	M5904
Peanut oil	Huilerie beaujolaise	
Trimethylamine solution	Sigma-Aldrich	92262
2,4,5-Trimethylthiazole	Sigma-Aldrich	W332518
Sucrose	Sigma-Aldrich	S9378
Quinine hydrochloride dihydrate	Sigma-Aldrich	Q1125
Experimental Models: Organisms/Strains		
Mice: C57BL6/6NTac	Taconic Farms	
Software and Algorithms		
Noldus 3.0	EthoVision	<a href="https://www.noldus.com/ethovision-xt">https://www.noldus.com/ethovision-xt</a>
Prism	Prism-GraphPad	<a href="https://www.graphpad.com/scientific-software/prism/">https://www.graphpad.com/scientific-software/prism/</a>
R	CRAN	<a href="https://cran.r-project.org/">https://cran.r-project.org/</a>
Deposited Data		
Datasets	Mendeley Data	<a href="http://dx.doi.org/10.17632/7rhz66hfw.1">http://dx.doi.org/10.17632/7rhz66hfw.1</a>

## Supplemental Tables and Figures

<b>Odor stimulus</b> \ <b>Group</b>	<b>Sal (n = 10)</b>	<b>GBR (on) (n = 9)</b>	<b>GBR (off) (n = 9)</b>
<b>Peanut oil</b>	t = 1,26, p = 0,238	t = 0,66, p = 0,525	t = 0,37, p = 0,722
<b>Female urine</b>	t = 5,56, ***p < 0,001	t = 0,28, p = 0,783	t = 2,78, *p = 0,024
<b>TMA</b>	W = -51,00, **p = 0,006	W = -45,00, **p = 0,004	t = 10,94, ***p < 0,001
<b>TMT</b>	W = - 55,00, **p = 0,002	W = -45,00, **p = 0,004	t = 11,79, ***p < 0,001

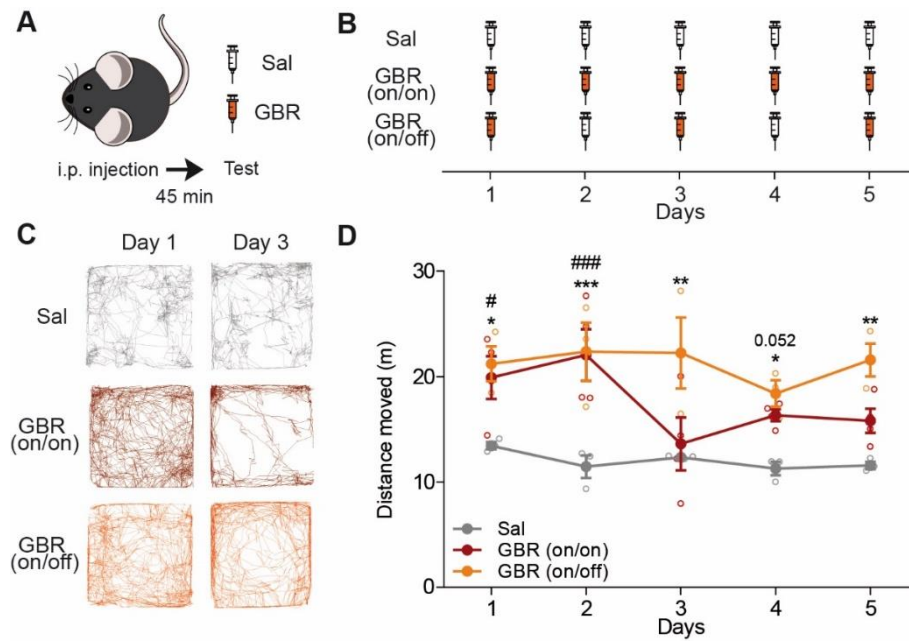
**Table S1 : Attractiveness or aversiveness of olfactory stimuli shown in Figure 3C**

The preference index for each odor and group was compared to a theoretical value of 0, by one sample Student or Wilcoxon tests depending on the normality of the data.

<b>Taste stimulus</b> \ <b>Group</b>	<b>Sal (n = 17)</b>	<b>GBR (n = 16)</b>
<b>Sucrose – 0-24h</b>	t = 14,40, ***p < 0,001	t = 2,89, *p = 0,011
<b>Quinine – 0-24h</b>	t = 4,59, ***p < 0,001	t = 13,80, ***p < 0,001
<b>Sucrose – 24-48h</b>	t = 3,28, **p = 0,005	t = 4,83, ***p < 0,001
<b>Quinine – 24-48h</b>	t = 1,28, p = 0,220	t = 13,57, ***p < 0,001

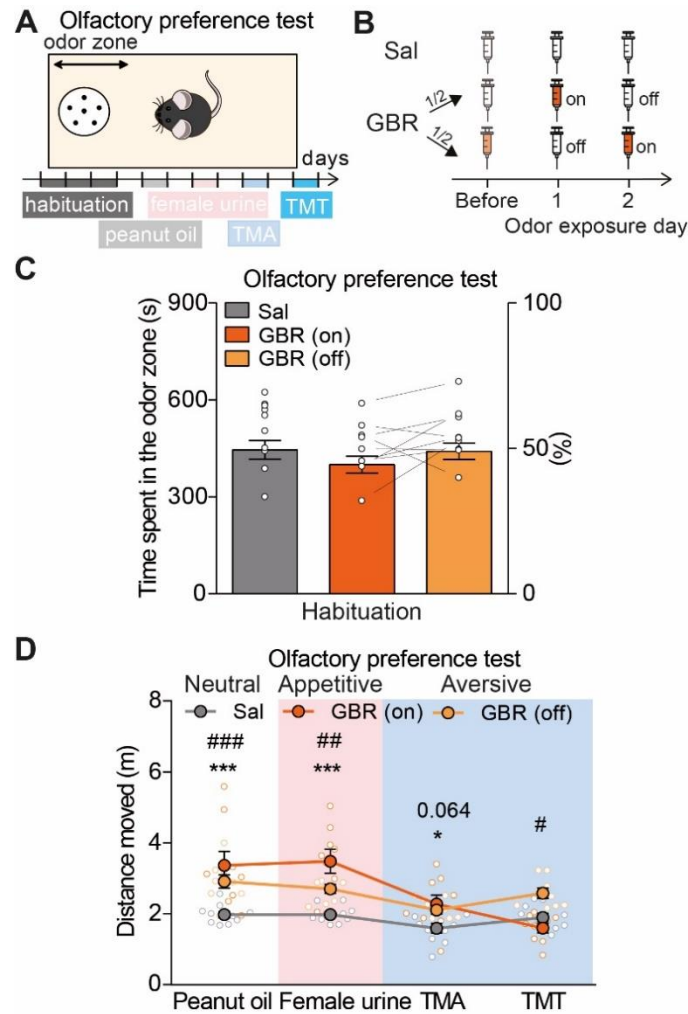
**Table S2 : Attractiveness or aversiveness of gustatory stimuli shown in Figure 5C-D**

The preference index for each tastant, period and group was compared to a theoretical value of 0, by one sample Student or Wilcoxon tests depending on the normality of the data.

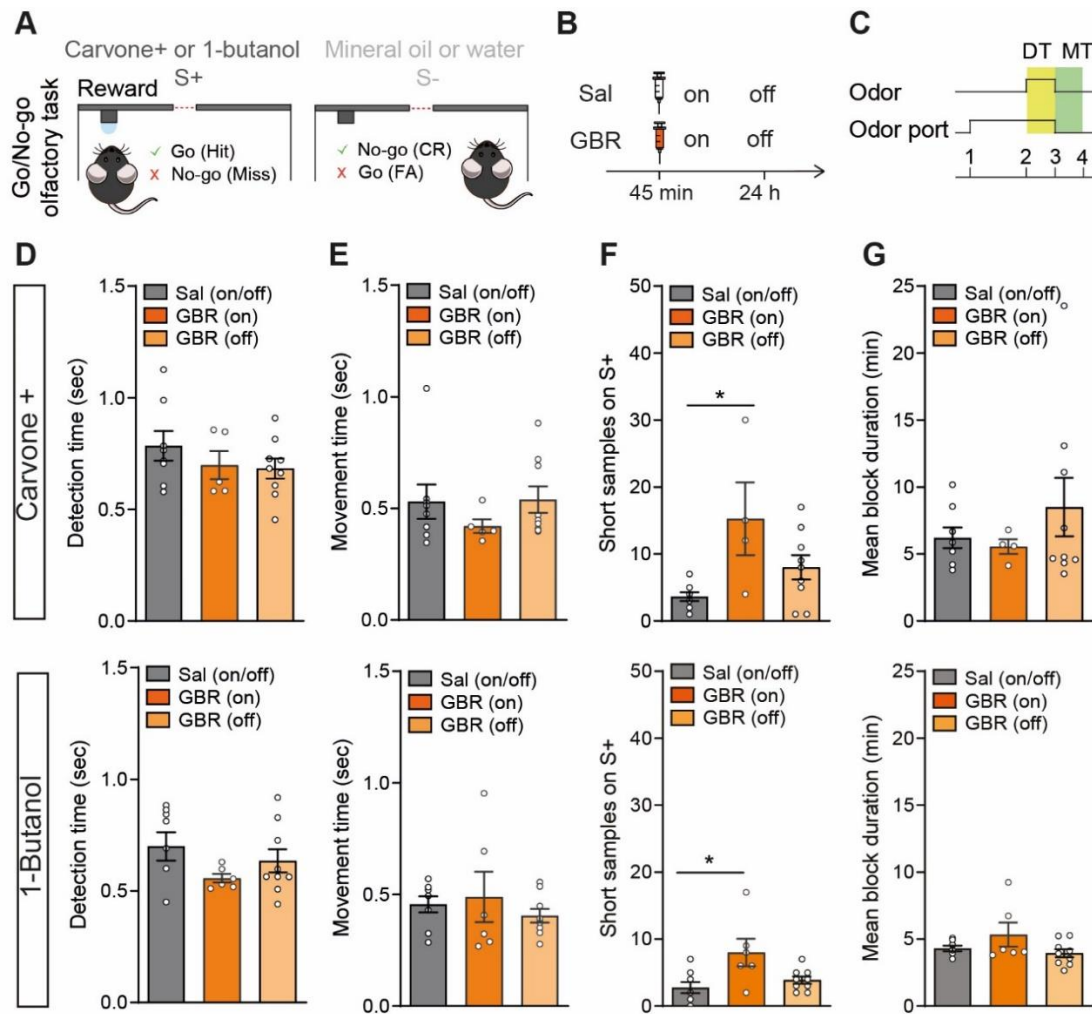


**Figure S1 : Injecting GBR everyday induces tolerance regarding the hyperlocomotion phenotype.**

**A**, Either GBR 12909 (GBR) or saline (Sal) was injected intra-peritoneally (i.p.) 45 min before the Open field test (OF). **B**, Timeline for two different injection protocol: GBR (on/on) mice were injected everyday whereas GBR (on/off) received GBR every other day (“on”), alterned with saline (“off”) during 5 days. **C**, Representative animal track for the 5 first minutes of the OF. One same animal is represented for Sal, GBR (on/on) and GBR (on/off) groups on days 1 and 3. **D**, OF revealed sustained hyperlocomotion in the GBR (on/off)-treated mice compared with the Sal-treated mice ( $n = 3$ ) over 5 days, whereas the GBR (on/on) group did not exhibit anymore hyperlocomotion from the day 3 (Two-way repeated measures ANOVA, Group :  $F_{(4, 28)} = 3.04$ ,  $p = 0.034$ , Days :  $F_{(2, 7)} = 11.38$ ,  $p = 0.006$ , Interaction :  $F_{(8, 28)} = 2.34$ ,  $p = 0.046$  followed by Holm-Sidak post-hoc test : # $p < 0.05$  and ### $p < 0.001$ , GBR (on/on) vs Sal ; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , GBR (on/off) vs Sal,  $n = 3-4$ ). Data are shown as mean  $\pm$  SEM and individual data points.



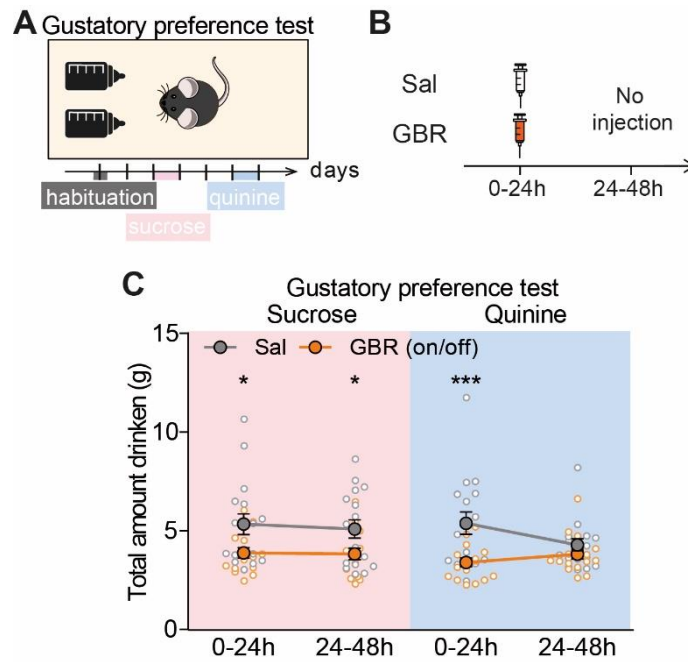
**Figure S2 : The olfactory preference test.** **A**, Scheme of the olfactory preference test protocol. The test occurred in a standard housing cage, with a covered petri dish installed on one side delimitating the odor zone. After four days of habituation without odorants, each odor was presented on two consecutive days inside the petri dish. Peanut oil and female urine (both pure) are used as neutral and innate attractive odors. TMA (trimethylamine, 6.75 % in water) and TMT (trimethylthiazoline, 5 % in mineral oil) are synthetic predator odors triggering innate aversion. **B**, Timeline of the injections protocol for the olfactory preference test. GBR-treated mice were divided into two groups, alternately injected each day with either Sal or GBR. **C**, The time spent in the odor zone during the habituation phase of the olfactory preference test is the same across Sal, GBR (on) and GBR (off) mice (Sal vs GBR (on) or GBR (off) : One-way ANOVA,  $F_{(2, 25)} = 0.87$ ,  $p = 0.432$ ,  $n = 9-10$  ; GBR (on) vs GBR (off) paired Student test,  $t = 1.42$ ,  $df = 8$ ,  $p = 0.193$ ,  $n = 10$ ). **D**, The TMT aversive odor prevented the hyperlocomotion observed during “on”, but not during “off” phase after GBR administration (Two-way repeated measures ANOVA, Group :  $F_{(2, 25)} = 9.05$ ,  $p = 0.001$ , Odor :  $F_{(3, 75)} = 25.92$ ,  $p < 0.001$ , Interaction :  $F_{(6, 75)} = 10.47$ ,  $p < 0.001$  followed by Holm-Sidak post-hoc test : \* $p < 0.05$ , \*\*\* $p < 0.001$ , GBR (on/off) vs Sal ; # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$ , GBR (off) vs Sal,  $n = 10$ ). Data are shown as mean  $\pm$  SEM and individual data points.



**Figure S3. Detection and movement time are not affected by GBR administration.** **A** Schematic of the Go/no-go learning task. In response to S+, licking the water port triggered water delivery. This constitutes a correct response (Go, a hit), whereas not going to lick is considered an error (No-go, a miss). In response to S-, trained mice refrained from licking, thus constituting the correct response (No-go, correct rejection, CR), whereas going to lick constitutes an error (Go, false alarm, FA). **B**, Experimental timeline and injection protocol. All mice were first trained in a detection task without any treatment (carvone + ( $10^{-2}$ ) vs mineral oil; 1-butanol ( $10^{-3}$ ) vs water, data not shown). **C**, Schematic of the times measured in the go/no-go task. (1) The mice initiated each trial by breaking the light beam positioned across the odor sampling port. The odor sampling time (detection time) was the latency from odor onset (2) to withdrawal of the nose from the odor port (3) (yellow shading). The movement time was the latency from nose withdrawal from the odor port (3) to licking of the water port (4) (green shading). **D**, Mean detection time in hit trials for the first five blocks above the criterion for  $10^{-2}$  carvone + dilution (top) and  $10^{-3}$  1-butanol dilution (bottom). The detection time remained unchanged among groups, supporting the notion that GBR treatment did not change olfactory performance (Kruskal–Wallis test, carvone+ (top):  $p = 0.647$ , 1-butanol (bottom):  $p = 0.311$ ). **E**, Same that in E for the mean movement time (Kruskal–Wallis test, carvone+ (top):  $p = 0.354$ ; 1-butanol (bottom):  $p = 0.703$ ). **F**,

Number of short samples in S+ trials for the first five blocks above the criterion increase in GBR (on) group respect to Sal-injected mice for both tasks (Kruskal–Wallis test, carvone+ (top):  $p = 0.032$ ; 1-butanol (bottom):  $p = 0.026$ , followed by Dunn’s test :  $*p < 0.05$ ) reflecting an increase on impulsivity. **G**, No difference were found in the mean block duration for the first five blocks above the criterion increase, suggesting no main difference in task engagement or motivation during the tasks (Kruskal–Wallis test, carvone+ (top):  $p = 0.953$  ; 1-butanol (bottom):  $p = 0.397$ ). Data are shown as mean  $\pm$  SEM and individual data points.





**Figure S4 : The gustatory preference test.** **A**, Scheme of the gustatory preference test. The test occurred in a standard housing cage, with two bottles containing either water or the tastant solution. After one day of habituation with two water bottles, each tastant solution is presented along with water for 48 h. **B**, Timeline of the injections protocol for the gustatory preference test. Mice were injected at the beginning of the test. **C**, GBR-treated mice drank significantly less solution than Sal-treated mice during the 48 h of sucrose presentation (left) (Two-way repeated measures ANOVA, Group :  $F_{(1, 31)} = 5.90$ ,  $p = 0.021$ , Period :  $F_{(1, 31)} = 1.01$ ,  $p = 0.324$ , Interaction :  $F_{(1, 31)} = 0.43$ ,  $p = 0.519$ , followed by Holm-Sidak post-hoc test :  $*p < 0.05$ ,  $n = 16-17$ ). For quinine presentation, GBR mice drank significantly less solution only during the first 24h of quinine presentation (right) (Two-way repeated measures ANOVA, Group :  $F_{(1, 31)} = 6.42$ ,  $p = 0.017$ , Period :  $F_{(1, 31)} = 3.32$ ,  $p = 0.078$ , Interaction :  $F_{(1, 31)} = 16.97$ ,  $p < 0.001$ , followed by Holm-Sidak post-hoc test :  $***p < 0.001$ ,  $n = 16-17$ ). Data are shown as mean  $\pm$  SEM and individual data points.

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## 2.3 BLA circuits-related negative olfactory hedonic bias in depression (*in preparation*) ..... 123

### *Summary*

In this study, we confirmed and extended that depressive states in humans are correlated with a negative bias during odor valence assignment, performing the Sniffin' stick test in bipolar depressed patients. In parallel, we used the olfactory preference test described earlier as a pre-clinical translational test to assess behavioral hedonic responses reflecting spontaneous valence assignment of innate odors in mice. This allowed us to demonstrate negative olfactory hedonic bias in a mouse model of depression induced by chronic CORT administration, very similarly to what happens in depressed patients. In other words, we found that chronic CORT treatment is associated with a shift to more negative valence assignment of both pleasant (that become less pleasant) and unpleasant (that become more unpleasant) odors. This bias was associated with disturbances in brain circuits involved in valence assignment. Indeed, BLA-to-NAc neurons activity was reduced while BLA-to-CeA neurons activity was increased in CORT-treated mice. Furthermore, the activation of BLA-to-NAc circuit by a chemogenetic approach was sufficient to improve the negative olfactory hedonic bias on the appetitive odors but not on the aversive odors observed in our model. Taken together, our results highlight the interest of olfactory hedonic evaluation in mouse models of depression and demonstrate the causal role of BLA circuits in hedonic biases associated with depressive-like states.



## BLA circuits-related negative olfactory hedonic bias in depression

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## **Abstract**

Despite the importance of emotional biases in depression, few studies investigated the disruption of related limbic basolateral amygdala (BLA) circuits in animal models of depression. Here, we show that depressed bipolar disorders patients display olfactory valence negative bias in the Sniffin' sticks test. Spontaneous behavioral response to innate odors, reflecting valence assignment in mice, uncovered similar negative bias in the corticosterone-induced depression model, with decreased appetitiveness of appetitive odors and increased aversiveness of aversive odors. Besides, corticosterone administration reduced BLA-to-nucleus accumbens neurons activity while increasing BLA-to-central amygdala neurons activity, circuits previously involved respectively in positive and negative valence encoding. Chemogenetically activating the BLA-to-nucleus accumbens circuit partially reversed the negative bias induced by corticosterone administration. Together, our results highlight the interest of olfactory hedonic evaluation as a translational assay in mouse models of depression and demonstrate the causal role of BLA circuits in hedonic biases associated with depressive-like states.

## **Introduction**

Depression is the single largest contributor to disability worldwide, affecting as many as 300 million people annually. One out of five individuals will experience depression at least once during their lifetime (World Health Organization, 2017). Depressive episodes occur in patients suffering from major depressive disorder (MDD) and bipolar disorders (BD) (DSM-5, APA, 2013). Beyond reduced activity and motivation, disturbed emotional processing plays an important role in the causation and maintenance of the persistent low mood associated with depressive episodes (Roiser et al., 2012). Emotional biases are an essential component leading depressed patients to attribute more negative valence to events or objects (Leppänen, 2006; Clark et al., 2009; Harmer et al., 2009; Bigot, 2020). In patients, these emotional biases are mainly evaluated using visual stimuli, particularly during facial expression recognition tests. Olfactory valence biases have already been reported in depressive episodes (Atanasova et al., 2010; Naudin et al., 2012; Kohli et al., 2016; Kazour et al., 2020; Colle et al., 2020). In addition, human models suggest that successful antidepressant treatment should restore the negative emotional bias associated with depression (Harmer et al., 2017; Colle et al., 2020).

Conversely to mood which is a subjective symptom not evaluable in animal, emotional processes can also be explored in pre-clinical studies. For instance, approach or avoidance behaviors are quantifiable motor readouts indicating what hedonic valence the animal assigned to the presented stimuli and expressing an indirect measure of the triggered emotion (Anderson & Adolphs, 2014; Tye, 2018; Zych & Gogolla, 2021). Therefore, we investigated whether behavioral hedonic responses reflecting innate olfactory valence assignment could be used to characterize depressive-like state in rodents.

Robust pre-clinical results show the crucial role of the basolateral nucleus of the amygdala (BLA) in both positive and negative valence assignment. BLA neurons preferentially respond either to positive or negative stimuli, and can be distinguished according to their projection targets, as well as spatial and genetic characteristics (Pignatelli & Beyeler, 2019). The BLA neurons projecting to the nucleus accumbens (NAc), the ventral part of the striatum, mainly respond to positive stimuli and trigger approach behaviors, while BLA neurons targeting the centromedial nucleus of the amygdala (CeA) mainly respond to negative stimuli and trigger defensive behaviors (Namburi et al., 2015; Beyeler et al.,



2016). However, the mechanisms underlying disturbed innate valence assignment have never been studied in animal models of depression.

Here, we replicate the fact that negative emotional bias is displayed by depressed bipolar patients akin previously reported in unipolar depression. Furthermore, olfactory preference test reveals negative hedonic bias exhibited by the corticosterone (CORT)-induced mouse model of depression. From there, we studied the mechanisms that could underlie this emotional bias. We demonstrate alterations of BLA circuits activity in the CORT-treated mice and investigate their causal relationship with negative olfactory hedonic bias and depressive-like behaviors using chemogenetic manipulation of these specific BLA circuits.

## Results

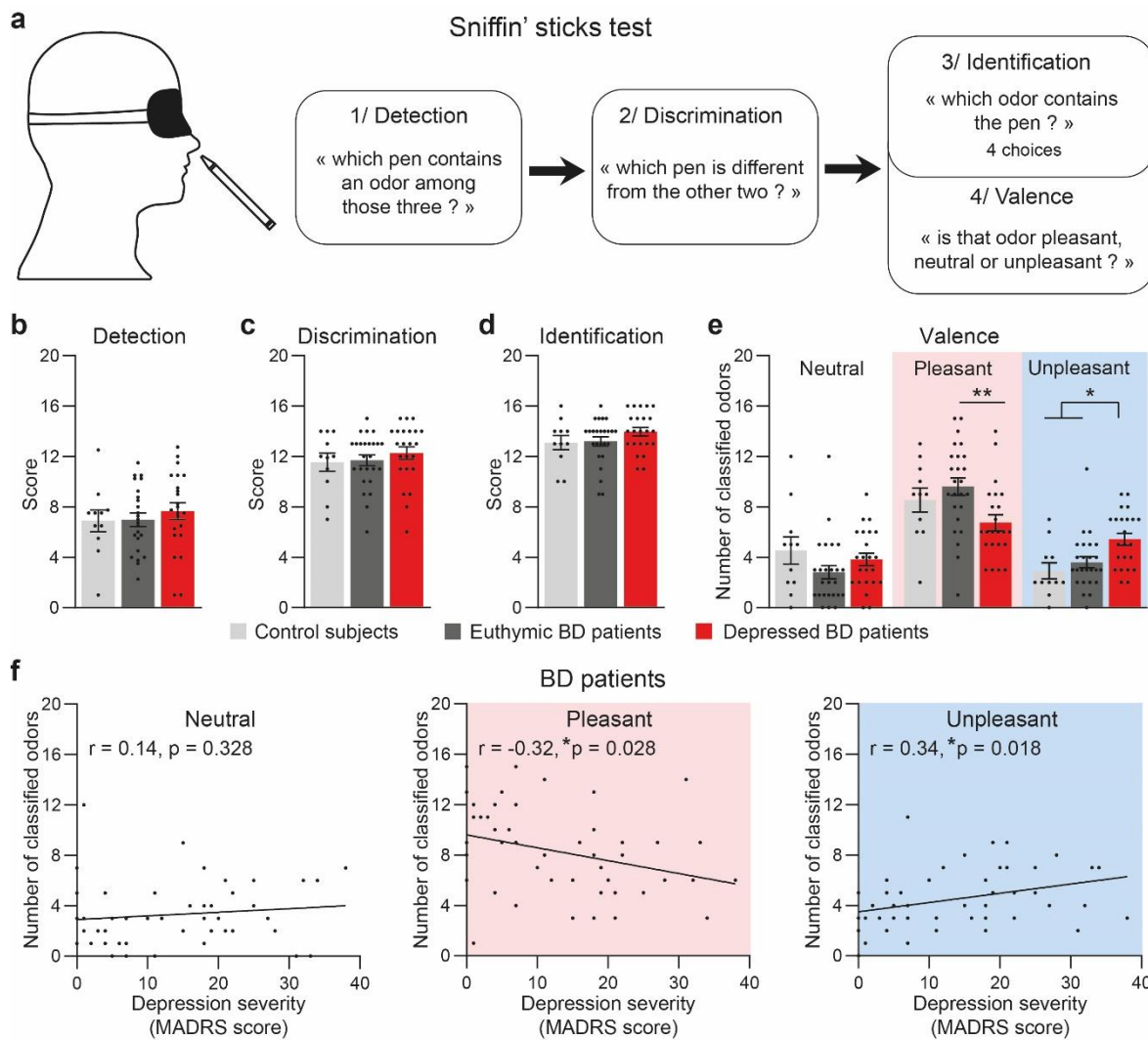
### Negative olfactory valence bias in depressed BD patients

Olfactory disturbance and negative olfactory bias have been reported as markers for unipolar depressive patients (Naudin & Atanasova, 2014; Kohli et al., 2016; Kazour et al., 2020; Colle et al., 2020). To confirm and extend these results, we evaluated different olfactory parameters using the Sniffin' sticks test in a cohort of control subjects and BD patients. Demographic and further clinical characteristics are presented in **Table 1**. The type of mood episode in BD patients was clinically determined, and patients were classified based on the DSM-IV-R (American Psychiatric Association, 1980) as euthymic on one side, and depressed, dysthymic or mixed (referred to collectively as “depressed BD patients”) on the other side. The group of depressed BD patients had significantly higher MADRS score, measuring depression severity, than both control subjects and euthymic BD patients, while euthymic BD patients did not differ from control subjects (**Table 1**). Of note, no difference were found between groups in other potential confounding factors (age, sex, *etc.*).

Variable	Control subjects (n = 11)	Euthymic BD patients (n = 25)	Depressed BD patients (n = 23)	F, H or $\chi^2$	p
Female, n (%)	7 (63.63)	13 (52)	12 (52.17)	0.48	0.786
Age, years mean (SD)	33.55 (10.8)	38.2 (12.55)	43.09 (11.33)	2.61	0.083
Education, years mean (SD)	16 (3.03)	14.65 (2.48)	15.86 (3.51)	1.21	0.306
Unemployed, n (%)	1 (9.09)	11 (44)	7 (31.82)	4.24	0.12
Current smoking, n (%)	2 (18.18)	12 (48)	7 (30.43)	3.4	0.183
Type BD, n (%)					
BD I		16 (64)	13 (56.52)		
BD II		9 (36)	9 (39.13)		
BD NOS		0 (0)	1 (4.35)		
MADRS, mean (SD)	1.36 (2.38)	4.92 (5.82)	22.61 (6.97)	41.15	< 0.001
Control subjects vs Euthymic BD patients					0.339
Control subjects vs Depressed BD patients					< 0.001
Euthymic BD patients vs Depressed BD patients					< 0.001
YMRS	0.36 (0.81)	1.12 (2.57)	3.83 (5.46)	12.26	0.002
Control subjects vs Euthymic BD patients					0.459
Control subjects vs Depressed BD patients					0.003
Euthymic BD patients vs Depressed BD patients					0.004

**Table 1.** Demographic and clinical characteristics of BD patients and control subjects. BD NOS: bipolar disorder not otherwise specified; MADRS: Montgomery Asberg Depression Rating Scale; YMRS: Young Mania Rating Scale.

The Sniffin' sticks test was performed following three steps as previously described (Rumeau et al., 2016; Colle et al., 2020), evaluating olfactory performances for detection, discrimination and identification (**Figure 1a**). Furthermore, once an odor identified, subjects were asked about the odor valence, *i.e.* if they would rate it as neutral, pleasant or unpleasant. Control subjects and BD patients regardless of their mood episode had similar detection, discrimination and identification scores (**Figure 1b-d**), suggesting no olfactory dysfunction in BD patients. However, depressed BD patients rated less odors as pleasant than euthymic BD patients, and more odors as unpleasant than both control subjects and euthymic BD patients (**Figure 1e**). This negative olfactory valence bias was associated with depression severity, as the MADRS score correlated negatively with the number of classified pleasant odors, and positively with the number of classified unpleasant odors (**Figure 1f** middle and right, respectively). No correlation was found between the number of classified neutral odors and depression severity (**Figure 1f** left). These results could not be explain by the tobacco use, as the smoking status did not modified olfactory valence assignement (Two-way RM ANOVA; Smoking status:  $F(1,57) = 0.00, p > 0.999$ , Valence:  $F(2,114) = 33.96, p < 0.001$ , Interaction:  $F(2,114) = 2.27, p = 0.108$ ). Our data confirm that bipolar depressive patients assign more negative valence to olfactory stimuli compared to control subjects or euthymic BD patients, in the same way that has been reported for unipolar depression (Atanasova et al., 2010; Naudin et al., 2012; Kohli et al., 2016; Kazour et al., 2020; Colle et al., 2020). We show for the first time negative emotional bias affecting not only pleasant, but also unpleasant odor ratings in depressed BD patients.



**Figure 1.** Negative olfactory valence bias in depressed BD patients. (a), Scheme of the Sniffin' sticks test evaluating olfactory detection, discrimination and identification. Each of the presented odors for identification were then classified among neutral, pleasant or unpleasant valence. (b-d), BD patients (euthymic,  $n = 25$  (except in (b):  $n = 24$ ); depressed,  $n = 23$ ) and control subjects ( $n = 11$ ) did not differ on olfactory performances for detection (b,  $F(2,55) = 0.42, p = 0.659$ ), discrimination (c,  $F(2,56) = 0.54, p = 0.586$ ), and identification (d,  $H = 2.26, p = 0.323$ ). (e), Depressed BD patients classified less odors as pleasant than euthymic BD patients ( $**p < 0.01$ ), and more as unpleasant odors compared to euthymic BD patients and control subjects ( $*p < 0.05$ ) (Group:  $F(2,56) = 0.00, p > 0.999$ , Valence:  $F(2,112) = 30.1, p < 0.001$ , Interaction:  $F(4,112) = 4.15, p = 0.004$ ). (f) BD patients with higher depression severity (*i.e.* higher MADRS score) classified less odors as pleasant (middle), and more odors as unpleasant (right). The number of classified neutral odors did not change with depression severity (left). Bars are mean  $\pm$  sem.

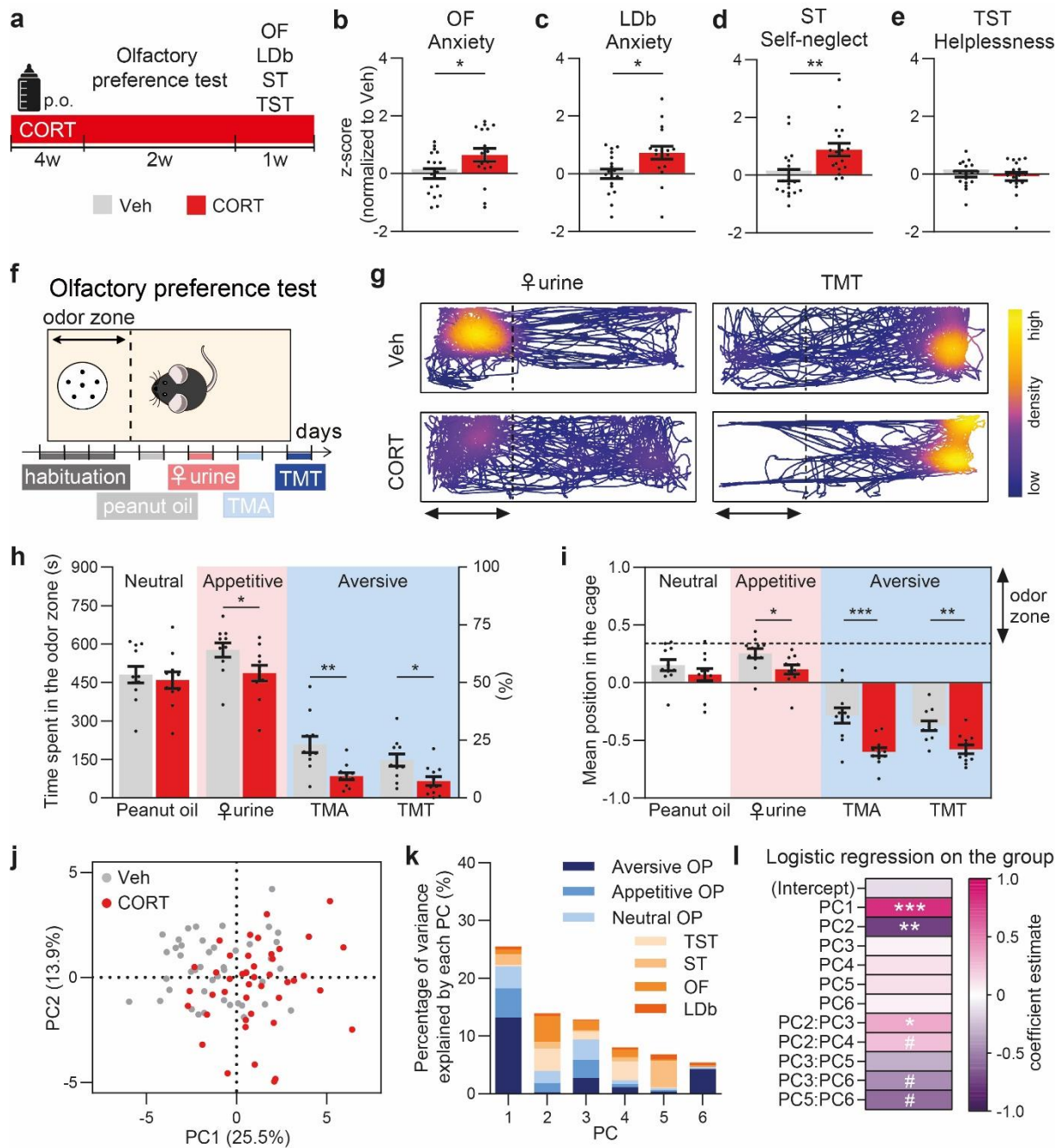
### Negative olfactory valence bias in a CORT-induced mouse model of depression

We then wondered if this negative olfactory valence bias could be also observed in mouse model of depression. We chose the well-described mouse model of anxiety/depressive-like state induced by four weeks of CORT administration in drinking water (David et al., 2009, **Figure 2a** and **Supplementary Figure 1a-b**). To validate the mice anxiety- and depressive-like phenotypes, we submitted the mice to

open field (OF), light and dark box (LDb), splash test (ST) and tail suspension test (TST). We then calculated z-scores for each behavioral test based on the different parameters measured, as well as a global emotionality score (see Materials and Methods). We confirmed the anxiety-like phenotype in the OF and LDb (**Figure 2b-c** and **Supplementary Figure 1c-g**), and the depressive-like behavior in the ST (**Figure 2d** and **Supplementary Figure 1h-j**). As previously reported, CORT-treated mice did not express despair-like features in the TST (Siopi et al., 2016, **Figure 2e** and **Supplementary Figure 1k-m**). Further parameters confirmed the expected phenotype like the body weight gain, altered coat state and global altered emotionality score (**Supplementary Figure 1b,n,o**).

To assess olfactory valence assignment in mouse, we set up an olfactory preference test, adapted from Pérez-Gómez et al. (2015). Briefly, mice were first habituated four days to the clean housing cage serving as testing arena. On one side of the cage, a petri dish with hooled cover defined the odor zone (**Figure 2f**). Each odor was presented two consecutive days, and its valence was defined by increased, decreased or not different odor zone exploration time compared to habituation in Veh-treated controls (One-way RM ANOVA:  $F(4,40) = 44.86$ ,  $p < 0.001$ , followed by FDR post-hoc comparisons,  $n = 11$ ). Peanut oil was then neutral in our experimental conditions ( $t(40) = 1.36$ ,  $q = 0.183$ ), whereas ♀urine was appetitive ( $t(40) = 3.85$ ,  $q < 0.001$ ) and predator urine synthetic compounds trimethylamine (TMA) and 2,4,5-trimethylthiazole (TMT) were aversive (TMA:  $t(40) = 5.68$ ,  $q < 0.001$ , TMT:  $t(40) = 7.27$ ,  $q < 0.001$ ).

Interestingly, CORT-treated mice investigated less appetitive ♀urine and aversive TMA and TMT, as revealed by decreased time in the odor zone and farther mean location to the odor zone (**Figure 2g-i**). This negative olfactory valence bias was specific for non-neutral odors, as we did not observe differences in the peanut oil exploration (**Figure 2h-i**), or during habituation (**Supplementary Figure 2a-c**). In addition, exposition to aversive odors decreased the distance moved compared to habituation in Veh controls (One-way RM ANOVA:  $F(4,40) = 18.17$ ,  $p < 0.001$ , followed by FDR post-hoc comparisons, TMA:  $t(40) = 4.78$ ,  $p < 0.001$ , TMT:  $t(40) = 2.55$ ,  $p = 0.019$ ), and this effect was accentuated in CORT-treated mice (**Supplementary Figure 2d**).



**Figure 2.** Negative olfactory valence bias in mouse depression model. **(a)** Mice received vehicle (grey,  $n = 18$ ) or chronic CORT (red,  $n = 17$ ) to model depression. **(b-e)** CORT mice presented anxiety-like phenotypes in the open field (**b**, OF,  $t(33) = 2.26$ ,  $p = 0.030$ ) and light and dark box (**c**, LDb,  $t(33) = 2.62$ ,  $p = 0.013$ ), and depressive-like behaviors in the splash test (**d**, ST,  $U = 59$ ,  $p = 0.001$ ) but not in the tail suspension test (**e**, TST,  $U = 149$ ,  $p = 0.909$ ). **(f)** Scheme of the olfactory preference test protocol using neutral (grey), appetitive (pink) and aversive (blue) odors. **(g)** Representative mouse tracks colored by the density of position points. **(h-i)** CORT mice explored less ♀ urine, TMA and TMT than Veh controls (**h**: Group:  $F(1,20) = 10.63$ ,  $p = 0.004$ ; Odor:  $F(3,60) = 163.50$ ,  $p < 0.001$ ; Interaction:  $F(3,60) = 1.54$ ,  $p = 0.214$ ; **i**: Group:  $F(1,20) = 17.98$ ,  $p < 0.001$ , Odor:  $F(3,60) = 150.80$ ,  $p < 0.001$ , Interaction:  $F(3,60) = 3.03$ ,  $p = 0.036$ ;  $n = 11$ ). **(j)** PCA on the 23 behavioral parameters evaluated in 87 mice from both CORT (red) and Veh (grey) mice. **(k)** The olfactory preference test (OP) stood for 86% of contributions to PC1 (blue bar parts). The OF (32%) and TST (28%) contributed mostly to PC2. **(l)** PC1, PC2 and PC2:PC3 significantly predicted the Veh/CORT status. Bars are mean  $\pm$  sem. # $0.05 \leq p < 0.1$ , \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

We were interested to know if this olfactory valence bias separating CORT-treated mice from Veh-treated controls revealed variability already measured by other behavioral tests, or could be considered as a new behavioral dimension. To do so, we applied principal component analysis (PCA) on a set of data containing 87 mice (44 Veh and 43 CORT) evaluated with all the tests presented in **Figure 2a**, *i.e.* through 23 behavioral parameters (**Figure 2j**). We selected the six first PCs, representing 72.6% of explained variance. Among each PC, we summed for each test the contributions of the different behavioral parameters (**Figure 2k**), so that the neutral, appetitive and aversive parts of the olfactory preference test (OP) contributed respectively to 15%, 20% and 51% to the PC1, 27%, 24% and 21% to PC3, and 7%, <1% and 78% to PC6. Contributions to PC2 were mainly driven by the OF (32%) and the TST (28%). The TST was also the major contributor to PC4 (40%). The ST contributed to 7% of PC1 and 8% of PC2, but 68% of PC5, as the LDb contributing to 13% of PC5. The relative segregation of olfactory preference test measurements from other tests in the PC1, PC3 and PC6 suggest that these olfactory parameters did not correlate with the LDb, OF, ST and TST, but rather captured variability on another dimension. We then performed a logistic regression on the group (Veh/CORT status) using the PCs and their second order interactions as predictors. AIC-based stepwise regression allowed us to select the minimal number of predictors while keeping the most information (see Material and Methods, **Figure 2l**). Predictors significantly associated with the group were PC1, PC2 and the interaction PC2:PC3, whereas PC2:PC4, PC3:PC6 and PC5:PC6 showed a trend to association with the group. In summary, these results suggest that olfactory valence assignment is a behavioral measurement independent and suitable to predict differences between control and depressive-like states in mice.

### **BLA circuits activity alterations in CORT-induced mouse model of depression**

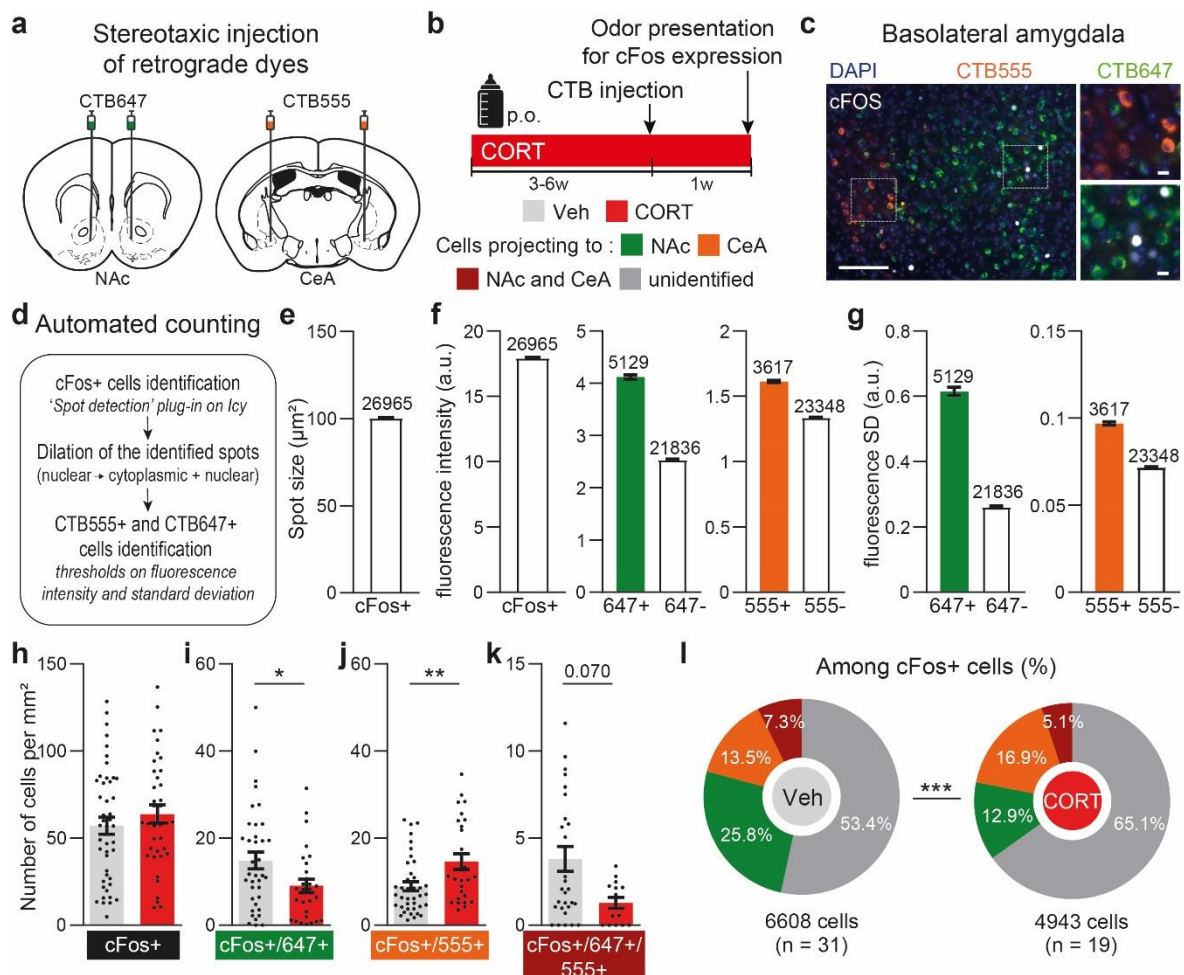
The BLA is a major structure involved in valence processing, with different neuronal population coding for attractiveness and aversiveness (Pignatelli & Beyeler, 2019). BLA projecting neurons to the NAc are thought to encode preferentially positive valence stimuli whereas projecting neurons to the CeA would encode preferentially negative valence stimuli (Namburi et al., 2015; Beyeler et al., 2016). Therefore, we wondered if alterations in the activity of these circuits could be involved in the negative olfactory valence bias observed in CORT mice, hypothesizing that the activity of BLA-to-NAc

projecting cells will be reduced in a depressive state while BLA-to-CeA cells will be more active. To test this, we injected retrograde CTB647 and CTB555 dyes in the NAc and the CeA (targeting the centromedial part) respectively (**Figure 3a**) to label BLA projecting cells to these structures. The stereotaxic surgery took place while chronically administering either CORT or Veh to mice, one week before presenting odors to trigger cFos expression, an immediate-early gene used as a proxy for neuronal activation (**Figure 3b**). We only included accurately injected mice (**Supplementary Figure 3**). We used Icy software to automatically detect cFos+, colocalizing cFos+/CTB647+, cFos+/CTB555+ and cFos+/CTB647+/CTB555+ cells setting up cut-offs on fluorescence intensity and standard deviation (**Figure 3d-g**, see Material and Methods). Even though odorless mineral oil, appetitive ♀ urine and aversive TMT recruited different proportion of the BLA-to-NAc, BLA-to-CeA and BLA-to-NAc-and-CeA neurons both in Veh control and in CORT mice, the total density of cFos+ and colocalizing cFos+/CTB+ cells were mostly similar across odors and groups (**Supplementary Figure 4** and **Supplementary Table 1**). Interestingly, when compared regardless of the odor used for cFos stimulation, BLA-to-NAc cells were less activated in CORT mice compared to Veh controls, and BLA-to-CeA cells more active (**Figure 3i-j**), possibly underlying the negative olfactory valence bias displayed in this model of depression. The total cFos+ cell number did not differ between both groups in the BLA (**Figure 3h**). BLA cells projecting to both NAc and CeA tended to be less recruited in CORT mice than in Veh controls (**Figure 3k**). Finally, the number of BLA projecting cFos+ cells were differently distributed between groups, with less BLA-to-NAc cFos+ cells, and more BLA-to-CeA and unidentified cFos+ cells in the CORT relative to the Veh group (**Figure 3l**).

Previous reports suggest specific roles of the basolateral and lateral amygdala subregions (BLA and LA, respectively) in valence assignment processing based on their different projection patterns (Beyeler et al., 2018). We performed similar analyses in the LA, showing some variations compared to the BLA. Interestingly, no difference were found on both LA-to-NAc and LA-to-CeA neuronal activation between Veh controls and CORT mice while the LA-to-NAc-and-CeA cells were more recruited in CORT mice. (**Supplementary Figure 5** and **Supplementary Table 2**).



Even though inconsistent throughout studies, topographical gradients of cells have been suggested to distinguish their preferential positive or negative encoding role (Kim et al., 2016; Beyeler et al., 2018). We then wondered if spatial location of active cells in response to odor presentation throughout the BLA and LA could be altered in CORT mice. When we analyzed the spatial coordinates of cFos+ and cFos+/CTB+ cells on antero-posterior, medio-lateral and dorso-ventral axes, we observed that all types of cells were similarly distributed between odors along axes (except for cFos+/CTB555+ which were more recruited in the anterior part of the LA/BLA in response to ♀urine compared to TMT; **Supplementary Figure 6 and Supplementary Table 3**). Overall, in our experimental approach, very few differences could be observed in the topography of these cells between CORT and Veh groups.



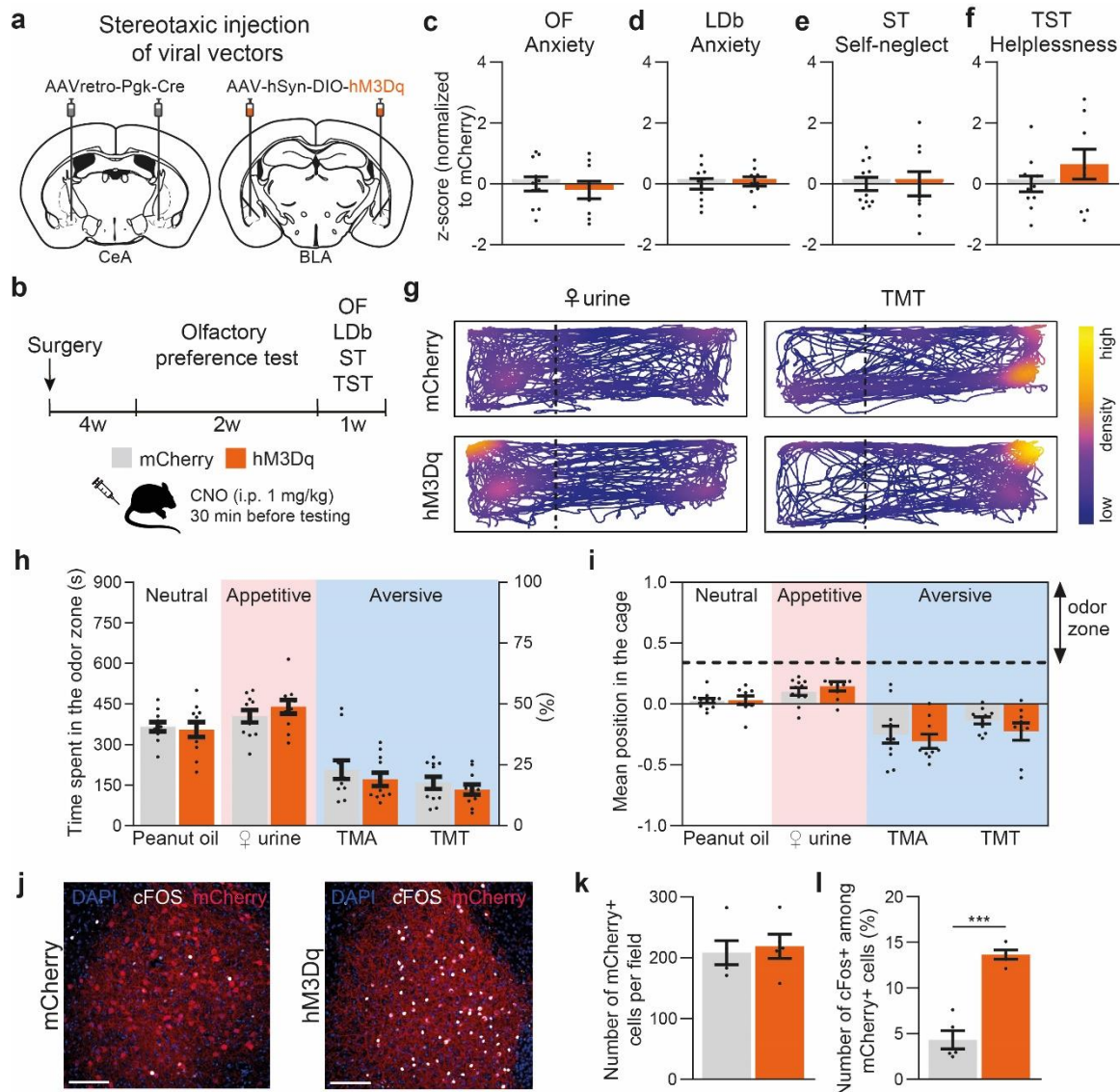
**Figure 3.** Altered BLA circuits activity after chronic CORT administration. (a-b) Retrograde fluorescent CTB647 and CTB555 dyes were injected in the NAc (green) and the CeA (orange) respectively. Odors were presented to trigger the immediate-early gene cFos expression in CORT (red) and Veh (grey) mice. (c) Representative image of BLA cFos expression colocalized with CTB647 and/or CTB555. Scale bars, 100  $\mu\text{m}$  (left) and 20  $\mu\text{m}$  (right). (d) Scheme of the protocol for automated cell counting. (e-g) Size (e) and fluorescence intensity (f left) of identified and dilated cFos+ spots, which were then sorted depending on their far red (647+) or red (555+)

fluorescence intensity (**f** middle and right) and standard deviation (SD, **g**) to determine the colocalization of cFos expression with CTB647 (**f** middle, **g** left) or CTB555 (**f,g** right). (**h-k**) Quantification of cFos+ (**h**), cFos+/CTB647+ (**i**), cFos+/CTB555+ (**j**) and cFos+/CTB647+/CTB555+ cell number (**k**) in the BLA. BLA cFos+ cell number was similar between groups (**h**,  $t(81) = 0.93$ ,  $p = 0.356$ ,  $n = 38-45$ ). BLA cFos+/CTB647+ cell number decreased in CORT mice (**i**,  $U = 356$ ,  $*p = 0.032$ ,  $n = 28-37$ ), whereas cFos+/CTB555+ cell number increased (**j**,  $U = 328$ ,  $**p = 0.006$ ,  $n = 27-40$ ). BLA cFos+/CTB647+/CTB555+ cell number tended to decrease in CORT mice (**k**,  $U = 144.5$ ,  $p = 0.070$ ,  $n = 15-29$ ) (**l**) Distribution of CTB647 and/or CTB555 colocalization among the total number of cFos+ cells in the BLA ( $\chi^2(3) = 340.0$ ,  $***p < 0.001$ ). Bars are mean  $\pm$  sem; a.u. : arbitrary unit.

Altogether, chronic CORT administration induced, irrespective of the olfactory valence of stimuli, decreased recruitment of BLA-to-NAc cells and increased recruitment of BLA-to-CeA cells, circuits already implicated in opposite valence encoding. We then wondered if such BLA circuits disturbances could be responsible for the negative olfactory bias observed in our mouse model of depression.

### **Chemogenetic BLA-to-CeA activation in control mice**

To know if these BLA circuits alterations had a causal role in the behavioral phenotype of CORT mice, we first attempted to elicit anxiety- and depressive-like behaviors as well as negative olfactory valence bias by chemogenetic BLA-to-CeA cells activation in control mice. For that purpose, we injected a retrograde AAVr-Pgk-Cre viral vector in the CeA, in order to express the Cre-recombinase in all the cells projecting to the CeA, and an anterograde AAV-hSyn-DIO-hM3Dq-mCherry (or AVV-hSyn-DIO-mCherry) in the BLA to express hM3Dq, an activator designer receptor exclusively activated by designer drugs (DREADD), coupled with mCherry protein (or mCherry alone) specifically into BLA-to-CeA cells (**Figure 4a**). Intraperitoneal (i.p.) clozapine-n-oxide (CNO) injection activated the cells (**Figure 4b**), as confirmed by increased cFos expression in mCherry+ cells (**Figure 4j-l**). Remarkably, chemogenetic BLA-to-CeA activation was not sufficient to trigger anxiety-like phenotypes in the OF and LDb, nor depressive-like phenotypes in the ST and TST (**Figure 4c-f** and **Supplementary Figure 7**). Consistently, it also failed to create negative olfactory valence bias in the olfactory preference test (**Figure 4g-i** and **Supplementary Figure 8**).

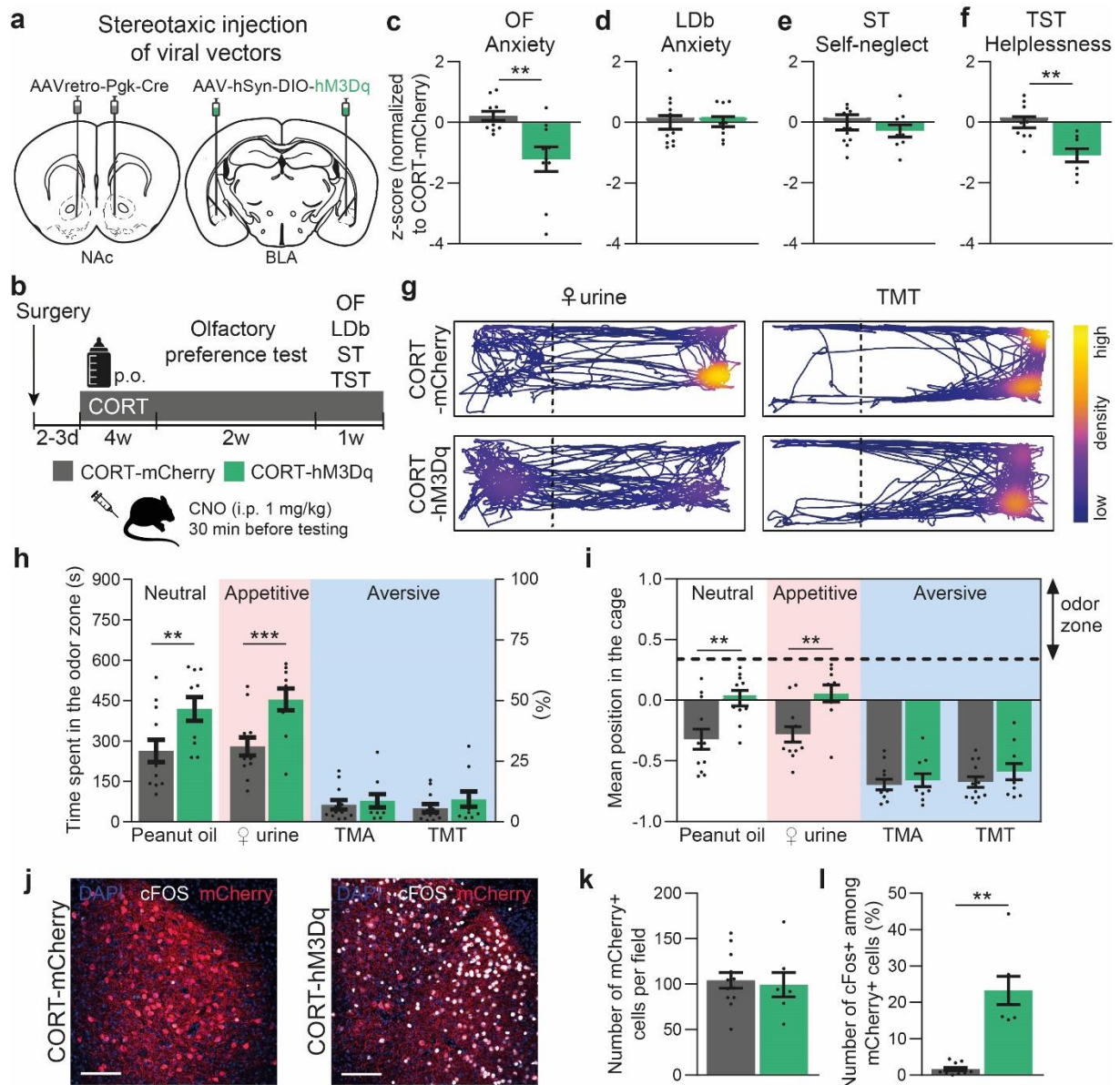


**Figure 4.** Chemogenetic BLA-to-CeA cells activation was not sufficient to mimic chronic CORT phenotype. **(a-b)** AAVr-Pgk-Cre in the CeA and AAV-hSyn-DIO-hM3Dq-mCherry (orange,  $n = 9$ , or AAV-hSyn-DIO-mCherry for the controls, grey,  $n = 11$ ) in the BLA were injected to activate BLA-to-CeA cells. **(c-f)** CNO intra-peritoneal (i.p.) injection in the hM3Dq mice had no effect on the behaviors measures in the OF **(c)**,  $t(18) = 0.54$ ,  $p = 0.598$ ), LDb **(d)**,  $t(18) = 0.35$ ,  $p = 0.733$ ), ST **(e)**,  $t(18) = 0.02$ ,  $p = 0.987$ ) or TST **(f)**,  $t(18) = 1.24$ ,  $p = 0.232$ ) compared to the mCherry controls. **(g)** Representative mouse tracks in the olfactory preference test. **(h-i)** Chemogenetic activation of BLA-to-CeA cells did not modify olfactory valence compared to mCherry controls **(h)**, Group:  $F(1,18) = 0.10$ ,  $p = 0.752$ ; Odor:  $F(3,54) = 75.02$ ,  $p < 0.001$ ; Interaction:  $F(3,54) = 1.16$ ,  $p = 0.336$ ; **(i)**, Group:  $F(1,18) = 0.28$ ,  $p = 0.605$ ; Odor:  $F(3,54) = 43.65$ ,  $p < 0.001$ ; Interaction:  $F(3,54) = 1.15$ ,  $p = 0.338$ ). **(j)** Representative images and magnifications of BLA cFos expression. Scale bar, 125  $\mu\text{m}$ . **(k-l)** Quantification of the mCherry+ cell number in mCherry and hM3Dq mice **(k)**,  $t(8) = 0.37$ ,  $p = 0.722$ ) and the percentage of cFos expression among mCherry+ cells after CNO injection **(l)**,  $t(8) = 8.31$ ,  $***p < 0.001$ ). Bars are mean  $\pm$  sem.

### Chemogenetic BLA-to-NAc activation in CORT mice

We then used the same strategy to activate BLA-to-NAc cells in CORT mice in order to alleviate anxiety- and depressive-like phenotype, as well as the negative olfactory valence bias. We injected a retrograde AAVr-Pgk-Cre viral vector in the NAc, in order to express the Cre-recombinase in all the

cells projecting to the NAc, and an anterograde AAV-hSyn-DIO-hM3Dq-mCherry (or AVV-hSyn-DIO-mCherry) in the BLA, to express hM3Dq coupled with mCherry protein (or mCherry alone) specifically into BLA-to-NAc cells (**Figure 5a**). We administered CORT to all animals for four weeks and then performed behavioral testing after CNO i.p. injection (**Figure 5b**). Chemogenetic BLA-to-NAc cells activation in CORT mice was sufficient to relieve anxiety-like behavior in the OF but not in the LDb, compared to CORT-mCherry mice (**Figure 5c-d** and **Supplementary Figure 9c-g**). It had no effect on the depressive-like phenotype measured by the ST, but had an antidepressant effect on the TST (**Figure 5e-f** and **Supplementary Figure 9h-m**). Overall, it decreased the global emotionality score in CORT-hM3Dq mice compared to CORT-mCherry controls (**Supplementary Figure 9n**). Strikingly, CNO injection also increased exploration of peanut oil and ♀urine in CORT-hM3Dq relative to CORT-mCherry, while leaving unchanged the aversive odors exploration (**Figure 5h-i** and **Supplementary Figure 10**). In addition, presentation of the appetitive female urine in Veh control mice increased the distance moved compared to the peanut oil (**Supplementary Figure 3d**,  $t(60) = 2.28$ ,  $q = 0.026$ ), suggesting increased locomotion as a marker of positive olfactory valence. We also observed an increased locomotor activity in presence of peanut oil and ♀urine in CORT-hM3Dq mice with respect to CORT-mCherry (**Supplementary Figure 10d**). It is worth noting that the time of exploration of the object during habituation also tended to be increased in CORT-hM3Dq mice (**Supplementary Figure 10c**). Therefore, BLA-to-NAc cells activation is sufficient to reverse negative olfactory bias and potentiate positive valence assignment of neutral or appetitive odors in our mouse model of depression.



**Figure 5.** Chemogenetic BLA-to-NAc cells activation partially improves the CORT phenotype. **(a)** AAVr-Pgk-Cre in the NAc and AAV-hSyn-DIO-hM3Dq-mCherry (green square, or AAV-hSyn-DIO-mCherry for the controls, grey square) in the BLA were injected to activate BLA-to-NAc cells. **(b)** All mice were treated with CORT. **(c-f)** CNO i.p. injection decreased anxiety-like phenotype in CORT-hM3Dq mice in the OF (**c**,  $t(11.46) = 3.30$ , \*\* $p = 0.007$ ,  $n = 10-12$ ) and was antidepressant on the TST (**f**,  $t(16) = 3.85$ , \*\* $p = 0.001$ ,  $n = 8-10$ ) compared to CORT-mCherry controls, but had no effect in the LDdb (**d**,  $t(20) = 0.09$ ,  $p = 0.926$ ,  $n = 10-12$ ) or ST (**e**,  $t(19) = 0.82$ ,  $p = 0.420$ ,  $n = 9-12$ ). **(g)** Representative mouse tracks in the olfactory preference test. **(h-i)** CORT-hM3Dq mice explored more peanut oil and ♀ urine relative to CORT-mCherry, but did not investigate differently aversive odors (**h**: Group:  $F(1,20) = 6.56$ ,  $p = 0.019$ ; Odor:  $F(3,60) = 11.2$ ,  $p < 0.001$ ; Interaction:  $F(3,60) = 7.31$ ,  $p < 0.001$ ; **i**: Group:  $F(1,20) = 7.37$ ,  $p = 0.013$ ; Odor:  $F(3,60) = 111.8$ ,  $p < 0.001$ ; Interaction:  $F(3,60) = 7.88$ ,  $p < 0.001$ ;  $n = 10-12$ ). **(j)** Representative images and magnifications of BLA cFos expression. Scale bar, 125 μm. **(k-l)** Quantification of the mCherry+ cell number in CORT-mCherry and CORT-hM3Dq mice (**k**,  $U = 36$ ,  $p = 0.650$ ,  $n = 7-12$ ) and the percentage of cFos expression among mCherry+ cells after CNO injection (**l**,  $t(6.135) = 5.51$ , \*\* $p = 0.001$ ,  $n = 7-12$ ). Bars are mean  $\pm$  sem.



## **Discussion**

In the present study, we show a negative bias in odor valence assignment in bipolar depressed patients. By using a pre-clinical translational test, we also demonstrate for the first time a negative olfactory hedonic bias in a mouse model of depression induced by chronic CORT administration. Indeed, we found that chronic CORT treatment is associated with a shift to more negative valence assignment of both pleasant and unpleasant odors. These biases are associated with disturbances in brain circuits involved in valence assignment. More specifically, BLA-to-NAc neurons activity is reduced while BLA-to-CeA neurons activity is increased in CORT-treated mice. Furthermore, the activation of BLA-to-NAc circuit is sufficient to improve at least partially the negative olfactory hedonic bias observed in our model.

### **Negative emotional bias in bipolar depressed patients**

Previous studies already demonstrated that emotional bias is an essential component of depressive episode leading depressed patients to attribute more negative valence to events or objects (Leppänen, 2006; Clark et al., 2009; Harmer et al., 2009; Bigot, 2020). Here we show that bipolar depression is associated with a negative bias on odor valence assignment measured by the Sniffin' sticks test. This bias is correlated with depression severity. In fact, depressed bipolar patients rated less odors as pleasant than euthymic BD patients, and more odors as unpleasant than both control subjects and euthymic BD patients. No difference was found in the allocation of neutral odors, suggesting a specific bias for salient stimuli. Interestingly, these olfactory emotional biases seem to regress during recovery of the depressive episode (namely in euthymic BD patients), indicating their role in the determinism and maintenance of the depressive mood.

These results confirm and extend previous data obtained in patients suffering from unipolar depressive episode (Atanasova et al., 2010; Naudin et al., 2012; Kohli et al., 2016; Kazour et al., 2020; Colle et al., 2020). Regarding bipolar depression, only one previous work showed that patients with bipolar depression exhibited a reduced hedonic score towards positive stimuli in agreement with our data (Kazour et al., 2020). However, in this study no difference was found in the hedonic score associated with negative odors and neutral olfactory cues were not analyzed. We also found that bipolar depressive

patients performed similarly than control subjects and euthymic patients concerning odor detection, discrimination and identification tests, consistently with the only comparable study (Kazour et al., 2020).

### **Negative olfactory hedonic bias in a mouse model of depression**

While mood is the main criterion for defining mood disorders in humans, this feature cannot be assessed in animals. Behavioral tests expected to explore mood in pre-clinical studies are far to be satisfactory. They are mostly limited to measuring the level of anhedonia with sucrose preference test or self-administration of psychostimulant drugs. Here, we developed an assay in mice based in the innate response to both positive and negative odors that does not require previous learning. Using this approach, we found that chronic CORT treatment is associated with a shift to a more negative valence assignment of both pleasant (that become less pleasant) and unpleasant (that become more unpleasant) odors. This hedonic bias is linked with a depressive-like phenotype similarly to what is found in humans. Of note, in these experiments, it is difficult to disentangle the role of valence *vs* intensity processing of emotional stimuli in the negative hedonic bias observed in CORT mice. Previous results have shown that chronic CORT induced deficits in olfactory acuity, fine discrimination of mixed odorants and olfactory memory (Siopi et al., 2016). However, in our study we analyzed the innate response to individual highly concentrated odors. In addition, the increased response to aversive odor in CORT-treated animals suggest that CORT-treated mice can effectively detect this type of odor stimuli. Importantly, our analysis also demonstrates that olfactory valence assignment is a powerful behavioral variable able to predict differences between control and depressive-like states in mice.

### **Alteration of BLA circuits after CORT administration**

In the BLA, projecting neurons to the NAc are thought to encode preferentially positive valence stimuli whereas projecting neurons to the CeA would encode preferentially negative valence stimuli (Namburi et al., 2015; Beyeler et al., 2016). Therefore, we wondered if alterations in the activity of these circuits could be involved in the negative olfactory valence bias observed in CORT mice, hypothesizing that the activity of BLA-to-NAc projecting cells will be reduced in a depressive state while BLA-to-CeA cells will be more active. As expected, we find the BLA-to-NAc neurons expressed less cFos in CORT-treated animals compared to the Veh control group, independently of the odor stimuli used to trigger

neuronal activation. On the contrary, the density of cFos BLA neurons projecting to the CeA is higher in animals with a depressive-like phenotype. Interestingly, some differences were found in BLA vs LA projecting neurons suggesting that both regions could be differently affected in depression.

What are the mechanisms behind BLA circuit dysregulation in depressive states? It has been shown that BLA-to-NAc and BLA-to CeA population neurons are highly interconnected and they mutually control their activity (Kim et al., 2016; Beyeler et al., 2018; Calhoon et al., 2018). In addition, although less abundant, local interneurons can tightly tune the functioning of the circuit (Prager et al., 2016). Increased peripheral levels of cortisol/corticosterone might disrupt molecular and cellular neuroplasticity in the BLA. Indeed, prolonged corticosteroid stimulation leads to atrophy of apical dendrites and granular cell death in the hippocampus (Bodnoff et al., 1995; de Quervain et al., 1998; Sapolsky, 2000). In this context, chronic stress might induce synaptic plasticity changes both in BLA principal neurons and local interneurons. Importantly, some evidence suggests that microglia activation in the amygdala could shape plasticity in these regions (VanRyzin et al., 2019). Even more, evidence of adult-neurogenesis in this region was also found, suggesting a high level of plasticity (Sanderson & Wilson, 1997; Bernier et al., 2002; Jhaveri et al., 2018).

Overall, our data show for the first time that olfactory biases in mouse model of depression induced by chronic administration of CORT are associated with reduced activation of BLA-to-NAc projecting neurons and increase activity of BLA-to-CeA cells. Further experiment will be required to identify the cellular types and the mechanisms behind the alteration in the activity of different BLA circuits.

### **Activation of BLA-to-NAc pathway partially improves negative hedonic value**

Our data show an increased activity of BLA-to-CeA cells in CORT-treated mice suggesting that depressive-phenotype and negative olfactory hedonic bias could be mimicked by over-stimulation of this pathway. However, we were unable to induce neither anxiety- or depressive-like phenotypes, nor a negative olfactory bias by chemogenetically stimulating the BLA-to-CeA pathway in control animals. Importantly, these data confirm that the anxiety/depressive-like phenotype is highly related to the olfactory bias. A possible explanation is the already described anxiolytic effect of clozapine, to which CNO rapidly converts *in vivo* (Gomez et al., 2017; Tran et al., 2020). This pharmacological effect could



compensate the activation of BLA-to-CeA neurons and attenuate the expression of anxiety/depressive-like phenotypes and negative emotional bias.

In contrast, activation of the BLA-to-NAc pathway allowed us to reduce the anxiety/depressive-like phenotype in CORT-treated mice, with statistically significant effects in the OF and the TST. Interestingly, in our hands, CORT treatment does not modify the performance on TST compared to the Veh-treated group. However, this test is sensitive to the activation of BLA-to-NAc projecting cells, supporting the idea that it would be better suited for measuring antidepressant effects than depressive-like behaviors in agreement with previous debates (Reardon et al, 2019). The exact opposite was observed for the ST, sensitive to the CORT pro-depressant effects but not the BLA-to-NAc activation antidepressant effects. Regarding the hedonic olfactory bias, activation of BLA-to-NAc neurons increases in the attractiveness for both neutral and pleasant odors. Importantly, BLA-to-NAc neurons are primarily suggested to be involved in reward processing and positive valence assignment, which is consistent with the effect we observed. However, it does not modify the response to negative odors with respect to the CORT-mCherry control group, indicating that other pathways could be required to restore the bias involving negative stimuli. Negative stimuli predicting danger, as the ones used in our study, might rely on multiple evolutionally selected and redundant mechanisms, and could be tougher to hijack. Importantly, we cannot completely rule out that a pharmacological effect of CNO could contribute to the improvement of depressive-like phenotypes by BLA-to-NAc neurons activation in CORT-hM3Dq mice, even though the CORT-mCherry control mice also received CNO in our experiment.

The BLA-to-NAc neuronal activation in a CORT-induced mouse model of depression was found to correct the anxiety/depressive-like behavior in the novelty suppressed feeding test (Dieterich et al., 2021). However, in this study, the CORT administration did not elicit clear anxiety- and depressive-like behaviors. In the same line, Ramirez et al. (2015) demonstrated that BLA-to-NAc inhibition could impede the antidepressant effect of positive-engram dentate gyrus cells activation. Present data demonstrate that BLA-to-NAc activation is able to improve positive valence assignment, which could be one mechanism for further alleviation of the anxiety- and depressive-like phenotypes associated with CORT treatment (Harmer et al., 2017).

Besides, Shen et al. (2019) showed that the BLA-to-NAc pathway could be subdivided into neurons triggering real-time place aversion, expressing the neuropeptide cholecystokin (CCK+), and CCK- neurons triggering real-time place preference. Sub-chronically inhibition of the “negative” CCK+ BLA-to-NAc neuronal population was sufficient to correct depressive-like behaviors induced by chronic social defeat stress, while activation of these neurons promoted depressive-like behaviors. To explain these opposite findings, above mentioned studies may have targeted mainly the CCK- BLA-to-NAc neurons. Overall, these results suggest that the BLA-to-NAc neurons might act as a common pathway for antidepressant treatment to restore negative hedonic bias and anxiety/depressive-like behaviors.

Futures steps will require to explore the effect of various classes of antidepressants (AD) on this process. Identifying a common final behavioral and neuronal pathway for AD with very different mechanisms of action would be an essential element in better understanding the pathophysiology of depression. Moreover, deciphering the role of specific brain circuits with genetically identified neurons in mice can open new avenues for developing specifically targeted drugs. Biomarkers that capture how effective drugs modulate the brain functional activity could prioritize candidate compounds, thus improving the productivity and cost-effectiveness of drug development. If reversibility of emotional negative bias is mandatory to expect an AD action, new molecules should be tested using the olfactory preference test that we have developed to assess olfactory valence assignment in the very early phase of development of new drugs. This would complement the gold standard but challenged forced swim and tail suspension tests for developing AD molecules. Moreover, new tests in patients to assess rapid improvement of emotional bias, using for instance olfaction, could also be implemented as an early prediction of AD response, facilitating treatment selection.

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## **Author contributions**

MB: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – Original Draft, Writing – Review and Editing; AB, EV, CHDB, MM: Investigation, Formal analysis, Visualization; PML, JH: Writing – Review and Editing, Supervision, Funding acquisition; MA: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – Original Draft, Writing – Review and Editing, Supervision; CH: Conceptualization, Methodology, Writing – Original Draft, Writing – Review and Editing, Supervision, Funding acquisition.

## **Material and Methods**

### **Human subjects**

The study was conducted in a tertiary-care psychiatric hospital located in Créteil, France. Consecutive sampling over a 36-month period was used to recruit patients. Forty-eight patients were included in our study, aged from 18–65 years, with a current bipolar disorder diagnosis according to the DSM-5 criteria. Potential participants were independently diagnosed by two board-certified psychiatrists with the aid of a structured interview based on DSM-IV criteria (American Psychiatric Association, 1980). Among the recruited patients, twenty-five subjects presented an euthymic state and twenty-three in a depressive, dysthymic or mixed state at the moment of the evaluation. Depression severity was measured by MADRS scale.

The same two psychiatrists classified the BP individuals as BP I (n = 29) or BP II (n = 18) according to the DSM-IV criteria. A control group (n = 11 subjects) was recruited through word-of-mouth from local community.

### **Olfactory assessment**

The Sniffin' sticks test (Burghardt®, Wedel, Germany) comprises 3 subtests, resulting in 3 scores: detection threshold, discrimination and identification scores (Hummel et al., 2007; Rumeau et al., 2016).

The detection threshold score was assessed using 16 dilutions prepared from a 4% n-butanol solution (dilution ratio 1:2). Three pens (two containing the solvent and the third the odorant) were presented in a randomized order using a single staircase of increasing concentration [16 (lower concentration) to 1 (higher concentration)]. Subjects had to identify the odor-containing pen. Reversal of the staircase was triggered when the odorant was correctly identified in two successive trials. The detection threshold score was defined as the mean of the last four of seven staircase reversals, scores ranging from 1 to 16 (the higher, the better). Subjects were blindfolded during this test.

To assess the discrimination score, triplets of pens were presented in a randomized order (two containing the same and one a different odorant). Subjects had to determine which of three pens smelled differently, scores ranging from 1 to 16. Subjects were blindfolded during this test as well.

The identification score was assessed for 16 common odors (orange, leather, cinnamon, peppermint, banana, lemon, liquorice, turpentine, garlic, coffee, apple, cloves, pineapple, rose, anise and fish). Using a multiple-choice task, the identification index of individual odors was performed from lists of four descriptors each, scores ranging from 1 to 16. Moreover, during the Sniffin' sticks identification task, patients and control subjects were asked for the pleasantness of the 16 selected odors smelling, as it was previously published (Swiecicki et al., 2009). The number of odors rated as 'pleasant', 'unpleasant' or 'neutral' range from 0 to 16 and the sum of these scores was equal to 16.

## **Animals**

All animal care and experimental procedures followed national and European (2010/63/EU) guidelines and were approved by the French Ministry of Research (APAFiS: #16380-2018080217358599\_v1). C57BL/6N male mice (7–8 weeks old) purchased from Taconic Farms (Denmark) were used for all behavioral tests and immunohistochemistry studies (n = 151). Once arrived in the animal facility, they were given at least one week to accommodate. Mice were socially housed, 4-6 per cage, and maintained under standard housing conditions (23 ± 1 °C; humidity 40%) on a 14/10 h light/dark cycle (lights were on from 6:30 AM to 9:30 PM every day) with food and water *ad libitum*, except during behavioral experiments and odor exposures. All behavioral tests were conducted during the period of light (10 AM-7 PM). We used the minimum number of animals, estimated from our previous knowledge in performing the same type of experiments.

## **Drugs**

### **Chronic corticosterone administration depression model**

Corticosterone (CORT) purchased from Sigma-Aldrich (France) was sonicated for 2 h in a vehicle made of 10% (2-hydroxypropyl)-beta-cyclodextrin ( $\beta$ -CD; Sigma-Aldrich, France) in water. After complete dissolution, the solution was added to the appropriate amount of water to reach the final concentration of 35  $\mu$ g/ml CORT and 0.45%  $\beta$ -CD. CORT (35  $\mu$ g/ml, equivalent to about 5 mg/kg/day) or vehicle (0.45%  $\beta$ -CD) was available *ad libitum* in the drinking water in bottles wrapped with aluminum to protect it from light. All the bottles were changed every 3-4 days in order to prevent any possible degradation, as previously described (David et al., 2009). Animal were weighted twice a week to verify the increase in body mass already described in this model (David et al., 2009).

### **Clozapine n-oxide**

Clozapine n-oxide (CNO) dihydrochloride (Tocris, France) was dissolved in sterile saline (NaCl 0.9%) and adjusted to a final concentration of 0.2 mg/ml. On behavioral testing days, mice received intra-peritoneal (i.p.) injection at a concentration of 1 mg/kg, 30 min before testing. Fresh CNO solution was prepared every 1-3 days.

### **Stereotaxic surgery**

Buprenorphine (0.05 mg/kg sub-cutaneous (s.c.) injection) was administered 30 minutes before the surgery. Mice were anesthetized with a ketamine/xylazine mix (150 mg/kg and 5 mg/kg respectively, i.p. injection) and placed in a stereotaxic frame, with their body temperatures maintained using a heating pad. The cranial skin was shaved and disinfected with povidone iodine, and craniotomies were performed above the injection sites. Droplets of lidocaine were administered on the cranial skin before aperture and suture. At the end of the surgery, meloxicam (1 mg/kg s.c. injection) was administered to help recovering.

### **CTB injection**

In order to label the neurons of the Basolateral Amygdala (BLA) projecting to the Nucleus Accumbens (NAc) or the Central Amygdala (CeA), the retrograde tracer Cholera Toxin Subunit B conjugated with Alexa Fluor 555 or 647 (CTB 555 or CTB 647, 1 mg/ml, Invitrogen, USA), was bilaterally injected with a glass micropipette connected to a Nanoject III microinjector (Drummond Scientific) at a rate of 5 nl/s in the NAc (AP : +1.4 mm ; ML :  $\pm 0.87$  mm ; DV : -4.35 mm, 300 nl) or in the CeA (AP : -0.8 mm, ML :  $\pm 2.35$  mm, DV : -4.35 mm, 100 nl, targeting the centromedial part). Distances were measured from theoretical bregma and brain surface. The needle was left in place one minute before each injection and 2-5 min after, and then slowly withdrawn. Allen Brain atlas was used as reference to report injection site in each animal (Supplementary Figure 5). Brain hemispheres where the injection site was outside the analyzed regions were discarded for the analysis (NAc: Hemispheres, n = 50/166, Mice, n = 17/83; CeA: Hemispheres, n = 71/166, Mice, n = 17/83).

### **Viral vector injection**

AAV5-hSyn-DIO-hM3Dq-mCherry, AAV5-hSyn-DIO-mCherry and AAVretro-PGK-Cre recombinant adeno-associated virus were used in order to manipulate the BLA neuronal activity (Atasoy et al, 2017). All AAV were purchased from Addgene (USA). The viral vectors were bilaterally injected into the BLA (AP: -1.75 mm, ML:  $\pm 3.15$  mm, DV: -4.25 mm, 100-150 nl) and the NAc (same coordinates as mentioned above, 100-150 nl) or the CeA (same coordinates as mentioned above, 50 nl) as previously mentioned. Animals whom both hemispheres were injected outside the analyzed regions were discarded for the analysis (BLA-to-NAc: n = 2/24; BLA-to-CeA: n = 2/22).

### **Behavioral assessment**

A battery of behavioral tests was used to assess anxiety and depression-like phenotypes, and olfactory preference. Before starting behavioral testing, mice were handled ~30 s at least twice a day for 3 days to habituate to the experimenter.

**Open-field.** Animals were placed in grey Plexiglas containers (43 x 43 cm<sup>2</sup>) and their behavior was recorded by a video camera during 20 min. A tracking system (Noldus Ethovision 3.0, Netherlands) was used to map center and periphery zones and to calculate the time spent and distance moved in each zone. The time spent in the center, the number of entries in the center and the total distance traveled were calculated as measures of anxiety behavior and ambulatory activity respectively. The arenas were cleaned with water between each trial.

**Light and dark box.** A two-compartment box containing a dark chamber (black walls with upper lid) and a light chamber (~300 lux, white Plexiglas walls, no upper lid) was used. The chambers were connected by a 10 × 10 cm door in the middle of the wall. Animals were placed in one corner of the light chamber facing the wall and were allowed to freely explore for 6 min. The Noldus Ethovision 3.0 tracking system was used to record behavior. The number of entries and time spent in the light

chamber were estimated as measures of anxiety. Between each trial, the light/dark compartments were cleaned with water.

**Splash test.** The test consisted in squirting ~ 200  $\mu$ l of a 10% sucrose solution on the dorsal coat of the mouse and placing it in its home cage without cage mates. The test was performed in a quiet, dimly lit room (~30 lux). Grooming latency, frequency, and duration were assessed during 6 min, as measures of self-neglect and depressive-like behaviors.

**Tail suspension test.** Mice were suspended at approximately one-third from the end of the tail, using regular tape, to a metal rod about 30 cm from the table, for 6 min. Upon viewing of the video recordings blindly to the treatment, the latency to immobility, the number of immobility episodes and the total time spent in an immobile posture was measured. Longer periods of immobility are associated with depressive-like states.

**Coat state test.** The total coat state score resulted from the sum of the score of five different body parts: neck, dorsal/ventral coat, tail, forepaws and hindpaws. For each body area, a score of 0 was given for a well-groomed coat and 1 for an unkempt coat (Griebel et al., 2002; Santarelli et al., 2003). The measurements of the coat state were done by an experimenter blind to treatments.

**Olfactory preference test.** The test was adapted from Pérez-Gómez et al. (2015). The test was performed in a quiet and dimly lit room (~ 40 lux), around 3 to 6 pm. Clean housing cages (17 x 32 cm) with regular bedding material were used as testing arenas, covered by transparent Plexiglas lids. Each testing arena received mice socially housed in the same cage. The first day, all the mice from the same cage were placed together to overcome neophobia, in each testing arena for habituation during 15-20 min. The second day, a petri dish (94 mm diameter) with a hooled cover was placed and adhered to one side of the arena, for defining an odor zone (one-third of the cage). For 12 consecutive days, the behavior was recorded by a video camera for 15 min and the Noldus Ethovision 3.0 system was used to track the position of the mice. The time spent in an odor zone and the locomotor activity were used as measures of olfactory valence. During the first 4 days, only a Whatman paper filter (GE Healthcare Life Sciences, USA) was placed into the petri dish, to assess the baseline exploration. Then, 2 days were dedicated to each odor, placed on a paper filter, in the following order: peanut oil (pure, 400  $\mu$ l), female urine (pure, 100  $\mu$ l), trimethylamine (Sigma-Aldrich, 6.75% in water, 400  $\mu$ l) and 2,4,5-trimethylthiazole (Sigma-Aldrich, 5% in mineral oil, 400  $\mu$ l). Peanut oil and female urine are classical appetitive odorants, whereas trimethylamine and 2,4,5-trimethylthiazole are predator urine synthetic compounds, aversive at these concentrations (Li et al., 2013; Pérez-Gómez et al., 2015; Root et al., 2015). However, in our experimental conditions, animals were not food deprived before the test and consequently peanut oil had a neutral value and did not trigger attraction. Repeating the measurement of each odor twice allowed to decrease the inter-subject variability of this spontaneous behavior.

### **Behavioral tests z-scores**

z-scores were calculated by standardization of the values measured (subtracted by the mean of the control group [Veh mice in Figure 2, BLA-to-CeA mCherry mice in Figure 4 and BLA-to-NAc CORT mCherry mice in Figure 5], and then divided by the control group standard deviation) for each parameter of each behavioral test. The directionality (*e.g.* the positive or negative sign) of the z-scores was adjusted so that an increased value corresponds to more anxiety- or depressive-like phenotype (Guilloux et al., 2011). It included the time spent and the number of entries in center and the distance moved for the open field, the time spent and the number of entries in the light box for the light and dark box, the latency to first grooming, the number of grooming episodes and the time spent grooming for the splash test, and the latency to immobility, the number of immobility episodes and the time spent immobile for the tail suspension test. The global z-scores for each behavioral test were calculated as the mean of z-scores of each parameter measured in the test, and the global emotionality score as a mean of all the z-scores for each behavioral test.

### **Odor stimulation for cFos activation**

Exposure sessions were conducted in custom-made device that allow a specific and controlled odor stimulation avoiding odor contaminations. Animal were located in small cages (0.5 L) that could be rapidly saturated with odorant vapor (pump air flux 3.5 L/min; total saturation time ~ 9 s). The odor input was located on the top of the cage and an extraction fan was located on the bottom to evacuate the odorant vapor (fan speed, 147 L/min; total evacuation time ~ 200 ms). Mice were first habituated to the device for 1 h for 3 consecutive days. During these habituation sessions, animals were subjected the first day to deodorized air with the extraction fan operating continuously. During the second and third days of habituation, the protocol alternating the functioning of fan and pump as in the day of test was used. The day of test, mice were subjected to (1) deodorized air for 60 min to minimize basal cFos expression, (2) odorant-containing air pulses (0.3 L/min pump odor flux; 8.5% final odor dilution) for 60 min (36 pulses of 40 s each, interleaved with 60 s applications of deodorized air to avoid sensory habituation), (3) additional 2 h in the small cages with pump and fans off. The mice were then quickly perfused. Animals from Veh and CORT-treated groups were exposed with only one odor solution: mineral oil (pure, 10 ml), female urine (pure, 5 ml) or TMT (10% in mineral oil, 10 ml). For female urine, a cotton stub impregnated with 100 µl of female urine was located on the top of the cage and the bottle containing the female urine solution was heated at 37 °C to increase odor stimulation.

### **Immunohistochemistry**

Mice were deeply anesthetized with a ketamine/xylazine mix (*i.p.*, 150 mg/kg and 50 mg/kg respectively) and perfused transcardially with a solution containing 0.9% NaCl, followed by 4% paraformaldehyde in phosphate buffer, pH 7.3. Brains were removed and postfixed by incubation in the same fixative at 4°C for additional 2 hours. Brain were then cryoprotected by incubation in 30% sucrose + 0.02% azide in PBS for 24-48 h. Forty-micrometer-thick coronal brain sections were



obtained using a sliding microtome (Leica SM 2010 R). Immunostaining was performed on free-floating sections. Non-specific staining was blocked by 0.2% PBS-Triton and 10% normal donkey serum (Sigma-Aldrich, Germany). Sections were incubated with 0.2% PBS-Triton, 4% bovine albumin serum (Sigma-Aldrich), 3% normal donkey serum and the following primary antibodies at 4°C : guinea pig anti-cFos (1:2000, Synaptic Systems, Germany) and rabbit anti-RFP (1:4000, Rockland ) for 48 h. Sections were then incubated with secondary antibodies (Alexa-conjugated secondary antibodies at 1:1000, Jackson ImmunoResearch Laboratories, United Kindom) at room temperature for 2 h. Fluorescent sections were stained with the nuclear dye DAPI (1:5000, Invitrogen) and then mounted using Fluoromount aqueous mounting medium (Sigma-Aldrich).

### **Image acquisition**

For slices stained for cFos expression after CTB647 and CTB555 injections, tissue sections from the whole BLA (basolateral and lateral amygdala, 10-20 per mouse) were collected using an Axioscan microscope (Zeiss, 10X objective). cFos+ cells, CTB647+ and CTB555+ cells, cFos+/CTB647+ and cFos/CTB555+ double-positive cells, and cFos+/CTB647+/CTB555+ triple-positive cells in the BLA were counted automatically using Icy, an open community platform for bioimage analysis (Institut Pasteur, see Icy software protocol). Values were given as cell density (number of cells per mm<sup>2</sup>). For cFos+ cell density analysis, mean values obtained from individual mice were reported (Figure 3, Figure S4).

To verify neuronal activation of hM3Dq-expressing cells (Figure 4 and 5), one week after the end of behavioral experiments, animals were injected with CNO 1 mg/kg i.p. 2 h before transcerebral perfusion. Immunohistochemistry for cFos and mCherry was performed as previously described. Images of slices were collected using a confocal laser-scanning microscope (LSM 700; Zeiss; 25X objective; 6-10 slices per mice). cFos+ cells and cFos+/mCherry+ double-positive cells were manually counted throughout the entire stack of optical slices (4 stacks spaced by 5 µm) by an experimenter blind to the image condition. Values were reported as total number of mCherry+ cells per field and percentage of cFos+ cells among mCherry+ cells averaged for each animal.

### **Icy and R softwares**

Image analyses were performed using the Icy open source platform (<http://www.icy.bioimageanalysis.org>; De Chaumont et al., 2012; Olivo-Marin, 2002). Regions of interest (ROI) were first drawn manually around the lateral and the basolateral amygdala. Identification of the antero-posterior coordinates of each slice was also performed manually. An Icy protocol was designed to automatically count cFos+ cells and their colocalization with CTB555 or CTB647. To do so, the “Spot Detector” plug-in was applied to each ROI (LA and BLA), creating small ROI (“spots”) around each cFos+ nucleus. These spots were then dilated by 3 pixels on X and Y to include the soma of the cells. In these enlarged spots, thresholds on red (CTB555) and far red (CTB647) fluorescence intensity and standard deviation were applied to determine if the spots were

CTB555+ or CTB555- and CTB647+ or CTB647-. Parameters were set manually and were identical for all the images from one experiment replicate (n = 3 experiment replicates). Collected data sheets contained information about spot size, fluorescence intensity and standard deviation, as well as X and Y coordinates relative to the center of the ROI (LA/BLA taken together). Spatial distribution of detected cells in medio-lateral and dorso-ventral axes were calculated with respect to the center of the drawn ROI corresponding to the LA/BLA), on each coronal slice whose antero-posterior position along the LA/BLA was manually determined

The R software (CRAN project, v3.6.3) was used to manage the data sheets generated by Icy software and produce final tables.

### **Statistical analysis**

Statistical analyses were performed with GraphPad Prism v9 software (USA). Normality was assessed using the Shapiro-Wilk test. The Barlett test was then used to statistically compare the variances.

Parametric or non-parametric tests were used accordingly: unpaired Student with or without Welch correction or Mann-Whitney tests, One-way ANOVA or Kruskal Wallis tests with repeated measures when suitable, Two-way ANOVA and Three-way ANOVA or Mixed-effect model when some values were missing, with repeated measures when suitable. Following post-hoc analyses were always applied with the False Rate Discovery method of Benjamini-Hochberg. Correlation analyses in Figure 1 were performed with the Pearson or the Spearman coefficient depending on the normality of the data. Chi-square tests were performed when suitable with contingency tables. Log-rank tests were performed on survival tables.

Principal Component Analysis (PCA) was performed with R (package FactoMineR) on a dataset containing 87 animals (44 Veh-treated and 43 CORT-treated) assessed on 23 behavioral parameters (3 parameters of the open field, 2 of the light and dark box, 3 of the splash test, 3 of the tail suspension test (reported in Supplementary Figure 1) and 12 of the olfactory preference test including for all the odors the time exploring, the mean position and the distance moved). Each behavioral parameter was z-scored as explained previously based on the Veh-treated control group of the experiment replicate (n = 3 experiment replicates). Missing values were completed using the missMDA package from R. The values were standardized so that the final mean and the standard deviation of each parameter equal 0 and 1 respectively. Six PCs were kept in order to attend >70% of explained variance (72.6%). The contributions of each behavioral parameter to each PC were computed and summed by behavioral test. The contributions of each test to each PC are represented by the proportion of the total bar plotted in Figure 2k. A logistic principal component regression (PCR) on the group (Veh vs CORT) was then performed using the PCA scores of each animal for the six first PCs, and their second-level interactions. The stepAIC function from the MASS package was used to perform a stepwise algorithm in order to choose the final model based on the best Akaike information criterion (AIC), an estimator of the model quality taking into account the goodness of fit (amount of variance explained) and the

simplicity of the model (number of explaining variables). The direction of the stepwise search was set on both backward and forward, meaning that removed explaining variables could be add again on each step. The final coefficient estimates and the associated p-values for the retained PCs and their second-level interactions are reported in Figure 21.

All datasets were described using the mean; error bars in the figures represent standard error mean (SEM), Differences were considered significant for  $p < 0.05$ .

The datasets and codes that support the findings of this study are available from the corresponding author upon reasonable request.

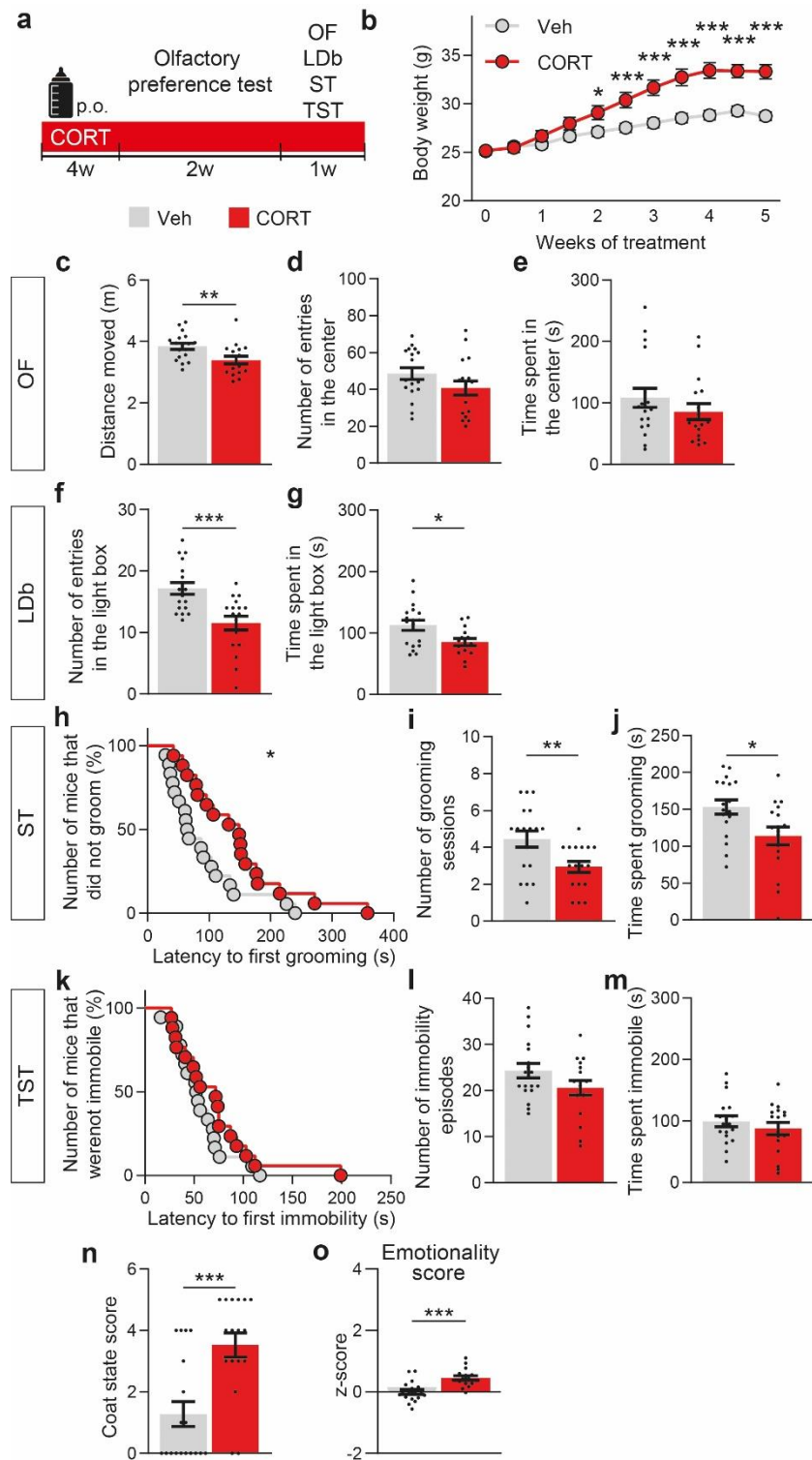
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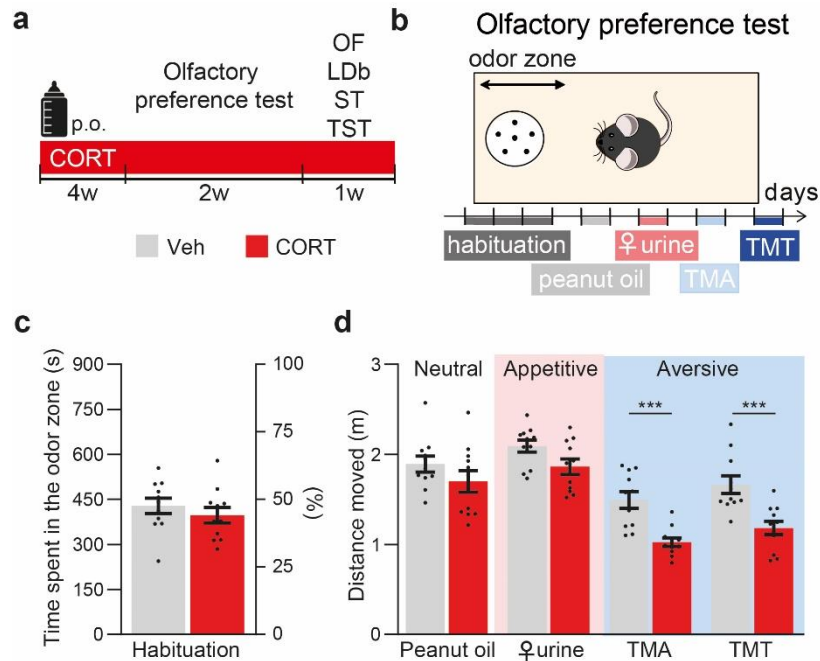
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## Supplementary Figures and Tables



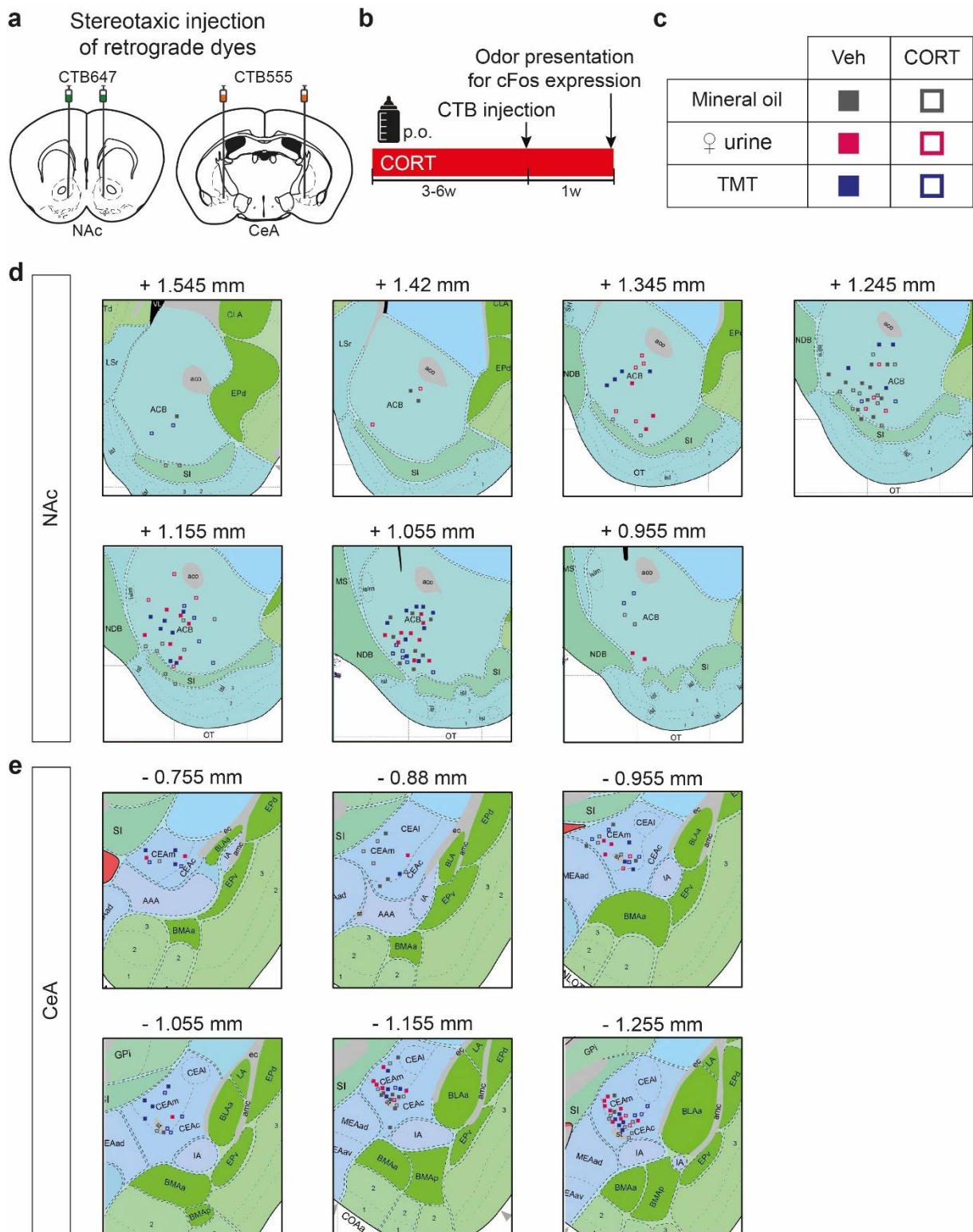
**Supplementary Figure 1.** Chronic CORT administration triggered anxiety-like and depressive-like behaviors in mice. (a) Mice receive vehicle (grey,  $n = 18$ ) or chronic CORT (red,  $n = 17$ ) to model depression. (b) Chronic CORT induced more body weight gain than Veh (Group:  $F(1,33) = 10.61$ ,  $p = 0.003$ ; Weeks:  $F(10,330) = 316.3$ ,  $p < 0.001$ ; Interaction:  $F(10,330) = 48.10$ ,  $p < 0.001$ ). (c-e) CORT mice moved less in the OF compared to Veh controls (c,  $t(33) = 2.79$ ,  $**p = 0.009$ ), but did not explore differently the center (d,  $t(33) = 1.58$ ,  $p = 0.123$ ; e,  $t(33) = 1.11$ ,  $p = 0.274$ ). (f-g) CORT mice explore less the light box during LDb (f,  $t(33) = 3.84$ ,  $***p < 0.001$ ; g,  $t(31) = 2.58$ ,  $*p = 0.015$ ,  $n = 15$  CORT). (h-j) Grooming is reduced in CORT mice during ST, with higher latency to

first grooming (**h**,  $\chi^2(1) = 4.51$ ,  $*p = 0.034$ ) and lower grooming sessions number (**i**,  $t(33) = 2.76$ ,  $**p = 0.009$ ) and time spent grooming (**j**,  $t(33) = 2.58$ ,  $*p = 0.015$ ). (**k-m**) Neither the latency to first immobility (**k**,  $\chi^2(1) = 1.19$ ,  $p = 0.275$ ), nor the number of immobility episodes (**l**,  $t(33) = 1.67$ ,  $p = 0.104$ ) nor the time spent immobile (**m**,  $t(33) = 0.87$ ,  $p = 0.393$ ) are different between CORT mice and Veh controls in the TST. (**n**) The CORT mice coat is deteriorated compared to Veh controls ( $U = 57.50$ ,  $***p < 0.001$ ). (**o**) Global emotionality score is increased in CORT mice ( $t(32) = 4.23$ ,  $***p < 0.001$ ). Bars are mean  $\pm$  sem.

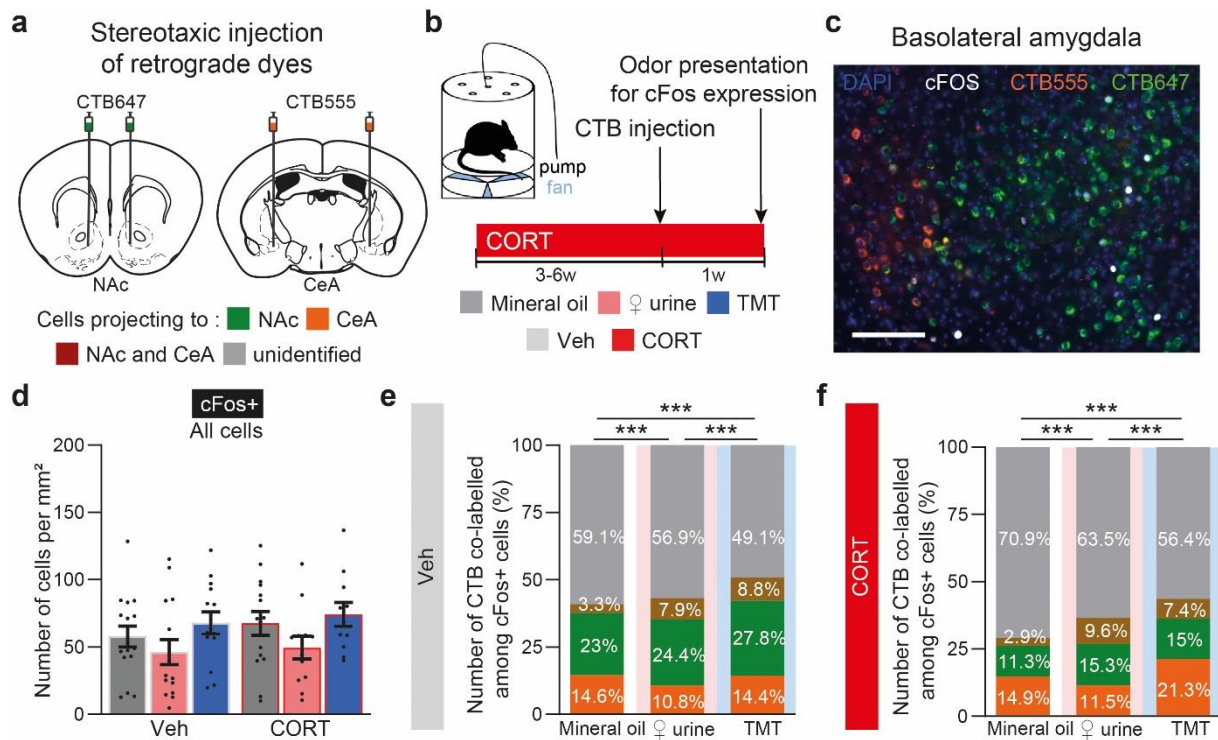


**Supplementary Figure 2.** Reduced locomotor activity in CORT mice upon aversive odor presentation in the olfactory preference test. (**a**) Chronic CORT (red,  $n = 11$ ) administration is used to model depression. Control mice only receive vehicle (grey,  $n = 11$ ). (**b**) Scheme of the olfactory preference test protocol. (**c**) Chronic CORT administration does not alter exploration of the “object” zone habituation without odor. (**d**) Aversive odors reduce the distance moved compared to peanut oil in Veh controls (Peanut oil vs TMA:  $t(60) = 4.50$ ,  $q < 0.001$ ; Peanut oil vs TMT:  $t(60) = 2.58$ ,  $q = 0.019$ ), and this effect is accentuated in CORT mice (Group:  $F(1,20) = 12.78$ ,  $p = 0.002$ ; Odor:  $F(3,60) = 56.24$ ,  $p < 0.001$ ; Interaction:  $F(3,60) = 3.09$ ,  $p = 0.034$ ;  $***p < 0.001$ ). Bars are mean  $\pm$  sem.





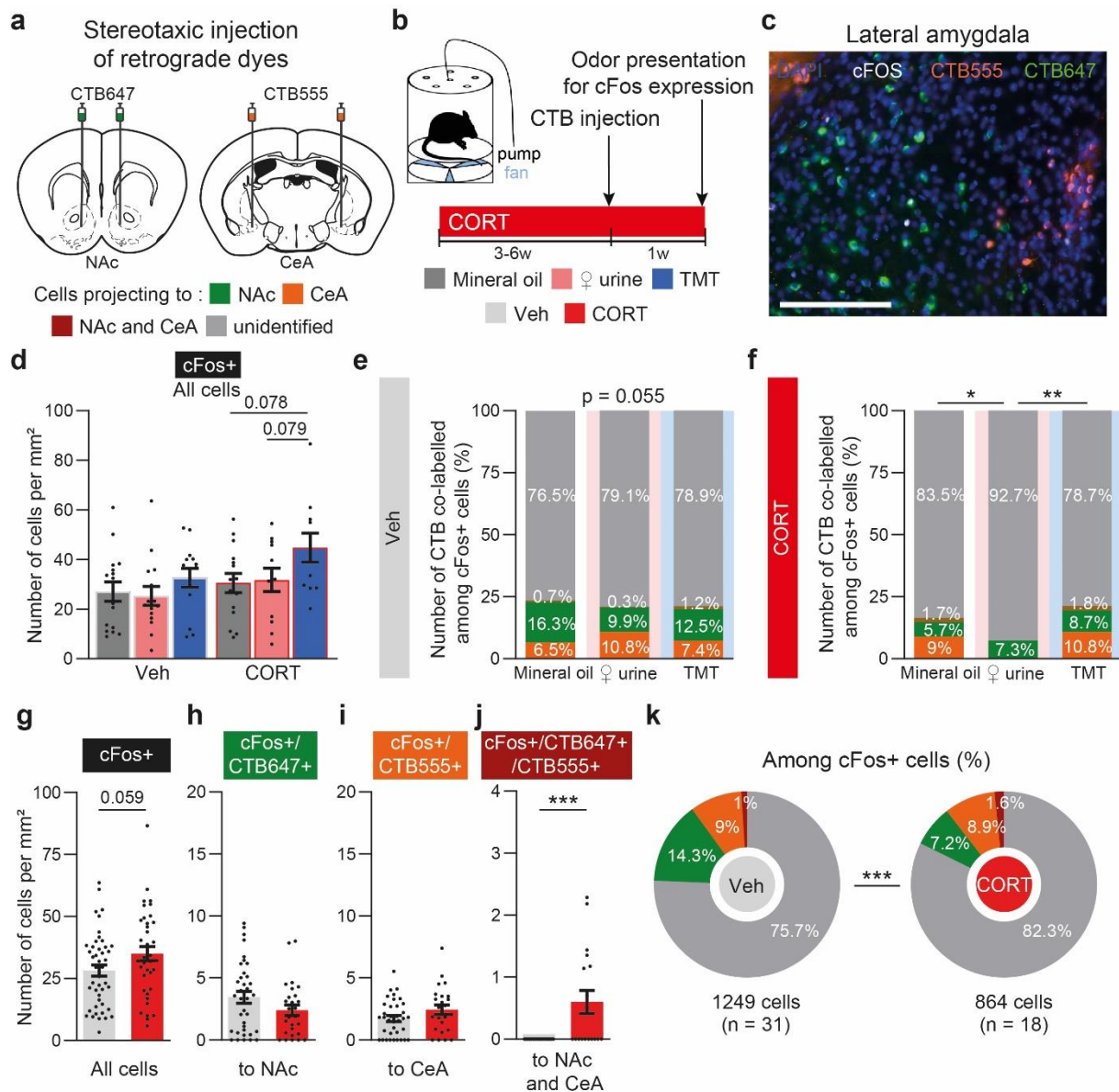
**Supplementary Figure 3.** Location of stereotaxic CTB injection sites. **(a-b)** Retrograde fluorescent CTB dyes were injected in the NAc and the CeA before presenting odors to trigger cFos expression. **(c)** Table presenting the symbol code used in **(d-e)**. **(d)** Injection sites for the NAc (Hemispheres,  $n = 116$ ; Mice,  $n = 66$ ). **(e)** Injection sites for the CeA (Hemispheres,  $n = 95$ ; Mice,  $n = 66$ ).



**Supplementary Figure 4.** Differential BLA circuits activation in response to appetitive and aversive odors in Veh and CORT mice (**a-b**) Retrograde fluorescent CTB647 and CTB555 dyes were injected in the NAc (green) and the CeA (orange) respectively. Appetitive (pink), neutral (dark grey) and aversive (blue) odors were presented to trigger the immediate-early gene cFos expression in CORT and Veh mice. (**c**) Representative image of BLA cFos expression colocalized with CTB647 and/or CTB555. Scale bar, 100  $\mu$ m. (**d**) The density of cFos+ cells was similar across groups and odors (Group:  $F(1,77) = 0.82$ ,  $p = 0.368$ ; Odor:  $F(2,77) = 3.45$ ,  $p = 0.037$ ; Interaction:  $F(2,77) = 0.07$ ,  $p = 0.930$ ,  $n = 12-16$ ; post-hoc analyses with FDR correction for the Odor effect were performed on each group separately and no statistically significant difference was found). (**e-f**) Proportion of activated BLA projecting cells to the NAc, the CeA or to both structures in Veh (**e**) and CORT (**f**) groups changes upon presentation of odorless mineral oil (1412 and 2777 cells respectively), ♀ urine (1574 and 417 cells respectively) and TMT (3575 and 1749 cells respectively) (**e**,  $\chi^2(6) = 88.35$ ,  $p < 0.001$ ; Mineral oil vs ♀ urine:  $\chi^2(3) = 37.20$ ,  $p < 0.001$ ; Mineral oil vs TMT:  $\chi^2(3) = 69.50$ ,  $p < 0.001$ ; ♀ urine vs TMT:  $\chi^2(3) = 29.11$ ,  $p < 0.001$ ; **f**,  $\chi^2(6) = 142.60$ ,  $p < 0.001$ ; Mineral oil vs ♀ urine:  $\chi^2(3) = 53.29$ ,  $p < 0.001$ ; Mineral oil vs TMT:  $\chi^2(3) = 117.40$ ,  $p < 0.001$ ; ♀ urine vs TMT:  $\chi^2(3) = 21.69$ ,  $p < 0.001$ ). \*\*\* $p < 0.001$ . Bars are mean  $\pm$  sem.

Type of cells	Veh			CORT		
	Mineral oil	♀urine	TMT	Mineral oil	♀urine	TMT
cFos+/CTB647+	12.15 ± 2.35 (12)	9.43 ± 2.45 (10)	19.51 ± 3.37 (13)	5.99 ± 2.31 (10)	10.43 ± 3.11 (9)	14.80 ± 4.40 (10)
Group: F(1,58) = 1.67, p = 0.202; Odor: F(2,58) = 4.22, p = <b>0.020</b> ; Interaction: F(2,58) = 0.70, p = 0.502						
Veh ♀urine vs Veh TMT: t = 2.365, q = 0.064						
cFos+/CTB555+	10.33 ± 2.07 (13)	6.13 ± 1.28 (13)	10.94 ± 1.72 (13)	13.58 ± 2.10 (13)	13.94 ± 5.43 (5)	16.50 ± 3.71 (9)
Group: F(1,60) = 7.33, p = <b>0.009</b> ; Odor: F(2,60) = 0.97, p = 0.386; Interaction: F(2,60) = 0.41, p = 0.667						
cFos/CTB647+/ CTB555+	0.73 ± 0.29 (7)	1.76 ± 0.54 (8)	6.39 ± 1.20 (12)	1.77 ± 0.73 (9)	4.27 ± 2.76 (3)	4.72 ± 2.22 (7)
Group: F(1,40) = 0.30, p = 0.588; Odor: F(2,40) = 6.22, p = <b>0.005</b> ; Interaction: F(2,40) = 1.19, p = 0.314						
Veh Mineral oil vs Veh TMT: t = 3.36, q = <b>0.005</b>						
Veh ♀urine vs Veh TMT: t = 2.87, q = <b>0.010</b>						

**Supplementary Table 1.** The density of cFos+/CTB647+, cFos/CTB555+ and CTB647+/555+ cells (calculated as number of cells per mm<sup>2</sup>) in the BLA is mostly similar across groups and odors. Data are reported as mean ± sem (n). Two-way ANOVA statistical analysis was followed by post-hoc FDR comparisons performed either on each group separately when the main Odor effect was statistically significant, or on each odor separately when the main Group effect was statistically significant. Only post-hoc comparisons with statistical trend or significance are indicated here.

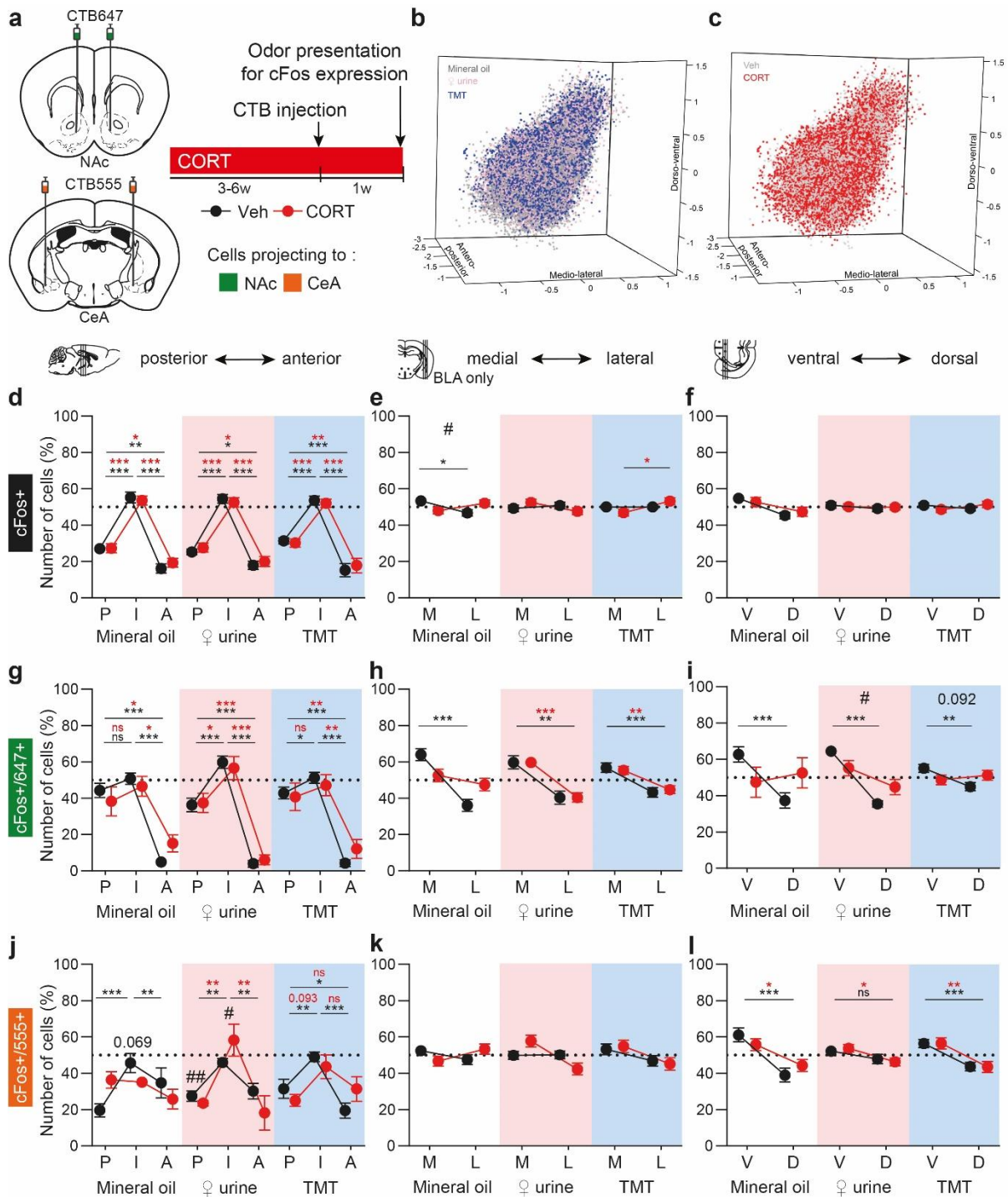


**Supplementary Figure 5.** LA circuits activity upon presentation of appetitive and aversive odors in Veh and CORT mice. (a-b) Retrograde fluorescent CTB647 and CTB555 dyes were injected in the NAc (green) and the CeA (orange) respectively. Appetitive (pink), neutral (dark grey) and aversive (blue) odors were presented to trigger the immediate-early gene cFos expression in CORT and Veh mice. (c) Representative image of LA cFos expression colocalized with CTB647 and/or CTB555. Scale bar, 100  $\mu$ m. (d) The density of cFos+ cells tended to increase in CORT mice in response to TMT compared to mineral oil and  $\bar{\text{f}}$ urine (Group:  $F(1,77) = 4.40$ ,  $p = 0.036$ ; Odor:  $F(2,77) = 3.47$ ,  $p = 0.036$ ; Interaction:  $F(2,77) = 0.52$ ,  $p = 0.594$ ,  $n = 11-16$ ; post-hoc analyses with FDR correction for the Odor effect were performed on each group separately and for the Group effect on each odor separately, for which no difference was found statistically significant). (e-f) Proportion of activated LA projecting cells to the NAc, the CeA or to both structures in Veh mice (e) tends to change and CORT mice (f) changes upon presentation of odorless mineral oil (307 and 401 cells respectively),  $\bar{\text{f}}$ urine (344 and 82 cells respectively) and TMT (758 and 381 cells respectively) (e,  $\chi^2(6) = 12.32$ ,  $p = 0.055$ ; f,  $\chi^2(6) = 14.32$ ,  $p = 0.026$ ; Mineral oil vs  $\bar{\text{f}}$ urine:  $\chi^2(3) = 9.75$ ,  $p = 0.021$ ; Mineral oil vs TMT:  $\chi^2(3) = 3.53$ ,  $p = 0.317$ ;  $\bar{\text{f}}$ urine vs TMT:  $\chi^2(3) = 12.09$ ,  $p = 0.007$ ). (g-j) Regardless of which odor was used for cFos stimulation, CORT mice display a trend to increased density of LA cFos+ (g,  $t(81) = 1.92$ ,  $p = 0.059$ ,  $n = 38-45$ ) and increased cFos+/CTB647+/CTB555+ (j,  $U = 105$ ,  $p < 0.001$ ,  $n = 18-21$ ) cell number relative to Veh controls, but no difference regarding LA cFos+/CTB647+ (h,  $U = 377.5$ ,  $p = 0.133$ ,  $n = 27-36$ ) and cFos+/CTB555+ (i,  $t(58) = 1.645$ ,  $p = 0.106$ ,  $n = 24-36$ ) density. (k) Distribution of CTB647 and/or CTB555 colocalization among the total number of cFos+ cells in the LA differs between CORT and Veh groups ( $\chi^2(3) = 27.61$ ,  $p < 0.001$ ). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Bars are mean  $\pm$  sem.

Type of cells	Veh			CORT		
	Mineral oil	♀urine	TMT	Mineral oil	♀urine	TMT
cFos+/CTB647+	2.78 ± 1.03 (12)	2.91 ± 0.74 (11)	4.55 ± 0.62 (13)	1.25 ± 0.52 (9)	2.90 ± 0.74 (9)	4.52 ± 1.64 (10)
Group: F(1,58) = 0.45, p = 0.507; Odor: F(2,58) = 3.68, p = <b>0.031</b> ; Interaction: F(2,58) = 0.41, p = 0.667						
CORT Mineral oil vs CORT TMT: t = 2.29, q = 0.077						
cFos+/CTB555+	1.43 ± 0.34 (12)	1.98 ± 0.43 (13)	1.80 ± 0.52 (11)	2.60 ± 0.33 (12)	1.44 ± 1.01 (5)	4.96 ± 1.55 (9)
Group: F(1,58) = 4.47, p = <b>0.039</b> ; Odor: F(2,56) = 2.95, p = 0.061; Interaction: F(2,56) = 2.91, p = 0.063						
Veh TMT vs CORT TMT: t = 3.14, p = <b>0.008</b>						
cFos/CTB647+/ CTB555+	0.00 ± 0.00 (6)	0.00 ± 0.00 (8)	0.31 ± 0.15 (11)	0.67 ± 0.30 (8)	0.00 ± 0.00 (3)	0.78 ± 0.32 (7)
Group: F(1,37) = 4.00, p = <b>0.053</b> ; Odor: F(2,37) = 2.65, p = 0.083; Interaction: F(2,37) = 0.92, p = 0.409						

**Supplementary Table 2.** The density of cFos+/CTB647+, cFos/CTB555+ and CTB647+/555+ cells (calculated as a number of cells per mm<sup>2</sup>) in the LA is mostly similar across groups and odors. Data are reported as mean ± sem (n). Two-way ANOVA statistical analysis was followed by post-hoc FDR comparisons performed either on each group separately when the main Odor effect was statistically significant, or on each odor separately when the main Group effect was statistically significant. Only post-hoc comparisons with statistical trend or significance are indicated here.



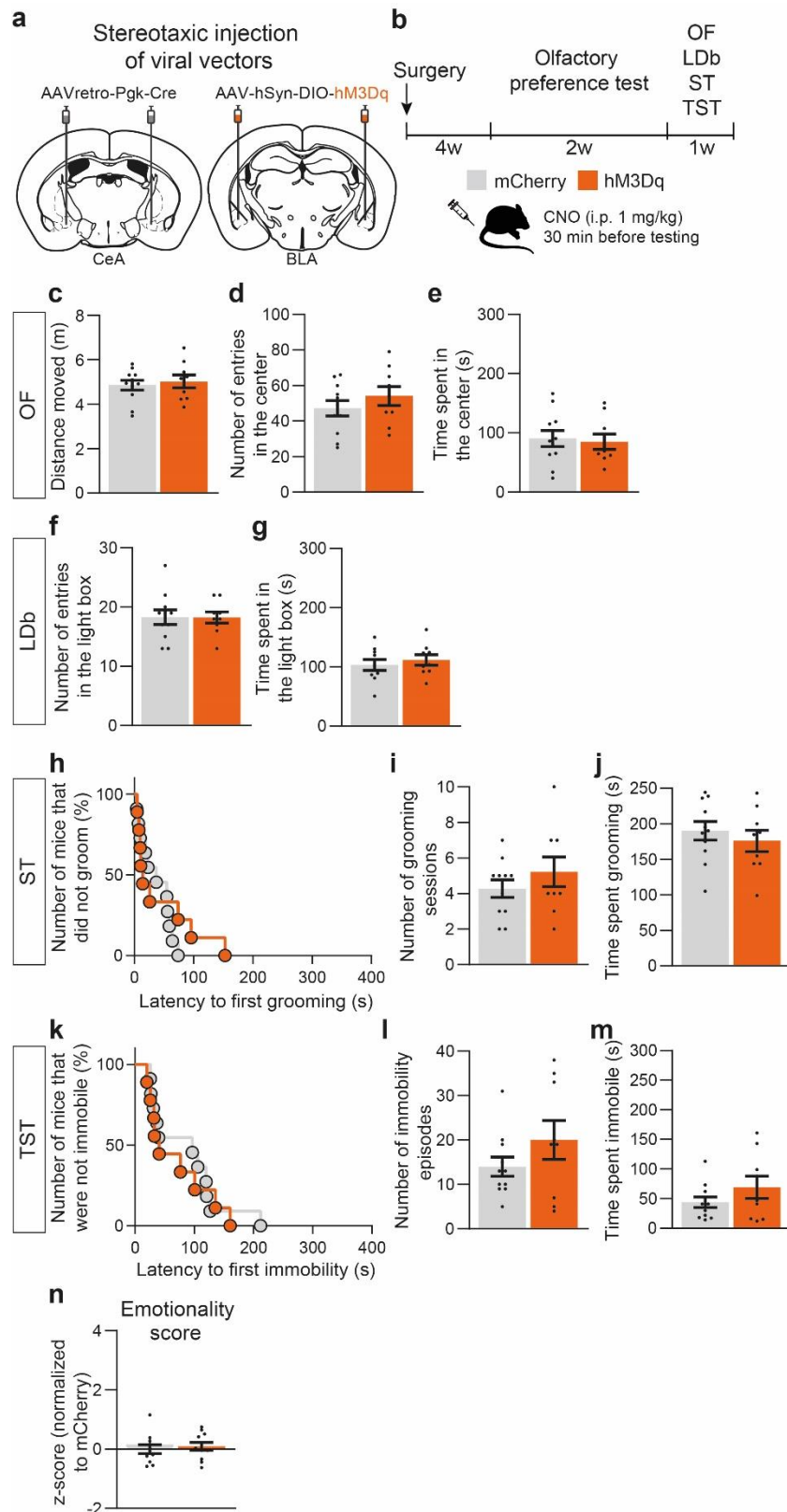


**Supplementary Figure 6.** Spatial location of cFos+ and colocalizing cFos+/CTB+ cells through LA/BLA in response to appetitive and aversive odors in both Veh and CORT mice. **(a)** Odorless mineral oil, appetitive ♀ urine and aversive TMT were presented to stimulate the immediate-early gene cFos expression, one week after stereotaxic injection of retrograde dyes CTB647 and CTB555 in the NAc and the CeA (centromedial part) respectively, in chronically CORT or Veh-treated mice. **(b-c)** 3-dimensional plot of all the cFos+ cells responding to different odors **(b)** in the different groups **(c)** (n = 11-16, corresponding to 32,288 cFos+ cells). **(d-l)** Spatial distribution of cFos+ **(d-f)**, cFos+/CTB647+ **(g-i)** and cFos+/CTB555+ **(j-l)** cells along the antero-posterior **(d,g,j)**, medio-lateral **(e,h,k)** and dorso-ventral **(f,i,l)** axis. Along the antero-posterior axis, the anterior part (A) corresponds to [-0.6;-1.4] mm respective to bregma, the intermediate part (I) to [-1.4;-2] mm and the posterior part (P) to [-2;-3] mm. For the medio-lateral axis, only BLA cells were included. The medial part (M) corresponds to [-1.4;-0.2] mm respective to the center of the drawn region of interest (ROI) around the LA/BLA while the lateral part (L) corresponds to ]-0.2;1.4] mm to the ROI center. Concerning the dorso-ventral axis, the cut was made around the BLA/LA separation. The ventral part (V) corresponds to [-1.6;-0.2] mm to the ROI center, while the

dorsal part corresponds to  $[-0.2;1.6]$  mm to the ROI center. Three-way repeated measures ANOVA analyses are reported in **Supplementary Table 3**. Black and red stars (\*) represent post-hoc statistically significant differences along axes for the Veh and CORT groups respectively. Hashtags (#) represent post-hoc statistically significant differences between Veh and CORT, also reported in **Supplementary Table 3**. \*#p < 0.05, \*\*/#p < 0.01, \*\*\*p < 0.001, ns: not significant. Data are points  $\pm$  sem.

Type of cell	cFos+		cFos+/CTB647+		cFos+/CTB555+	
Axis		p-value		p-value		p-value
<b>Antero-posterior</b>						
Axis	F (1.67,125.52) = 221.16	<b>&lt;0.001</b>	F (1.43,77.19) = 95.85	<b>&lt;0.001</b>	F (1.68,92.6) = 23.64	<b>&lt;0.001</b>
Odor	F (2,75) = 0.00	1.000	F (2,54) = 0.00	1.000	F (2,55) = 0.00	1.000
Group	F (1,75) = 0.00	1.000	F (1,54) = 0.00	1.000	F (1,55) = 0.00	1.000
Axis x Odor	F (3.35,125.52) = 0.76	0.531	F (2.86,77.19) = 1.78	0.161	F (3.37,92.6) = 0.94	0.434
Axis x Group	F (1.67,125.52) = 1.08	0.332	F (1.43,77.19) = 1.64	0.206	F (1.68,92.6) = 0.22	0.762
Odor x Group	F (2,75) = 0.00	1.000	F (2,54) = 0.00	1.000	F (2,55) = 0.00	1.000
Axis x Odor x Group	F (3.35,125.52) = 0.07	0.98	F (2.86,77.19) = 0.20	0.890	F (3.37,92.6) = 3.08	<b>0.026</b>
cFos+/CTB555+; Veh MO I vs CORT MO I: t = 3.19, q = <b>0.005</b> Veh MO P vs CORT MO P: t = 2.60, q = <b>0.017</b> Veh FU I vs CORT FU I: t = 1.96, q = 0.069 Veh FU A vs Veh TMT A: t = 1.78, q = 0.087 CORT MO I vs CORT FU I: t = 3.26, q = <b>0.006</b> CORT MO P vs CORT FU P: t = 1.80, q = 0.092 CORT MO P vs CORT TMT P: t = 1.92, q = 0.070						
<b>Medio-lateral</b>						
Axis	F (1,77) = 0.00	0.952	F (1,53) = 39.72	<b>&lt;0.001</b>	F (1,60) = 3.89	0.053
Odor	F (2,77) = 0.00	1.000	F (2,53) = 0.00	1.000	F (2,60) = 0.00	1.000
Group	F (1,77) = 0.00	1.000	F (1,53) = 0.00	1.000	F (1,60) = 0.00	1.000
Axis x Odor	F (2,77) = 1.08	0.344	F (2,53) = 0.71	0.498	F (2,60) = 1.53	0.225
Axis x Group	F (1,77) = 1.52	0.222	F (1,53) = 2.90	0.095	F (1,60) = 0.25	0.622
Odor x Group	F (2,77) = 0.00	1.000	F (2,53) = 0.00	1.000	F (2,60) = 0.00	1.000
Axis x Odor x Group	F (2,77) = 3.17	<b>0.048</b>	F (2,53) = 1.95	0.152	F (2,60) = 2.47	0.094
cFos+; Veh MO M vs CORT MO M: t = 2.07, q = <b>0.047</b>						
<b>Dorso-ventral</b>						
Axis	F (1,77) = 3.15	0.080	F (1,54) = 9.19	<b>0.004</b>	F (1,60) = 18.66	<b>&lt;0.001</b>
Odor	F (2,77) = 0.00	1.000	F (2,54) = 0.00	1.000	F (2,60) = 0.00	1.000
Group	F (1,77) = 0.00	1.000	F (1,54) = 0.00	1.000	F (1,60) = 0.00	1.000
Axis x Odor	F (2,77) = 2.66	0.077	F (2,54) = 1.54	0.225	F (2,60) = 1.24	0.296
Axis x Group	F (1,77) = 1.25	0.267	F (1,54) = 7.78	<b>0.007</b>	F (1,60) = 0.20	0.656
Odor x Group	F (2,77) = 0.00	1.000	F (2,54) = 0.00	1.000	F (2,60) = 0.00	1.000
Axis x Odor x Group	F (2,77) = 0.11	0.899	F (2,54) = 0.52	0.596	F (2,60) = 0.65	0.525
cFos+/CTB647+; Veh FU V vs CORT FU V: t = 2.13, q = <b>0.048</b> Veh TMT V vs CORT TMT V: t = 1.78, q = 0.092						

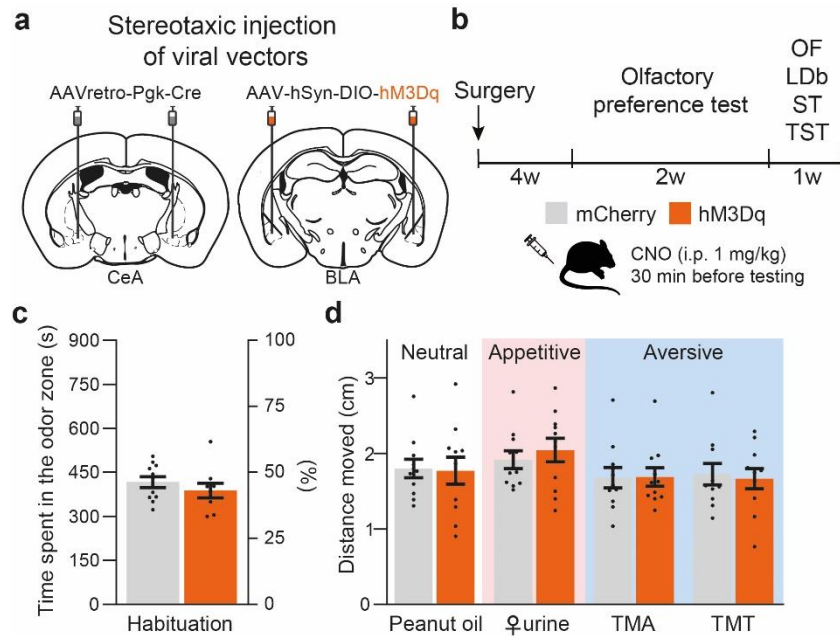
**Supplementary Table 3.** Corresponding statistical analyses for **Supplementary Figure 6** (Three-way repeated measures ANOVA or Mixed-effect model). For the medio-lateral and dorso-ventral axes, post-hoc analyses comparing Veh and CORT are reported for the medial part (M) and ventral part (V), but they have respectively identical adjusted p-values (q) than the lateral and dorsal parts. A: anterior part, I: intermediate part, P: posterior part.



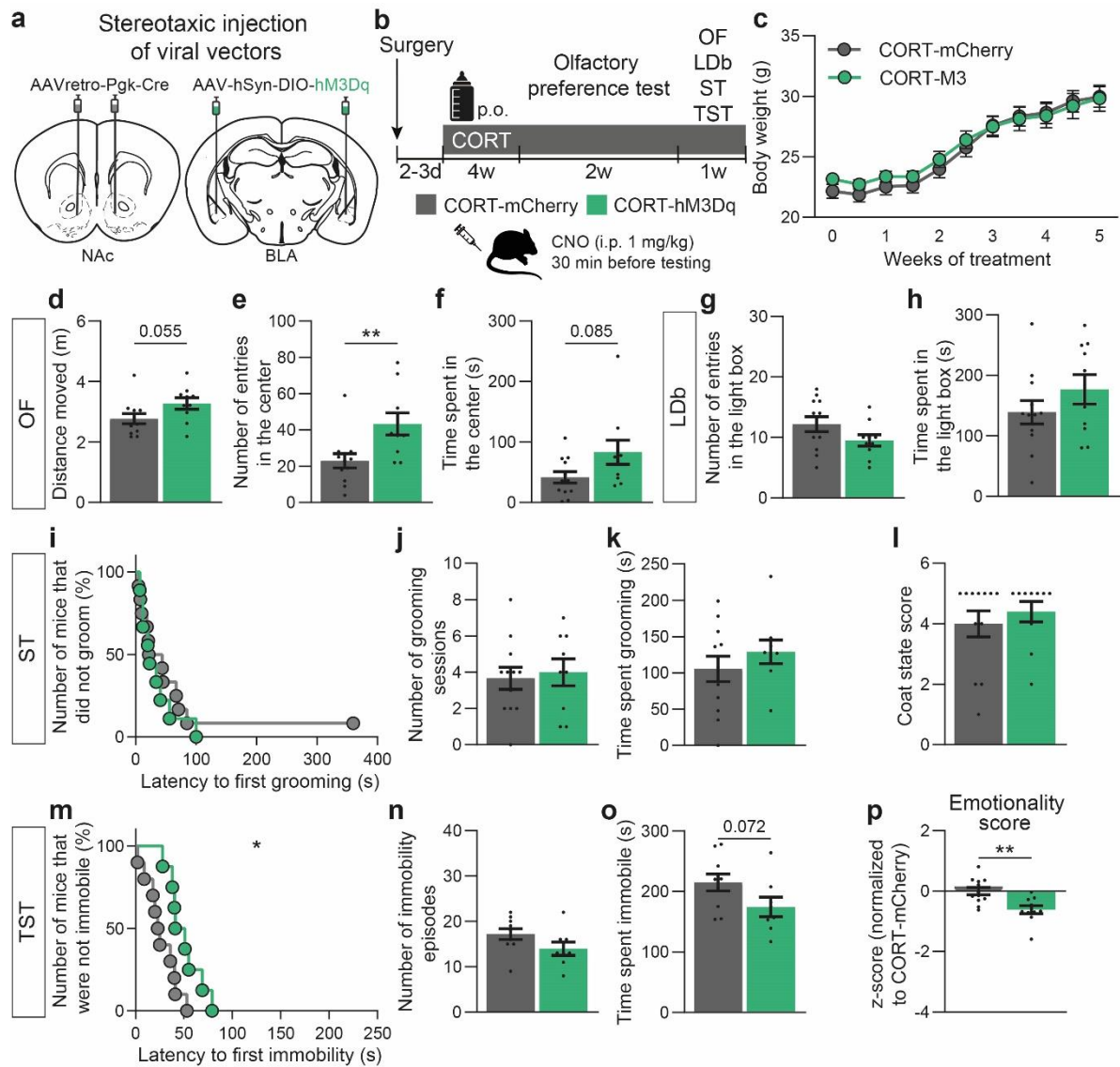
**Supplementary Figure 6.** Chemogenetic BLA-to-CeA cells activation does not promote anxiety- and depressive-like behaviors. **(a-b)** AAVr-Pgk-Cre in the CeA and AAV-hSyn-DIO-hM3Dq-mCherry (orange,  $n = 9$ , or AAV-hSyn-DIO-mCherry for the controls, grey,  $n = 11$ ) in the BLA were injected to activate BLA-to-CeA cells. **(c-e)** CNO injection had no effect in the OF in hM3Dq mice relative to mCherry controls (**c**:  $t(18) = 0.46$ ,  $p = 0.650$ ; **d**:  $t(18) = 1.02$ ,  $p = 0.321$ ; **e**:  $t(18) = 0.28$ ,  $p = 0.785$ ). **(f-g)** hM3Dq mice were not different than mCherry controls in the LDb (**f**:  $t(18) = 0.03$ ,  $p = 0.098$ ; **g**:  $t(17) = 0.65$ ,  $p = 0.522$ ,  $n = 10-11$ ) and ST (**h**:  $\chi^2(1) = 0.46$ ,  $p = 0.500$ ; **i**:



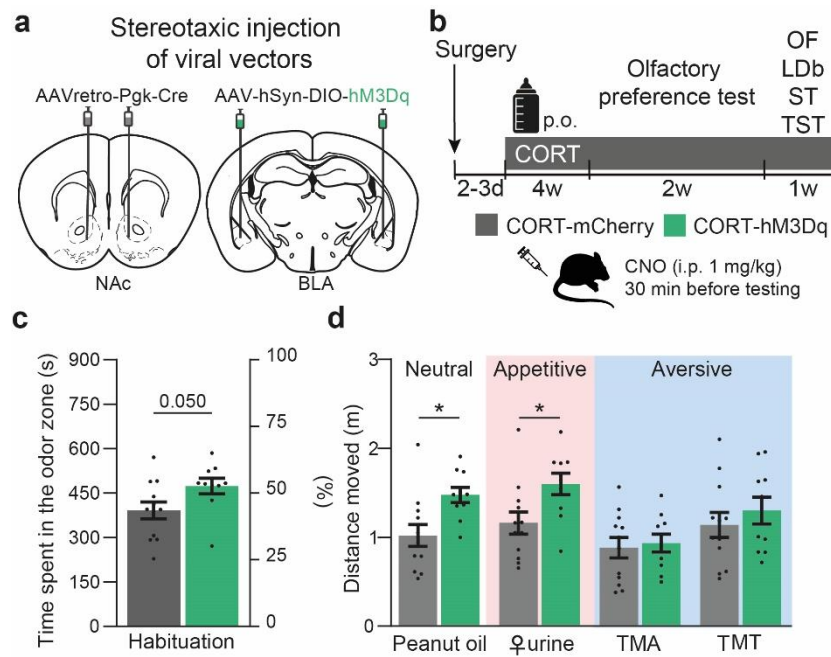
$t(18) = 1.03, p = 0.317$ ; **j**:  $t(18) = 0.73, p = 0.476$ ). (**k-m**) CNO injection did not change immobility behavior of hM3Dq compared to mCherry mice in the TST (**k**,  $\chi^2(1) = 0.19, p = 0.667$ ; **l**,  $t(18) = 1.30, p = 0.209$ ; **m**,  $t(18) = 1.28, p = 0.216$ ). (**n**) Overall, CNO injection did not affect the global emotionality score ( $t(20) = 0.49, p = 0.626$ ). Bars are mean  $\pm$  sem.



**Supplementary Figure 7.** Chemogenetic BLA-to-CeA cells activation did not affect the behavior in the olfactory preference test. (**a-b**) AAVr-Pgk-Cre in the CeA and AAV-hSyn-DIO-hM3Dq-mCherry (orange,  $n = 9$ , or AAV-hSyn-DIO-mCherry for the controls, grey,  $n = 11$ ) in the BLA were injected to activate BLA-to-CeA cells. (**c**) CNO injection in hM3Dq mice did not modify the time spent in the “object” zone during habituation without odor compared to mCherry controls ( $t(18) = 0.93, p = 0.366$ ). (**d**) hM3Dq mice moved similarly than mCherry controls (Group:  $F(1,18) = 0.05, p = 0.827$ , Odor:  $F(3,54) = 9.34, p < 0.001$ ; Interaction:  $F(3,54) = 0.91, p = 0.444$ ). Bars are mean  $\pm$  sem.



**Supplementary Figure 8.** Chemogenetic BLA-to-NAc cells activation has anxiolytic and antidepressant-like effects on CORT mice. **(a-b)** AAVr-Pgk-Cre in the NAc and AAV-hSyn-DIO-hM3Dq-mCherry (green, or AAV-hSyn-DIO-mCherry for the controls, grey) in the BLA were injected to activate BLA-to-NAc cells. **(c)** Body weight evolution was similar between CORT-mCherry and CORT-hM3Dq mice (Group:  $F(1,20) = 0.12$ ,  $p = 0.736$ ; Weeks:  $F(10, 200) = 181.80$ ,  $p < 0.001$ ; Interaction:  $F(10, 200) = 1.56$ ,  $p = 0.121$ ). **(d-f)** CNO injection had anxiolytic-like effects in the OF in CORT-hM3Dq mice relative to CORT-mCherry controls ( $n = 10-12$ ) (**e**):  $t(20) = 2.90$ ,  $**p = 0.009$ ; **d**):  $t(20) = 2.04$ ,  $p = 0.055$ ; **f**):  $t(12,81) = 1.87$ ,  $p = 0.085$ ). **(g-k)** CORT-hM3Dq mice were not different than CORT-mCherry controls in the LDb (**g**):  $t(20) = 1.67$ ,  $p = 0.111$ ; **h**):  $t(20) = 1.24$ ,  $p = 0.228$ ,  $n = 10-12$ ) and ST (**i**):  $\chi^2(1) = 0.40$ ,  $p = 0.529$ ; **j**):  $t(19) = 0.35$ ,  $p = 0.730$ ; **k**):  $t(19) = 0.95$ ,  $p = 0.354$ ,  $n = 9-12$ ). **(l)** The coat state was altered in both CORT-hM3Dq and CORT-mCherry mice ( $U = 51.50$ ,  $p = 0.529$ ,  $n = 10-12$ ) **(m-o)** CNO injection increased the first immobility latency in CORT-hM3Dq compared to CORT-mCherry mice in the TST ( $n = 8-10$ ) (**m**):  $\chi^2(1) = 6.16$ ,  $*p = 0.013$ ) and tended to decrease the immobility time (**o**):  $t(16) = 1.92$ ,  $p = 0.072$ ), but had no effect on the immobility episodes number (**n**):  $t(16) = 1.72$ ,  $p = 0.106$ ). **(p)** Overall, CNO injection decreased the global emotionality score ( $t(20) = 3.40$ ,  $**p = 0.003$ ). Bars are mean  $\pm$  sem.



**Supplementary Figure 9.** Chemogenetic BLA-to-NAc cells activation increases interest for neutral and appetitive odors in the olfactory preference test. **(a-b)** AAVr-Pgk-Cre in the NAc and AAV-hSyn-DIO-hM3Dq-mCherry (green,  $n = 10$ , or AAV-hSyn-DIO-mCherry for the controls, grey,  $n = 12$ ) in the BLA were injected to activate BLA-to-NAc cells. **(c)** CNO injection in CORT-hM3Dq mice tends to increase the time spent in the “object” zone during habituation without odor compared to CORT-mCherry controls ( $t(20) = 2.09$ ,  $p = 0.050$ ). **(d)** CORT-hM3Dq mice moved more upon presentation of peanut oil and ♀urine than CORT-mCherry controls (Group:  $F(1.20) = 3.20$ ,  $p = 0.089$ . Odor:  $F(3.60) = 18.26$ ,  $p < 0.001$ ; Interaction:  $F(3.60) = 4.66$ ,  $p = 0.005$ ; \* $p < 0.05$ ). Bars are mean  $\pm$  sem.

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## DISCUSSION

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The aim of this thesis was to study in animals the mechanisms underlying the positive and negative emotional biases characterizing respectively the manic and depressive states of mood disorders in humans. Preclinical and clinical data suggest that emotional biases observed in response to stimuli from different sensory modalities converge to particular brain circuits which could underpin the modification of hedonic values in mood episodes in patients. Therefore, we developed a rodent olfactory preference test to determine the hedonic values attributed to innately salient odors by mouse models of mania and depression, as it is done in humans.

### 3.1 Summary of the results

#### *3.1.1 Hedonic biases in mouse models of depression and mania*

Previous studies already demonstrated that emotional bias is an essential component of depressive episode leading depressed patients to attribute more negative valence to events or objects (Leppänen, 2006; Clark et al., 2009; Harmer et al., 2009; Bigot et al., 2020). Here we showed that bipolar depression was associated with a negative bias during odor valence assignment in the Sniffin' sticks test. Indeed, a group of depressed/dysthymic/mixed bipolar patients rated less odors as pleasant than euthymic BD patients, and more odors as unpleasant than both control subjects and euthymic BD patients (Figure 25). This olfactory emotional bias was correlated with depression severity. Interestingly, it seemed to regress during recovery of the depressive episode (in euthymic patients). We found

that bipolar depressive patients performed similarly than control subjects and euthymic patients concerning odor detection, discrimination and identification tests, consistently with the only comparable study (Kazour et al., 2020).

In parallel, we have developed a preclinical test to assess behavioral responses reflecting spontaneous valence assignment of innate odors in mice. This allowed us to demonstrate a negative olfactory hedonic bias in a mouse model of depression induced by chronic corticosterone administration, very similarly to what happens in depressed patients. Indeed, we found that chronic CORT treatment was associated with a shift to more negative valence assignment of both appetitive (that became less appetitive) and aversive (that became more aversive) odors (Figure 25). Importantly, our analysis also underscored that olfactory valence assignment was a powerful behavioral variable able to predict differences between control and depressive-like states in mice.

Besides, we used a pharmacological mania model to study emotional biases, an essential, yet overlooked dimension of manic state in humans. For that purpose, we evaluated behavioral hedonic responses to olfactory and gustatory stimuli with both positive and negative innate valences in rodents exposed to GBR 12909. To our surprise, apart from the classical features of mania models (hyperlocomotion and antidepressant-like phenotype), we found very strong negative hedonic biases in both olfactory and gustatory preference tests shortly following GBR administration, along with anxiety-like behaviors.

Our results emphasize the importance of multi-dimensional assessment of animal models. In the DSM-5 (American Psychiatric Association, 2013), the two main criteria to define a manic state are elevated and expansive mood with increased activity and energy. It is therefore particularly important to have translational evaluations of these two elements. While the second criterion has largely been retained to define mania models, based on the increased motor and exploratory activity of animals, the mood main criterion has to date not found an equivalent measure applicable to animals. Indeed, mood is a subjective symptom peculiar to human nature. As proposed in our emotional-based model for bipolar patients, emotional biases can be explored both in humans and animals as a proxy for mood (Bigot et al., 2020). So far, the only tests proposed in animals to measure the hedonic dimension independently of higher cognitive processes are the sucrose preference test and situations of self-stimulation or self-administration of psychostimulant drugs, which are far from satisfactory.

Our data support the notion that GBR-treated mice exhibit a mixed-like state in which

manic and depressive symptoms occur simultaneously, unlike classical mania models. Therefore, according to the idea that animal models are better suited for modeling only some facets of the disease instead of the whole symptomatology (see below “General avenues for better animal models research and translation”), GBR administration is a model that fits for studying the motor and motivational dimension of mania, but not the emotional valence system (Cosgrove et al., 2016).

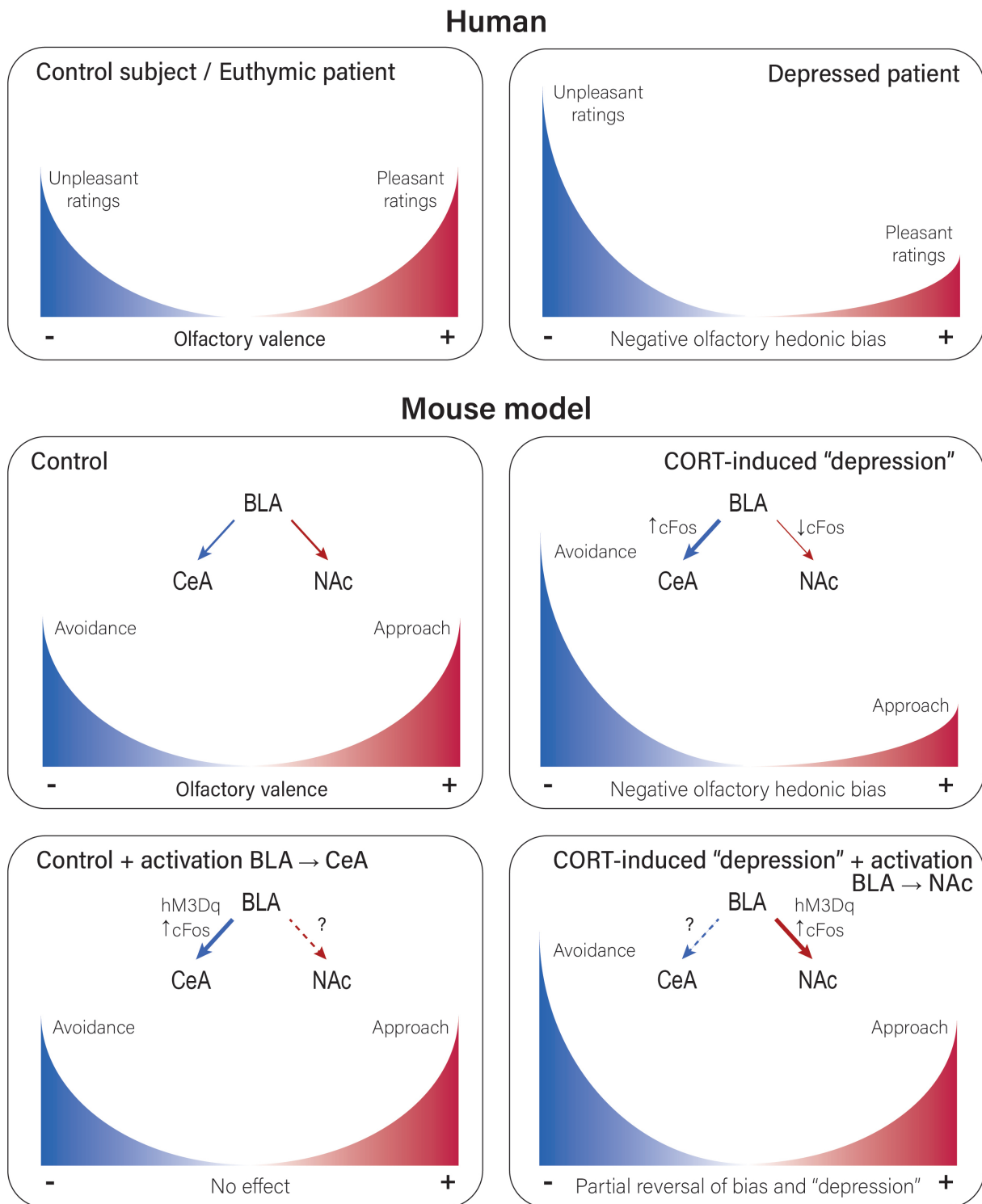
As restoration of emotional biases appears necessary for recovery from depression (Harmer et al., 2017), our assay might be useful for testing new potential antidepressants, replacing controversial tests (Reardon, 2019). GBR was first suggested as a new antidepressant drug, based on these classical preclinical depression tests (Hemby et al., 1997; Søggaard et al., 1990), although it induces negative biases. Consequently, our results highlight the need to test in preclinical phases how potential antidepressant drugs modify emotional biases.

### ***3.1.2 The role of BLA circuits in negative hedonic bias associated with depressive-like states***

The negative olfactory hedonic bias observed upon CORT administration was associated with disturbances in brain circuits involved in valence assignment. In the basolateral amygdala, projecting neurons to the NAc are thought to encode preferentially positive valence stimuli whereas projecting neurons to the CeA would encode preferentially negative valence stimuli (Namburi et al., 2015; Beyeler et al., 2016). Congruently with the negative hedonic bias observed, BLA-to-NAc neurons activity was reduced while BLA-to-CeA neurons activity was increased in CORT-treated mice, using a cFos immediate early gene expression proxy for neuronal activation (Figure 25).

These data suggest that depressive-like phenotype and negative olfactory hedonic bias could be mimicked by over-stimulation of the BLA-to-CeA neurons. However, we were unable to induce neither anxiety- or depressive-like phenotypes, nor negative olfactory bias by chemogenetically stimulating the BLA-to-CeA pathway in control animals (Figure 25).

In contrast, activation of the BLA-to-NAc pathway allowed us to reduce the anxiety-depressive-like phenotype in CORT-treated mice. Regarding the hedonic olfactory bias, activation of BLA-to-NAc neurons increased in the attractiveness for both neutral and pleasant odors (Figure 25). Importantly, BLA-to-NAc neurons are primarily suggested to encode reward and positive valence, which is consistent with the effect we observed.



**Figure 25. Summary scheme of the findings from the “BLA circuits-related negative olfactory hedonic bias in depression” study.** BLA: basolateral amygdala, CeA: centromedial amygdala, NAc: nucleus accumbens.

However, it did not modify the response to negative odors with respect to the CORT-mCherry control group, indicating that other pathways could be required to restore this bias. Negative stimuli predicting danger as the ones we used might rely on multiple evolutionarily selected and redundant mechanisms, and could be tougher to hijack.

## 3.2 About the need and validity of animal models of mood disorders

### 3.2.1 *Are animal models relevant for psychiatry?*

Several researchers questioned the interest of animal models for the psychiatric field since few translational advances and clinical benefits have been achieved until now (Stanford, 2017, 2020; Papassotiropoulos and Dominique, 2015). Beyond cross-species barriers as differences in neuroanatomy and complexity, the mental illnesses seem uniquely human. Indeed, psychiatric disorders are defined by lots of symptoms not accessible in animals (hallucinations, delusion, sadness, guilt, racing thoughts, grandiosity, suicide ideation, *etc.*) or with approximate resemblance to the evaluated behaviors in animals (Of men, not mice, 2013; Stanford, 2017).

Poor experimental design and statistical analysis, subjective biases and flawed interpretation of behavioral data are other reasons given to explain the lack of new therapeutic options brought by preclinical research (Sjoberg, 2017; Stanford, 2017, 2020). Indeed, behavioral tests such as the forced swim and the tail suspension tests used for so-called “depressive-like phenotype” or “models of depression” have actually been developed since they were sensitive to the effect of pharmacological monoaminergic drugs discovered by serendipity. These tests might actually have impaired the discovery of new treatments, as their power to detect non-monoaminergic antidepressant effects is debated (Hyman, 2013, 2012; Berton and Nestler, 2006). In addition, reverse translational research based on the mechanisms of action of monoaminergic drugs has shown that mechanisms underlying the pro-depression and anti-depression states could be different.

The problem also raises from the definition of the diseases, only based on collection of symptoms that sometimes can be completely different between two patients diagnosed with the same disease, or overlap with symptoms of other psychiatric diseases. Moreover, no objective and broadly recognized biomarker in terms of molecular, synaptic, cellular or neural circuits alterations can confirm the diagnosis (Nestler and Hyman, 2010). In this context, failed attempts of animal models at reproducing all the facets of one psychiatric disorder are not surprising.

Disillusion with animal models for predicting successful drug development (either by severe side effects or very small therapeutic effects in humans) lead the therapeutic development



in psychiatry to be judged too difficult and risky by big pharmaceutical companies that retire themselves from the research of new treatments (Hyman, 2013; Insel, 2012; Abbott, 2011). This is despite the fact that actual therapies are not satisfactory for most of the patients, and that the psychotropic drug market is among the most profitable (Abbott, 2011).

### ***3.2.2 Alternatives to animal research in psychiatry***

Human research, including genetic and imaging studies for instance, remains limited to uncover the biological mechanisms underlying psychiatric disorders because of ethical and practical issues with studying living human brain. It needs to be complemented with preclinical research.

An example of alternative model is induced pluripotent stem cell technology (iPSC) that can bring insights about genetic mutations identified in patients and their impact on neurodevelopmental mechanisms involved in the diseases (Das et al., 2020). iPSC are derived from somatic cells taken from human tissues (*e.g.* from the skin) and have the potential to grow and develop in culture into different types of cells including excitatory and inhibitory neurons. They can spontaneously form networks and offer the possibility to study synaptic transmission. These cells also have the power, following a precise protocol, to form “brain organoids”. It means that embryonic stem cells develop into multiple discrete and inter-dependant brain regions, mimicking features of the human cortical development. Brain organoids allow to model many neurodevelopmental disorders including the autism spectrum disorder and schizophrenia, but seem less useful for understanding mood disorders (Di Lullo and Kriegstein, 2017; Adams et al., 2019; Stachowiak et al., 2017).

Despite their promising contribution, *in vitro* studies cannot fully replace *in vivo* behavioral examination to unveil the involvement of brain circuits, cellular, synaptic and molecular pathways in normal and pathological brain functions, especially given that psychiatric disorders diagnosis is exclusively based on behavioral and self-reported symptoms. As a consequence, it seems unthinkable to advance without animal research. However, progress should be made to improve translation and relevance of animal models.

### ***3.2.3 General avenues for better animal models research and translation***

Proposed avenues to improve translation first rely on better characterization and stratification of patients with mental illness, based on deep-phenotyping to identify reliable biomarkers (Insel and Cuthbert, 2015; Kas et al., 2019; Sokolowska et al., 2015). Big data analyses lean on multiomics approaches (genomics, transcriptomics, epigenomics, proteomics, *etc.*), but also on large multi-site and collaborative cohorts collecting large-scale biological and phenotypic data (including *via* digital phenotyping), and finally on unbiased computational analytic and model-generating tools, comprising machine learning methods (Ressler and Williams, 2021).

Besides, the dimensional approach should be applied to animal models, recognizing that no animal can recapitulate all the features of a disease. Animal models are instead useful to study a particular altered dimension, such as cognition, emotions, *etc.* (Anderzhanova et al., 2017; Cosgrove et al., 2016; Nestler and Hyman, 2010; Robinson, 2018).

Regarding the behavioral evaluation, researchers advocate for more ethological assessments in conditions closer to “natural” environment, instead of using very short and stressful paradigms that depends on stress coping abilities (Stanford, 2017). In this line, particular attention should be made on housing environment as confounding factor, for example avoiding single-housing. The broadest range possible of behavioral tests should be used to evaluate putative animal models (Nestler and Hyman, 2010; Kalueff et al., 2007). In addition, behavioral tests should use translational assays that can be done in humans and in animals whenever possible (Anderzhanova et al., 2017; Markou et al., 2009).

Finally, extended validity criteria have been proposed for animal models, including for instance the type of species chosen, or distinguishing among the face validity ethological (behavioral) and biomarker (biological) validity (Belzung and Lemoine, 2011).

### ***3.2.4 Specific sex issues***

Depression affects about twice more women than men, from the adolescence to the older ages (Kuehner, 2017). Sex differences in models of depression have been suggested, but with contradictory results (Ma et al., 2019). Experimental conditions and housing as well as estrus cycle could be responsible for these discrepancies. A variety of biological systems have been hypothesized to account for sex differences in mood disorders; most of them are

already studied in the pathophysiology of these diseases (*e.g.* monoamines, neuroplasticity and neurogenesis, HPA axis; Bale and Epperson, 2017; Simpson and Kelly, 2012; Ma et al., 2019). All these systems could develop differently between males and females and/or be modulated by sex hormones. But one major factor impeding the understanding of sex differences is that research in males still dominates (Zucker and Beery, 2010). Most of the rodent models of depression and mania have been developed using males. For instance, the social defeat model has been understudied in females, as aggression levels are low in most female rodent laboratory species (Greenberg et al., 2014). Concerning the CORT-induced model of depression, female submitted to the same administration protocol than males displayed anxiety- and depressive-like phenotypes, but to a lower and less robust extent (Mekiri et al., 2017). Therefore, it would be interesting to investigate whether our olfactory preference test would be sensitive to the effect of CORT administration also in females or follows the less strong differences observed in other tests.

The unpredictable chronic mild stress (UCMS) could be useful to extend our results, since female mice are more sensitive to this protocol (Guilloux et al., 2011), even though it seems to depend on strain and tests used (Ma et al., 2019; Franceschelli et al., 2014; Mineur et al., 2006).

Regarding bipolar disorders, despite similar prevalence between men and women, differences exist in the symptoms and course of the disease. Type I bipolar disorder affects equally men and women, whereas women are more susceptible to develop type II bipolar disorder (Nivoli et al., 2011). In addition, bipolar women are more likely to show a predominance of depressive polarity than men. However, very few studies investigated sex differences biological mechanisms due to the limited animal models available (Kokras and Dalla, 2014). To our knowledge, female mice presenting manic-like behaviors were only reported in genetic models, and the particular effects of GBR 12909 have never been tested in females. Further progress should be made to decipher if behavioral manifestations of putative manic-like states are the same between male and female rodents, and to examine the underlying biological mechanisms.

### ***3.2.5 Time-course, cyclicity and neuroprogression issues***

Face validity of models for depression and bipolar disorders are always limited by their poor similarity with the time-course of mood disorders. Among unipolar depression, after the first episode, the risk of recurrence is estimated around 35%, and 15% of patients

show no remission at all (Eaton et al., 2008; Belsher and Costello, 1988). On the other side, bipolar disorders are characterized by recurrent depressive and manic or hypomanic episodes, with a course dominated by the depressive state (Judd et al., 2002, 2003).

Barely no animal model displays spontaneous mood episodes (Kato et al., 2016). Some attempts have been made to model recurrent mood episodes. Clock mutant mice, which exhibit persistent hyperactivity, reduced anxiety- and depressive-like behaviors, sleep disturbances, increased intra-cranial self-stimulation and sucrose consumption, show a normalization of the mania-like phenotype during the night (Sidor et al., 2015). It has been suggested to model rapid mood cycling. Mutations on the alpha calcium/calmodulin-dependent protein kinase II induces in mice hyperactive periods that cycle over 1-2 weeks (Yamasaki et al., 2008). Chronic methamphetamine treatment also disturbs the circadian rhythm, with rats exhibiting a free-running period lasting more than 24h (Honma et al., 1986). Mice mutated on the prokineticin receptor 2 exhibit spontaneous and recurrent periods of hypolocomotion associated with hypometabolic state lasting about 8h (Jethwa et al., 2008). These results, described under the term of “daily torpor”, could be related to hibernation and seasonal affective disorder, a particular type of mood disorder (Kato et al., 2016). Some studies investigated the effect of repeating twice procedures to induce depressive-like behaviors, with an in-between recovery period (Remus et al., 2013; Isingrini et al., 2010). They observed either faster or stronger effects of the second chronic mild stress period. Finally, one potentially interesting protocol relies on the depressive-like behaviors induced by withdrawal of psychostimulants that could be informative of mood switches mechanisms in bipolar patients (Gururajan et al., 2019; Renoir et al., 2012; Barr and Markou, 2005). Indeed, manic or hypomanic states are often directly followed by a depressive episode (Salvadore et al., 2010; Vieta et al., 2009). It would be very interesting to investigate how brain circuits related to emotional valence coding, including the BLA, are structurally and functionally modified along these mood switches.

Because of the difficulty of modelling recurrent mood episodes, biological mechanisms underlying neuroprogression, defined by the progressive clinical and neurocognitive deterioration in the course of depression and bipolar disorders associated with pathological rewiring of the brain, can barely be investigated in animals (Ruiz et al., 2018; Passos et al., 2016; Berk et al., 2011). Currently, since a preponderant clinical aspect of neuroprogression is the increased risk of dementia, most preclinical research relies on learning and memory deficits without necessarily modelling the mood dimension.

### *3.2.6 Investigating hedonic biases to improve animal models of mood disorders and enhance new treatment discovery*

According to the all the propositions formulated in “General avenues for better animal models research and translation”, we have to acknowledge that the GBR-induced “model of mania” is useful for studying increased energy and locomotor dimensions of manic states (Young et al., 2010), but does not seem to reflect the elevated or expansive mood nor risk-taking behaviors. Indeed, we showed negative hedonic bias and anxiogenic effects of GBR administration, as opposed to what we predicted for a mania model. Alternatives for positive hedonic bias evaluation in attempt to study the underlying brain circuits related in mania models could go through certain genetic models that already displayed increased sucrose consumption (Kirshenbaum et al., 2011; Leussis et al., 2013; Roybal et al., 2007; van Enkhuizen et al., 2013). Acute but not chronic sleep deprivation, paradigms used to model mania, has also been associated with increased sucrose seeking (Liu et al., 2016; Arora et al., 2021).

Concerning the CORT-induced model of depression, we were able to measure negative hedonic bias in our olfactory preference test, but not in a gustatory preference test. Using the same protocol reported in “Negative hedonic bias in a mouse model of mania” consisting in 48h of a two-bottle choice paradigm, one bottle filled with water and the other with sucrose 1% as appetitive tastant or quinine 0.1mM as aversive tastant, we did not observe any difference in the gustatory preference of both sucrose and quinine between Veh control and CORT-treated mice (Sucrose, Veh: 63.30%  $\pm$  3.02, CORT: 65.6%  $\pm$  3.48; Quinine, Veh: 32.15%  $\pm$  3.96, CORT: 31.74%  $\pm$  3.20 (mean  $\pm$  SEM); Two-way repeated measures ANOVA, Group:  $F(1,33) = 0.07$ ,  $p = 0.790$ , Tastant:  $F(1,33) = 91.69$ ,  $p < 0.001$ , Interaction:  $F(1,33) = 0.16$ ,  $p = 0.692$ ,  $n = 17-18$ ). One study reported decreased sucrose preference with CORT administered orally, but the animals were water-deprived (Gourley et al., 2013). One may worry that water deprivation differentially affect Veh control and CORT-treated mice, particularly because chronic CORT administration modifies water intake through the mineralocorticoid pathway (Amount of solution drunk in 48h (g): Sucrose, Veh: 8.58  $\pm$  0.45, CORT: 11.13  $\pm$  1.28; Quinine, Veh: 10.15  $\pm$  0.56, CORT: 13.59  $\pm$  1.63 (mean  $\pm$  SEM); Two-way repeated measures ANOVA, Group:  $F(1,33) = 5.93$ ,  $p = 0.021$ , Tastant:  $F(1,33) = 5.12$ ,  $p = 0.030$ , Interaction:  $F(1,33) = 0.26$ ,  $p = 0.614$ ,  $n = 17-18$ ).

It would be interesting to replicate our results in the olfactory preference test using another model of depression, in particular in which female mice can be tested (see above “Specific sex issues”).

Several studies already demonstrated altered female urine sniffing in different kind of male depression models as measure of anhedonia (Malkesman et al., 2010; Lehmann et al., 2013; Finger et al., 2011; Zanos et al., 2016, 2017; Terrillion et al., 2017). However, our test further extends the olfactory valence assignment deficit to aversive odors, going beyond anhedonia to a global negative hedonic bias. We propose that the olfactory preference test we developed, assessing both innate positive and negative odors, could be a reliable and sensitive measure of hedonic bias in mouse models of mania and depression. It could be used to verify the ability of different paradigms to model the emotional valence feature, and further improve knowledge about this major dimension of mood disorders.

We also need to investigate whether the different families of antidepressants that have been clinically proven to be effective reverse this hedonic bias in the CORT-induced model of depression and in others. In other words, could the restoration of hedonic biases be a common final pathway of all antidepressants? Thus, screening of both monoaminergic and non-monoaminergic antidepressant drugs should be made to assess on their capacity to restore the negative olfactory hedonic bias observed in the CORT model of depression, as well as their effect on brain circuits encoding valence (see below “The basolateral amygdala, a crucial brain structure integrated in larger networks for valence encoding in mood disorders”). If the results are conclusive, we hope that the olfactory preference test could be used to identify new effective antidepressant treatments.

Depending on the possibility to observe positive hedonic bias in mouse models of mania, we could also envisage the use of the olfactory preference test to test mood stabilizers.

Several issues should be overcome first in order to apply this test at a larger scale. It should be easy to implement and reproducible. Despite its long duration (twelve days), our olfactory preference test does not require sophisticated material, since it is done in cages used for housing with a petri dish as odor recipient. Its duration could be shortened if the number of odors tested is reduced. Peanut oil, trimethylamine and trimethylthiazoline are relatively easy to find. However, the use of female urine is a major factor likely impacting scalability and reproducibility. The estrous cycle phase should be checked as much as possible prior to collection, and fresh samples are preferred. As the volume that can be sampled is limited, the number of mice that can be tested is restrained. This concern

further supports the use of female mice as model of depression, because male urine may be easier to manage. Reproducibility is also likely to be impacted by a broad range of factors such as housing conditions, since the olfactory preference test is based on a spontaneous behavior already quite variable. The number of animals to be tested for attaining good statistical power could be high because of this variability. Improvement could be also done to measure odor preference in more natural conditions by developing automatic devices measuring odor preference in housing conditions. This new type of devices presents the big advantage of studying animals living in bigger housing groups, through long periods of time and without intervention, respecting their wake/sleep cycle (*e.g.* Souris City, Torquet et al., 2018).

Moreover, one way to reduce variability could go through assessing learned instead of innate valence assignment, but this solution has the caveat of learning and memory as probable confounding factors. In addition, for conditioning protocols, animals are usually water- or food-deprived. One cannot exclude that internal state modifications could affect differentially mice under control conditions *versus* mice submitted to stress paradigms inducing depressive-like behaviors.

Uncovering hedonic biases in mouse models of depression and mania encourages us to investigate what brain circuits could underlie these emotional disturbances. We primarily focused on the basolateral amygdala because of the growing interest about its ability to encode emotional valence (Tye, 2018), even though other brain areas are probably also involved.

### 3.3 The basolateral amygdala, a crucial brain structure integrated in larger networks for valence encoding in mood disorders

#### 3.3.1 *BLA circuit-specific changes under stress*

The dense literature on the impact of stress on behavior and brain plasticity has already been briefly summarized in the introduction (see “Hypotheses concerning the pathophysiology” and “BLA alterations under stress”). It goes through plasticity at multiple brain levels, including cellular, synaptic and molecular changes that can affect all the cell populations in the BLA, meaning the principal neurons, the interneurons, the astrocytes but also the microglial cells (Sharp, 2017; Prager et al., 2016; Naskar and Chattarji, 2019; Munshi et al., 2020). However, most of these studies takes the neuronal population of the BLA in its entirety. All these findings start to be refined trying to distinguish BLA neurons involved either in positive or negative valence coding in terms of topography, connectivity or genetic expression (Zhang et al., 2021).

Only few studies have examined the effect of stress in topologically difference subregions of the BLA. For instance, chronic CORT treatment enhanced the frequency of spontaneous excitatory post-synaptic currents (EPSC) specifically in the anterior part and not the posterior part of the BLA (Morgan et al., 2019). It could depend on the cyclooxygenase 2 (COX-2), an enzyme involved in endocannabinoid metabolism and production of pro-inflammatory prostaglandins whose expression is limited to particular brain regions including the BLA, where it is up-regulated by stress. Indeed, lumiracoxib which is a specific inhibitor of COX-2 normalized the increased BLA glutamatergic transmission selectively in the anterior part of the BLA in parallel of its anxiolytic effects in the CORT-treated mice. This is of particular interest because of the potential role of inflammation in the genesis of mood disorders.

We only observed statistical trends analyzing cFos expressing-cell location differences between Veh and CORT-treated mice. In particular, CORT cFos responses were more lateral specifically upon presentation of the neutral odorless mineral oil compared to Veh controls. We did not observe any modification of the cFos expression along the antero-posterior or the dorso-ventral axes. As opposed to our results, Ma et al. (2021)



found that UCMS increased and decreased cFos expression in the anterior and posterior parts of the BLA respectively.

### **BLA-to-vHPC circuit**

More evidence is available on specific BLA circuit alterations under stress. Several studies from the Bing-Xing Pan laboratory showed structural and functional changes in the BLA-to-ventral hippocampus (vHPC) neurons following chronic restraint stress that were not present in BLA-to-dorsomedial prefrontal cortex (dmPFC) nor BLA-to-NAc neurons, such as increased firing rate and cFos expression, dendritic hypertrophy, spine enlargement and spine density elevation (Zhang et al., 2019b,c). The increased excitability of BLA-to-vHPC neurons was caused by downregulation of small-conductance, calcium-activated potassium (SK) channel under chronic restraint stress. Interestingly, while BLA-to-vHPC was thought to drive anxiety-like behavior (Felix-Ortiz et al., 2013), a recent publication specified that anterior BLA-to-vHPC neurons had this anxiogenic effect, while posterior BLA-to-vHPC neurons had an opposing anxiolytic effect (Pi et al., 2020). Further investigations demonstrated decreased activity of posterior BLA-to-vHPC neurons triggered in the unpredictable chronic mild stress model of depression, whose reversal by chemogenetic activation was sufficient to suppress the depressive-like behaviors (Ma et al., 2021). The altered posterior BLA-to-vHPC neuronal activity and depressive-like behaviors were notably mediated by decreased levels of synaptosomal AMPA-R and could be corrected by administration of cannabidiol (CBD), a promising treatment for depression acting on endocannabinoids receptor CB1.

### **BLA-to-PFC circuit**

BLA-to-prefrontal cortex (PFC) pathway seems disrupted under stress too. One proposed mechanism is an altered pre-synaptic glutamatergic release from the BLA projections to the dmPFC observed after chronic restraint stress, even though the effect could be strain-dependent and needs further clarification (Lowery-Gionta et al., 2018). In particular, it appeared that acute stress enhanced glutamate release at the BLA-to-PFC synapse, but only to the PFC neurons that send reciprocal inputs to the BLA (Marcus et al., 2020). Reduction of 2-arachidonoylglycerol (2-AG)-mediated endocannabinoid signaling under stress was found to cause the synaptic strengthening and anxiety-like behaviors. Dendritic hypertrophy of BLA-to-dmPFC neurons (corresponding mostly to the prelimbic cortex)

had also been observed following chronic restraint stress (Zhang et al., 2019b). In addition, resting state functional connectivity between the PFC and the BLA was reduced in adult rats submitted to early-life stress (Guadagno et al., 2018), but increased between the PFC and the total amygdala in mice following another kind of early-life stress (Johnson et al., 2018). More systematic distinction should be made between the prelimbic (PL) and the infralimbic (IL) parts of the PFC, as they could be involved in opposite valences (Senn et al., 2014; Felix-Ortiz et al., 2016; Burgos-Robles et al., 2017).

### **BLA-to-NAc circuit**

Concerning the BLA-to-NAc pathway, chronic social defeat stress increased excitatory inputs of cholecystokin (CCK)-BLA neurons onto D2-NAc neurons only in susceptible mice through reduced CB1 levels (Shen et al., 2019). Interestingly, these CCK BLA-to-NAc seemed to send projections to the vHPC as well. In this work, the authors did not measure the activity of non-CCK BLA-to-NAc neurons following chronic defeat stress. Consequently, it is complicated to compare this study with our findings that BLA-to-NAc neurons decreased their activity upon CORT administration.

By using electrophysiological recordings instead of immediate early gene expression, several studies reported no modification of BLA-to-NAc neuronal activity following chronic stress in disagreement with our results (Lowery-Gionta et al., 2018; Zhang et al., 2019b,c). Equally, Bagot et al. (2015) did not observe activity changes in BLA-to-NAc neurons in susceptible mice following chronic social defeat stress by using EGR1 immediate early gene RNA labeling. However, this same study found that acute activation of amygdala terminals in the NAc increased social interaction, locomotor activity in the open field and decrease immobility in the forced swim test following chronic social defeat stress, suggesting pro-resilient/anti-depressant effect of this pathway.

Besides, one very interesting study found that sleep deprivation reduced specifically glutamate release in the anterior BLA-to-ventrolateral NAc, while leaving unchanged the posterior BLA-to-medial NAc (Wang et al., 2020). Sleep deprivation is a paradigm to induce mania-like state in rodents, and the authors paralleled their observations in the BLA with an increased sucrose seeking after sleep deprivation. Enhancing anterior BLA-to-NAc neuronal activity by optogenetics decreased sucrose seeking in control mice and reversed the elevated sucrose seeking of sleep-deprived mice. On the contrary, optogenetic inhibition of these neurons was sufficient to mimic the sucrose seeking induced by sleep deprivation.

In summary, this study suggests that the anterior BLA-to-NAc neurons would be involved in suppressing the appetitive response.

This finding could be related with the above mentioned work of Shen et al. (2019), who showed that activating CCK BLA-to-NAc neurons increased susceptibility to social stress and further decreased sucrose preference, and the opposite when inhibiting CCK BLA-to-NAc neurons in a chronic social defeat model of depression. As a matter of fact, they mainly targeted the anterior part of the BLA. Altogether, these two studies suggest that the anterior BLA-to-NAc neurons would encode preferentially negative valence. It is worth noting that inhibiting BLA ensemble coding for the aversiveness of pain also induced increased sucrose preference, without knowing which BLA circuits are implicated (Corder et al., 2019).

### **BLA-to-CeA circuit**

Finally, few studies investigated the BLA-to-CeA pathway in rodents models of depression. Some insights come from genetically-engineered mice on the BDNF gene (Galvin et al., 2015). The BDNF Val66Met is a human single polymorphism that triggers anxiety-like phenotype in mice (Montag et al., 2010). Knock-in BDNF<sup>met/met</sup> mice displayed in the BLA-to-CeM synapse elevated non-NMDA receptor transmission, suppression of NMDA receptor transmission and therefore impaired synaptic plasticity (Galvin et al., 2015).

In this context, it would be very interesting to extend the measurements of BLA neuronal activity in CORT-treated mice to other projections targets such as the vHPC and the PFC (PL and IL parts), eventually separating the anterior and the posterior parts of the BLA for BLA-to-NAc and BLA-to-vHPC populations. Further work should investigate as well the synaptic and molecular changes to explain the circuit-specific altered activity we found after CORT treatment, and potentially provide therapeutic targets. The endocannabinoid system could be a promising research lead, as demonstrated by several above mentioned publications.

### ***3.3.2 Intra-BLA connectivity and the role of interneurons***

We found both increased BLA-to-CeA and decreased BLA-to-NAc neuronal activity following CORT administration. On the contrary, most of the studies comparing circuit-specific BLA alterations in stress-submitted mice reported changes in a single BLA pathway,

often leaving other pathways unchanged (Lowery-Gionta et al., 2018; Zhang et al., 2019b,c; Wang et al., 2020).

Recurrent collaterals between pyramidal cells of the BLA have been anatomically described (McDonald, 1984), and functionally studied using optogenetics, *in vivo* and/or *ex vivo* calcium imaging and electrophysiological recordings (Kim et al., 2016a; Beyeler et al., 2016, 2018; Calhoun et al., 2018). In summary, it appears that BLA-to-CeA neurons exert a strong inhibition drive onto their neighbors including the BLA-to-NAc neurons (by the intermediate of interneurons), but can also increase excitability of neuronal populations responding similarly than themselves to appetitive and aversive stimuli, meaning mostly other BLA-to-CeA neurons (Beyeler et al., 2018; Calhoun et al., 2018). On the contrary, optogenetic activation of BLA-to-NAc neurons increased the current-evoked firing rate of BLA-CeA neurons, and did not seem to increase the firing rate of other BLA-to-NAc neurons (Calhoun et al., 2018). Therefore, it would be interesting to test whether in our CORT-treated mice a stronger activation of BLA-to-CeA pathway preceded and triggered the decreased BLA-to-NAc neuronal activity.

The interneurons population of the BLA could also play a role in the dysregulated BLA circuits activity in CORT-treated mice. Although it represents only 15-20% of the BLA neurons (Sah et al., 2003), the interneurons population tightly regulates network excitability, synchronizing spiking rate and leads to low resting firing rate of BLA pyramidal neurons (Prager et al., 2016). Interestingly, Kim et al. (2016a) found that the *Rspo2+* and *Ppp1r1b+* BLA neuronal populations preferentially encoding opposite valences induced systematic reciprocal inhibition on each other. In other words, optogenetically activating one population always resulted in inhibitory post-synaptic currents (IPSC) measured in the other population. In some cases, activation also elicited excitatory responses, but in a more marginal way. These findings suggest a particular role of the interneurons in the coordination of pyramidal neurons “positive” and “negative” populations activity.

Nevertheless, evidence for the effects of chronic stress onto the GABAergic neurons of the amygdala, and in particular of the BLA, are scarce probably because it is understudied (Jie et al., 2018). Interestingly, a recent study reported neurogenesis in the BLA of adult mouse (Jhaveri et al., 2018), after being observed in the amygdala of monkeys and marsupials (Bernier et al., 2002; Sanderson and Wilson, 1997). New-born cells of the mouse BLA were found to give rise to mature and functional interneurons (Jhaveri et al., 2018). However, their function in modulating behavior is unknown. Since the fact that chronic stress shrinks

adult hippocampal neurogenesis in various rodent models of depression and that a broad range of antidepressant methods increases adult hippocampal neurogenesis (Planchez et al., 2020), it would be interesting to study how adult BLA neurogenesis reacts to stress or CORT administration as well as to antidepressant therapy, and relate it to the disturbed activity of specific BLA circuits.

### ***3.3.3 Basolateral amygdala dysfunction and emotional biases: cause or consequence of mood disorders?***

One fundamental question to elucidate is whether the emotional biases and BLA dysfunction are a cause or a consequence of the occurrence of mood episodes. Initially, the cognitive theory of depression from Beck (1967) implicating “high” and “low” level biases (*i.e.* affective cognitive control and affective perception) did not state about causality between these biases and the occurrence of depressive states. However, Roiser et al. (2012) renewed this theory, proposing a “cognitive neuropsychological model of depression”, and explicitly stating that negative information processing biases have a causal role in depressive disorders. They further proposed that these biases could be a hallmark of depression susceptibility, and so could represent a predictive or risk factor.

One possible way to answer the question of causality in animals would be to evaluate olfactory hedonic biases and BLA circuits activity at several points during the four weeks of CORT administration, to determine whether they precede depressive-like behaviors.

Even though we showed that manipulating the BLA-to-NAc neurons was sufficient to correct at least partially the negative hedonic bias caused by chronic CORT administration, this manipulation also exerted anxiolytic and antidepressant effects on some other behavioral tests. Consequently, we cannot assert that BLA-to-NAc activation directly induced the correction of negative hedonic bias, which in turn alleviated the anxiety- and depressive-like phenotypes. Instead, the negative hedonic bias correction could have only participated in the global alleviation of the anxiety- and depressive-like phenotype resulting from the restoration of BLA-to-NAc normal neuronal function.

Importantly, activating the BLA-to-CeA neurons in control mice was not sufficient to induce negative olfactory bias neither anxiety- or depressive-like behaviors, suggesting that alteration of this specific BLA circuit alone cannot cause a depressive state. It would be very interesting to investigate if inhibiting BLA-to-NAc neurons, alone or concomitantly

with BLA-to-CeA neuronal activation, could trigger negative olfactory bias and/or anxiety- and depressive-like behaviors. Similarly, inhibiting the BLA-to-CeA neurons, alone or in association with BLA-to-NAc neuronal activation in CORT-treated mice could bring additional insights on this question. In particular, one interesting question would be to see if manipulating the BLA-to-CeA pathway would reverse the negative hedonic bias towards aversive odors, while BLA-to-NAc activation only modified olfactory valence of neutral or appetitive odors.

All of these experiments could be performed using chemogenetics but also optogenetics, which rely on light-sensitive ion channels virally expressed in specific neurons to control their activity with the use of light. Chemogenetics and optogenetics could bring different results, given the different time-scale and biological pathways involved in these manipulations. Activating and inhibiting both BLA-to-NAc and BLA-to-CeA neuronal populations should give insights on the necessity and sufficiency properties of each BLA neuronal pathway in the expression of hedonic biases associated with mood disorders. In particular, it would be interesting to see if sequential optogenetic activation and inhibition of one pathway (or sequential activation - or independently inhibition - of the two pathways) in the same animal could bidirectionally induce positive and negative hedonic bias in opposite manner. This would be an attempt to model mood switch or cyclicality that characterizes bipolar disorders (Henry et al., 2018).

### ***3.3.4 The BLA-to-NAc neurons: a common pathway for antidepressant action?***

Accumulating evidence demonstrates the role of BLA-to-NAc neurons in the antidepressant effects of several manipulations. For instance, Ramirez et al. (2015) were able to reverse the depressive-like behaviors induced by chronic restraint stress by re-activation of hippocampal dentate gyrus neurons previously activated by a positive memory (*i.e.* female mouse exposure). This rescue was dependent on the BLA-to-NAc neurons, as their inhibition blocked the antidepressant effects of dentate gyrus activation. Optogenetic activation of BLA neurons terminals in the NAc also showed antidepressant effects in susceptible mice submitted to chronic social defeat stress (Bagot et al., 2015). Consistently, chemogenetic BLA-to-NAc neuronal activation in a CORT-induced mouse model of depression corrected the anxiety/depressive-like behavior in the novelty suppressed feeding test, while not affecting latency to eat the pellet in Veh-treated control mice (Dieterich et al., 2021). However,

the CORT administration did not elicit clear anxiety- and depressive-like phenotypes in the OF and novelty suppressed feeding compared to Veh-treated controls in this study, and other depressive-like behaviors were not assessed.

All of these findings are in accordance with our data showing anxiolytic and antidepressant effects of chemogenetic activation of BLA-to-NAc neurons in CORT-treated mice in the open field and the tail suspension test. Such manipulation was also able to correct the negative hedonic bias observed in this mouse model of depression by increasing the olfactory positive valence assignment, but only on neutral or pleasant odors. One important question is whether the anti-depressant effect of the BLA-to-NAc neuronal activation is specific of depressed-like mice, or could also be observed in control mice, potentially inducing an opposing manic-like state. In addition, adding a Veh control group to the experiment attempting at activating the BLA-to-NAc neurons in CORT mice would inform us about the complete or partial restoration of the appetitiveness of positive odors in CORT mice compared to these Veh controls. It would also be interesting to decipher if manipulating other BLA circuits would be sufficient to unlock the increased aversiveness of negative odors in CORT-treated mice.

As mentioned above (see “BLA circuit-specific changes under stress”), among the BLA-to-NAc neurons can be distinguished to two separated populations encoding preferentially appetitive and aversive motivated behaviors (Shen et al., 2019). In contrast to all the findings reported above about optogenetic and chemogenetic manipulations, sub-chronically inhibiting the “negative” CCK+ BLA-to-NAc neuronal population was sufficient to correct depressive-like behaviors in chronic social defeat submitted and susceptible mice, while activation of these neurons during sub-threshold social defeat stress paradigm promoted depressive-like behaviors. However, the authors did not measured the effects of manipulating the CCK- BLA-to-NAc neurons, which could correspond to the neuronal population targeted by Ramirez et al. (2015); Bagot et al. (2015); Dieterich et al. (2021) and ourselves. In particular, the antero-posterior coordinates for targeting BLA-to-NAc neurons could be crucial in selecting neurons preferentially encoding positive or negative valence.

An interesting study further supports this hypothesis. As reported above, Wang et al. (2020) found that sleep deprivation reduced specifically activity in the anterior BLA-to-ventrolateral NAc, while leaving unchanged the posterior BLA-to-medial NAc, in parallel to increased sucrose seeking reminiscent of mania-like state. Activation of this circuit had an anti-manic effect, by decreasing sucrose seeking in sleep deprived mice.

Contrary, optogenetic inhibition of these neurons was sufficient to mimic the sucrose seeking induced by sleep deprivation, and we could imagine that this manipulation may have an antidepressant effect on a model of depression. Anterior and posterior BLA-to-NAc neurons could mainly overlap with CCK+ and CCK- neurons, respectively. Further work is necessary to disentangle precisely the contribution of BLA-to-NAc neurons to positive and negative valence encoding, and the features separating “positive” and “negative” populations.

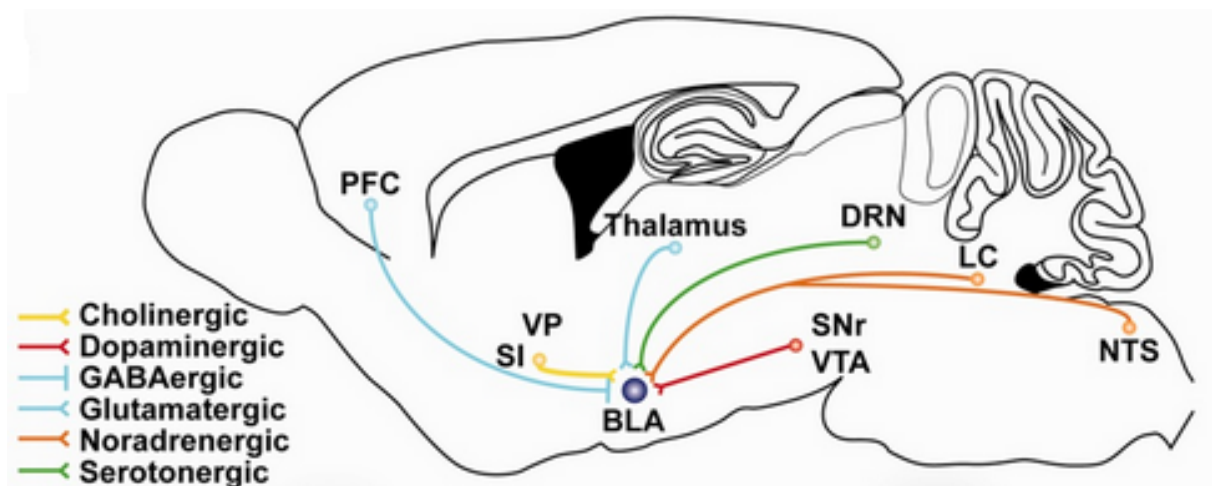
Altogether, these findings suggest a very important role of the BLA-to-NAc pathway in both manic and depressive states, notably by modifying emotional biases associated with mood episodes. As successful antidepressant response has been suggested to be predicted by early emotional bias restoration (Harmer et al., 2017), it would be very interesting to explore parallel modifications in BLA-to-NAc neuronal activity. Indeed, if the alleviation of hedonic biases in animals correlates with correction in the activity of BLA-to-NAc neurons, this pathway would possibly offer a brain biomarker for developing efficient antidepressant therapies in preclinical set-up, and for predicting antidepressant response in patients. Furthermore, if opposed BLA-to-NAc circuit activity reflects manic and depressive states, it would be very interesting to look into its role in mood switches that characterize bipolar disorders.



## 3.4 Perspectives

### 3.4.1 *Stress-sensitive modulatory inputs to the BLA and their involvement in valence coding*

We showed BLA circuits specific functional alterations, namely an increased BLA-to-CeA and a decreased BLA-to-NAc neuronal activity in the CORT-induced mouse model of depression. In addition to intra-BLA connectivity that could explain our results, there is a lot of evidence of the BLA activity control by long-range inputs targeting both BLA principal neurons and interneurons. Interestingly, among those inputs, several are modulated by stress. They are briefly reviewed below. Modulatory inputs in the BLA come from various brain regions, such as the PFC, the thalamus, the dopaminergic ventral tegmental area (VTA) and substantia nigra (SN), the serotonergic dorsal raphé nucleus (DRN), the noradrenergic locus coeruleus (LC) and nucleus of the tractus solitarius (NTS) and finally the cholinergic substantia innominata (SI) and ventral pallidum (VP) (Figure 26, Prager et al., 2016; Sah et al., 2003). Some of these inputs are already known to contain neurons preferentially encoding positive and/or negative valence (Correia and Goosens, 2016).



**Figure 26. Modulation of the synaptic transmission in the BLA.** Adapted from Prager et al. (2016). “Schematic representation of GABAergic projections from the PFC and glutamatergic projections from the thalamus. In addition, [glutamatergic neurons and] GABAergic interneurons in the BLA receive cholinergic projections from the SI and the VP, dopaminergic projections from the VTA and the SN, noradrenergic projections from the LC and the NTS, and serotonergic projections from the DRN.” BLA: basolateral amygdala, DRN: dorsal raphé nucleus, LC: locus coeruleus, NTS: nucleus of the tractus solitarius, PFC: prefrontal cortex, SI: substantia innominata, SNr: substantia nigra pars reticulata, VTA: ventral tegmental area, VP: ventral pallidum.

## Prefrontal cortex

In the prefrontal cortex, the prelimbic and the infralimbic cortices are two subregions that represent upstream regulators of the monoamine system (Lee and Han, 2019). Chronic stress differentially modulated activity in these regions, reducing firing rate of PL neurons but increasing activity of IL neurons, even though discrepancies exist in regard to the role of IL in fear conditioning, anxiety- and depressive-like behaviors, suggesting functional heterogeneity among this structure (Myers-Schulz and Koenigs, 2012). In addition, activation of PL neurons in chronically social defeat stress-submitted mice reversed depressive-like behaviors, whereas activation of IL neurons elicited social and sucrose avoidance (Covington et al., 2010; Ferenczi et al., 2016; Yizhar et al., 2011). The PL and IL are often taken as a whole, under the name of medial prefrontal cortex (mPFC), likely leading to inconsistent results.

The mPFC has an inhibitory top-down control over the amygdala activity, mostly contacting the BLA principal neurons and interneurons to limit its output (Rosenkranz and Grace, 2001; Arco and Mora, 2009; Hübner et al., 2014). This regulation has been found to develop along adolescence, and is sensitive to stress (Selleck et al., 2018; Arruda-Carvalho et al., 2017; Ferrara et al., 2021). It should be noted that BLA afferents could modulate differentially the distinct BLA projection neurons. Indeed, McGarry and Carter (2017) showed that PFC inputs to the BLA mainly target BLA-to-PFC and BLA-to-vHPC neurons, while sparing BLA-to-NAc neurons. In addition, specific neurotransmitters released by specific afferents can modulate differentially BLA outputs depending on the receptors expressed on each BLA pathway. For instance, dopamine has been shown to modulate BLA-to-PFC neuronal firing, as well as cortico-releasing factor, opioids, acetylcholine and serotonin (Floresco and Tse, 2007; Shekhar et al., 2005; Yarur et al., 2020; Tejeda et al., 2015; Hervig et al., 2017; Guo et al., 2017; Arco and Mora, 2009). Unfortunately, very few data are available for BLA circuit-specific and input-specific activity in rodents models of mania. However, it is interesting to note that chronic amphetamine administration abolished the dopamine modulation of BLA-triggered mPFC activity (Tse et al., 2011).

Under chronic stress, the prefrontal cortex loses its control abilities, leading to aberrant amygdala hyperactivity (Correll et al., 2005). Hultman et al. (2016) demonstrated that chronic social stress disrupted mPFC-dependent BLA-VTA synchrony and chemogenetic activation of mPFC-to-BLA neurons was sufficient to restore this synchrony and the depressive-like behaviors. In contrast to what was found in the global PL and IL neuronal

populations, one study recently reported that chronic restraint stress specifically enhances amplitude of ESPC from PL-to-BLA neurons, leaving unchanged the ISPC amplitude induced by these neurons, and the activity of IL-to-BLA and vHPC-to-BLA neurons (Liu et al., 2020). It increased the presynaptic release of glutamate onto the BLA neurons receiving inputs from the PL, but not sending back projections to the PL. Low-frequency optogenetic stimulation was set up to delete this increased presynaptic glutamate release, and reversed the anxiety-like phenotype.

The mPFC also comprise the anterior cingulate cortex (ACC) that sends projections to the BLA. Activation or inhibition of ACC-to-BLA neurons was recently demonstrated to decrease or increase respectively freezing response in front of innate aversive stimuli such as predator odors (Jhang et al., 2018).

### **Ventral tegmental area**

The dopaminergic fibers from the VTA densely innervate both principal neurons and interneurons. Overall, the effect of BLA dopamine release corresponds to enhanced pyramidal neurons activity (Sharp, 2017; Prager et al., 2016). BLA dopamine level increases following different kind of acute stress, but the effect is less known after chronic stress (Douma and de Kloet, 2020). In the VTA, chronic physical stress induced a reduction in the neurons soma size, as well as a degeneration or even loss of VTA neurons. It also decreased excitability of VTA DA neurons leading to decreased DA levels in the BLA upon chronic stress (Lee and Han, 2019; Cui et al., 2012). On the contrary, social stress seemed to increase VTA DA neuronal activity and DA release, notably in the NAc (Lee and Han, 2019). Further work needs to be done to understand the specific VTA-to-BLA pathway alterations following different kind of chronic stress.

Interestingly, a recent study observed bidirectional modulation of anxiety-like behaviors in control mice when optogenetically activating (anxiolytic effect) or inhibiting (anxiogenic effect) the DA VTA-to-BLA neurons (Nguyen et al., 2020). Inhibition of the DA VTA-to-BLA neurons also induced real-time place aversion.

### **Dorsal raphe nucleus**

Chronic physical and social stress in rodents induced serotonin cell death in the DRN and decrease in the spontaneous firing of 5HT neurons (Lee and Han, 2019). One study also reported increased inhibition of the 5HT neurons in the DRN by their GABA neighbors

(Challis et al., 2013). Consequently, 5HT levels were reduced in the whole amygdala, but the different nuclei were not distinguished (Cui et al., 2012). The various effects of 5HT onto BLA neurons in normal condition and during fear conditioning have been reviewed by Bocchio et al. (2016).

### **Locus coeruleus**

The locus coeruleus, containing neurons releasing noradrenalin, is sensitive to chronic stress (Lee and Han, 2019; Daviu et al., 2019). Its activity and number of neurons are reduced, resulting in decreased NA levels in the PFC and HPC. On the contrary, acute stress increases NA release by the LC in the BLA responsible at least partially of the firing rate increase of BLA neurons (Buffalari and Grace, 2007; Giustino et al., 2020). Of note, optogenetic activation of NA LC-to-BLA neurons induced conditioned place aversion and anxiety-like behaviors, preferentially recruiting BLA-to-CeA and BLA-to-vHPC neurons in comparison to BLA-to-NAc neurons (McCall et al., 2017). Recent data suggest that phasic or tonic activation of LC neurons, that seem to play a role in learning by enhancing salience of sensory stimuli, preferentially recruits BLA-to-NAc (at 10 Hz) or BLA-to-CeA (at 25 Hz) neuronal populations, respectively (Ghosh et al., 2020). In this study, phasic 10Hz activation increased rearings of rats, while tonic 25 Hz activation reduced distance moved and increased freezing. In addition, in both real-time and conditioned odor preference test, where one odor was coupled with optogenetic stimulation and the other not, 10 Hz phasic activation promoted a positive bias towards the odor associated with the stimulation, while 25 Hz tonic activation associated with one odor decreased its valence in the conditioned odor preference test, suggesting a negative valence assignment. Interestingly, high tonic activity of the LC is associated with stress (Valentino and Van Bockstaele, 2008).

### **Basal forebrain**

Acetylcholine (ACh) from the basal forebrain can modulate both principal neurons and interneurons expressing muscarinic and nicotinic receptors. The activation of basal forebrain inputs to the BLA elicited excitatory activity when BLA neurons were already strongly active, whereas it had an inhibitory effect during quiescence of BLA neurons, suggesting a role in signal-to-noise ratio improvement in the BLA (Unal et al., 2015; Aitta-aho et al., 2018). Another study with a different stimulation protocol found that activating cholinergic basal forebrain terminals in the BLA led to enhanced BLA neuronal

activity (Jiang et al., 2016). Ach is suggested to be released in the BLA particularly in response to salient stimuli to facilitate conditioned learning (Crouse et al., 2020; Morin et al., 2021). Finally, an antagonist of nicotinic receptors administered within the BLA was found to have anti-depressant effects (Mineur et al., 2016). Globally, cholinesterase inhibitors are proposed as potent antidepressants, as they restore depressive-like behaviors in several rodents models of depression even though evidence for increased cholinergic signaling in chronically stressed animals are scarce (Fitzgerald et al., 2021; Dulawa and Janowsky, 2019).

### ***3.4.2 Other brain areas of interest for valence coding disruption in mood disorders***

Of course, modulation of other brain areas involved in valence processing could arise independently from BLA disruptions and underly the hedonic biases we observed in the CORT-induced mouse model of depression.

The lateral habenula (LHb) has received a lot of interest in the past years for its role in encoding negative states and negatively motivated behaviors, and in particular in relation with depression (for a review, see Hu et al., 2020). Hyperactivity of this structure during depressive state has been highlighted both in humans and animals, and pilot studies using deep brain stimulation to inhibit the LHb found improvement of the depressive symptoms (Caldecott-Hazard et al., 1988; Mirrione et al., 2014; Andalman et al., 2019; Morris et al., 1999; Schmidt et al., 2017; Sartorius et al., 2010; Zhang et al., 2019a). The LHb particularly responds to aversive unexpected stimuli, and is inhibited by appetitive unexpected stimuli (Hu et al., 2020). Similarly, the corticotropin-releasing factor (CRF) neurons in the paraventricular nucleus of the hypothalamus, notably responsible of the CRF release controlling HPA response upon stress, were recently shown to be activated by negative stimuli and inhibited by positive ones (Kim et al., 2019). Optogenetic manipulation of their activity was sufficient to induce conditioned place aversion (when activated) or preference (when inhibited). Besides, the rostromedial tegmental nucleus was demonstrated to have the same pattern of activation upon aversive stimuli and inhibition following rewarding stimuli (Li et al., 2019).

These are only few examples of other brain areas involved in valence coding indirectly connected to the BLA, and the list is probably going to grow in the future years, unveiling large brain networks working together or independently (*e.g.* for redundancy) in order to

decode the appetitive or aversive features of sensory stimuli.

An important question is whether emotional biases are only underpinned by brain areas involved in the processing of emotions and motivated behaviors, or if they could also arise from direct alterations in the sensory systems. Disruptions in sensory cortices activity, structure and connectivity have been recently reported in bipolar patients (Shaffer et al., 2018; Thomas et al., 2019), and the hypothesis of sensory impairment in depression has been proposed several years ago (Fitzgerald, 2013). It has been investigated particularly for interoception, in relation with abnormal insula activity (Eggart et al., 2019) and for olfaction (Rochet et al., 2018). For instance, reduced olfactory bulb volume is associated with depression, and proposed as a biomarker (Rottstaedt et al., 2018; Croy and Hummel, 2017). However, few preclinical studies have tackle the question of olfactory function in animal models of depression and mania (Gao et al., 2018; Siopi et al., 2016; Li et al., 2015; Gusmão et al., 2012). Olfactory bulbectomy as an animal model for depression has further been invoked as an argument supporting the role of olfactory disturbances in the pathophysiology of depressive states, but this model obviously does not allow to investigate olfactory dysfunction in depression (Yuan and Slotnick, 2014). To our knowledge, although some studies investigated olfactory valence assignment in rodent depression and mania models (Malkesman et al., 2010; Lehmann et al., 2013; Finger et al., 2011; Zanos et al., 2017; Terrillion et al., 2017), there is no publication about modifications of the olfactory system involved in odor valence encoding in models of mood disorders. It would be interesting to investigate whether odor valence processing is modified in these models within the olfactory bulb, the olfactory tubercle, the cortical and medial amygdala, olfactory system regions known to encode either innate or learned olfactory valence (Kermen et al., 2021; Li et al., 2015; Midroit et al., 2021; Gadziola et al., 2015; Root et al., 2014; Bergan et al., 2014).

### ***3.4.3 Could emotional biases be useful for mood disorders care?***

#### **As help for diagnosis**

We showed that bipolar depression correlated with a negative bias during odor valence assignment using the Sniffin' sticks test. Depressed BD patients rated less odors as pleasant than euthymic BD patients, and more odors as unpleasant than both control subjects and euthymic BD patients. Interestingly, no effect was found in the allocation of neutral odors, suggesting a specific negative bias affecting emotionally salient stimuli. These results

are consistent with previous data obtained in patients suffering from unipolar depression (Colle et al., 2020; Kohli et al., 2016; Kazour et al., 2020; Atanasova et al., 2010; Naudin et al., 2012). Regarding bipolar depression, a previous work showed that patients had reduced hedonic score for pleasant odors, in agreement with our data (Kazour et al., 2020). However, in this study no difference were found in the hedonic score associated with unpleasant odors, and neutral olfactory cues were not analysed.

It would be very interesting to mirror these findings with the analysis of olfactory valence in manic patients. Unfortunately, in our dataset, only three patients were in manic or hypomanic state.

Importantly, we did not observe any deficit in olfactory performances, including odor detection threshold, discrimination and identification. Nevertheless, several discrepancies were found between unipolar and bipolar depressive episodes. For instance, Kazour et al. (2020) found that bipolar depressive patients performed equally than control and euthymic patients in odor detection, discrimination and identification tests, consistently with our results. On the contrary, several reports demonstrated that odor detection is altered in unipolar depression (Colle et al., 2020; Kazour et al., 2020; Kohli et al., 2016). The recurrence and duration of depressive states could be factors impacting olfactory performances, reminiscent of the early olfactory deficits observed in neurodegenerative disorders (Pabel et al., 2018; Doty, 2017).

Analyzing olfactory valence assignment could be a way to refine the knowledge about the emotional processing disturbances experienced by patients. In a dimensional perspective of psychiatric diseases, it could further detail the diagnosis, considering that precise alterations could help classifying patients into more homogeneous groups, likely sharing the same pathophysiology and sensitive to the same treatments (Insel and Cuthbert, 2015).

### **As help for treatment development and selection**

Unfortunately, 30% of depressions are resistant to currently available treatments, and the development of new drugs is hampered by the lack of knowledge about the pathophysiology (Hyman, 2013). Translational assessment of emotional biases through olfactory valence assignment offers the possibility to carry out clinical and preclinical studies allowing to determine the brain structures involved in emotional processes. Elucidating the mechanisms underlying emotional biases could provide new molecular, cellular or neuronal circuits targets for treatment development.

On the other side, if their restoration provides a common pathway determining the effectiveness of antidepressants, our olfactory preference test developed in the laboratory may be of great interest for the pharmaceutical industry in order to test new molecules on this dimension.

Early changes in emotional biases in depressed patients are suggested to be predictive of efficient but delayed response to antidepressants (Harmer et al., 2017). Therefore, evaluating emotional biases for instance using an olfactory valence based biomarker, could prioritize candidate compounds, thus improving the productivity and cost-effectiveness of drug development. Currently, it is necessary to wait six weeks to determine if a treatment is effective or not, which does not promote optimal management. The Sniffin' sticks test is probably too long to be implemented in routine practice, but simpler tests evaluating olfactory valence assignment could be developed to evaluate these emotional biases changes and guide efficient treatment selection.





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## ANNEXES

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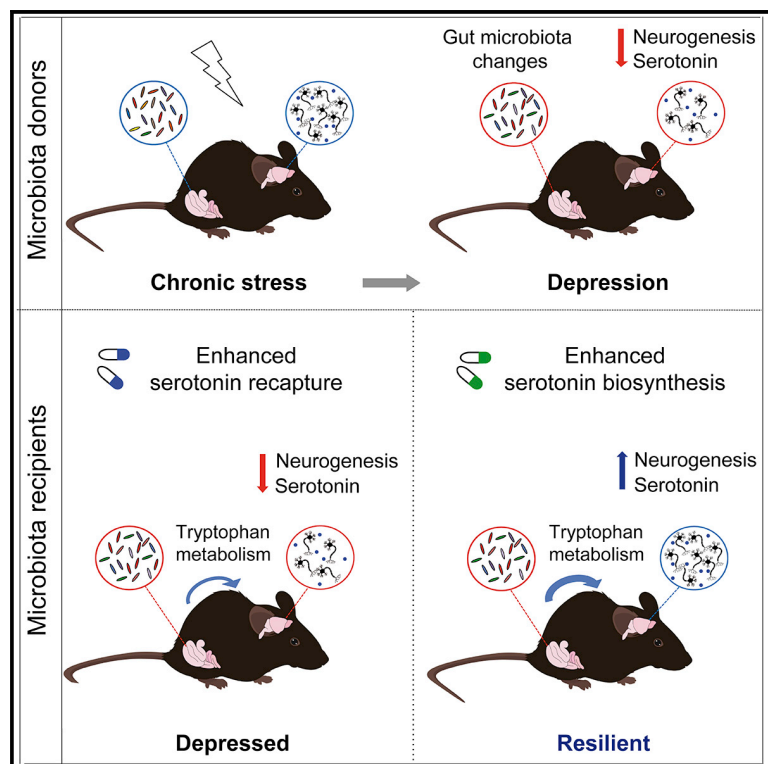
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# Cell Reports

## Changes in Gut Microbiota by Chronic Stress Impair the Efficacy of Fluoxetine

### Graphical Abstract



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### In Brief

Siopi et al. demonstrate that perturbations in the gut microbiota by chronic stress induce resistance to serotonergic antidepressants via impairments in serotonin biosynthesis and bioavailability. Supplementation with the immediate serotonin precursor 5-hydroxytryptophan restores serotonin levels and neurogenesis in the hippocampus and confers resilience.

### Highlights

- UCMS induces changes in the GM
- GM from UCMS mice induces depression and decreases HpC neurogenesis in healthy mice
- GM from UCMS mice impairs the serotonergic pathway of Trp metabolism
- Promoting 5-HT biosynthesis alleviates the symptoms of GM-induced depression



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# Changes in Gut Microbiota by Chronic Stress Impair the Efficacy of Fluoxetine

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## SUMMARY

Major depressive disorders (MDDs) constitute a leading cause of disability worldwide and current pharmacological treatments are partially effective. The gut microbiota (GM) has recently emerged as a target of therapeutic interest for MDDs. In this study, we transfer GM from mice that sustained unpredictable chronic mild stress (UCMS) to healthy recipient mice. The fecal transfer induces despair-like behavior, decreases neurogenesis in the hippocampus (HpC), and impairs the antidepressant and neurogenic effects of a standard selective serotonin (5-HT) reuptake inhibitor, fluoxetine (FLX). These effects are paralleled by deficits in 5-HT bioavailability, biosynthesis, and reuptake in the HpC. Treatment with 5-hydroxytryptophan restores the levels of 5-HT and its precursors in the HpC, improves HpC neurogenesis, and alleviates despair-like symptoms. Our results reveal that stress-induced changes in GM are involved in the pathogenesis of depressive disorders and minimize FLX efficacy via alterations in the serotonergic pathway of Trp metabolism.

## INTRODUCTION

Major depressive disorders (MDDs) constitute a leading cause of disability worldwide (Kassebaum and GBD 2015 DALYs and HALE Collaborators, 2016). Although current antidepressants (ADs), such as selective serotonin (5-HT) reuptake inhibitors (SSRIs), help many patients recover, high rates of partial or no response leave many patients inadequately treated, leading to debates about their efficacy (Ioannidis, 2008). The identification of new targets of therapeutic interest or factors that could ameliorate AD drug efficacy is therefore of crucial importance for health care.

The most significant risk determinant for MDDs is chronic stress (Nestler et al., 2002). One factor that is highly sensitive to chronic stress and that is impacted in MDDs is the gut microbiota (GM) (Naseribafrouei et al., 2014; De Palma et al., 2015;

Jiang et al., 2015; Zheng et al., 2016; Marin et al., 2017). Various studies have shown that the GM is strongly implicated in whole host metabolism (Velagapudi et al., 2010; Desbonnet et al., 2015), namely, tryptophan (Trp) availability (Clarke et al., 2013; O'Mahony et al., 2015). Importantly, the efficacy of SSRI treatments depends on the availability of synaptic 5-HT and, therefore, on Trp catabolism (Ressler and Nemeroff, 2000). However, no study so far has addressed the question of whether GM can affect SSRI drug efficacy.

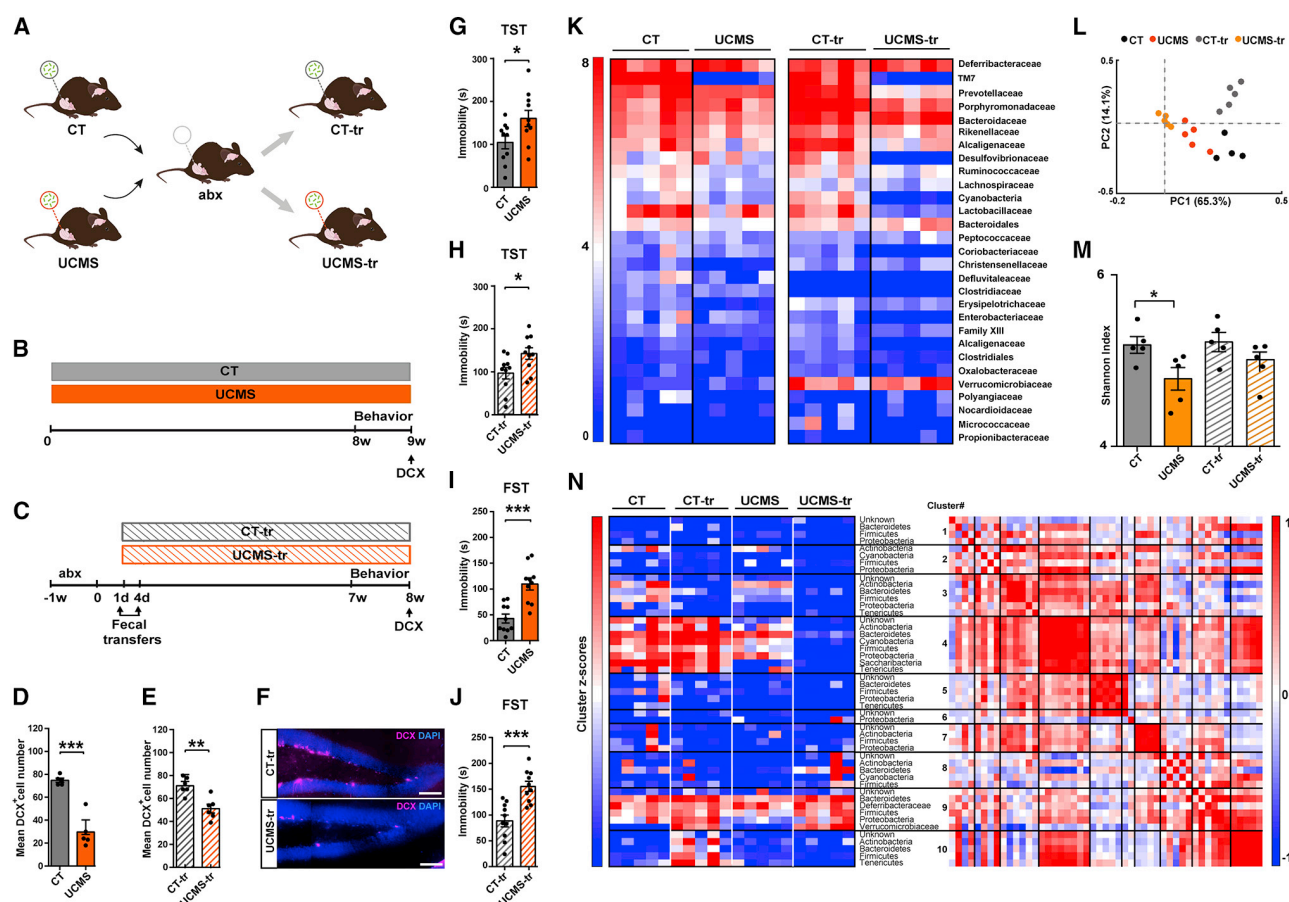
Here, we hypothesize that stress-induced GM perturbations could be responsible for metabolic changes impacting affective behavior and serotonergic drug responsiveness. To test this hypothesis, we used a model of chronic stress-induced depression to study the effect of GM on the antidepressant and neurogenic effects of a standard SSRI, fluoxetine (FLX).

## RESULTS

### GM Transfer from UCMS Mice Promotes Depression and Decreases HpC Neurogenesis in Host Mice

To determine whether the transfer of GM from stressed mice affects mood states and adult neurogenesis in healthy recipient mice, we used the unpredictable chronic mild stress (UCMS) model. Mice treated with antibiotics (abx) were inoculated with GM harvested from UCMS mice (Figures 1A–1C). UCMS induced despair-like behavior, as depicted by immobility in the tail suspension test (TST; Figure 1G) and forced swim test (FST; Figure 1I). Both tests measure the amount of time an animal struggles to escape an uncomfortable situation, a behavior typically affected in models of depression and corrected by AD treatment. We next looked at adult neurogenesis in the dentate gyrus (DG) of the hippocampus (HpC), which is consistently impaired in models of depression (Nestler et al., 2002). We found that the number of cells expressing DCX, a marker of transient proliferating mitotic neuronal progenitor cells, was significantly decreased (Figures 1D and 1F). Recipient mice adopted a similar phenotype, characterized by despair-like behavior (Figures 1H and 1J) and a decrease in the number of DCX<sup>+</sup> cells in the DG (Figures 1E and 1F). Abx treatment did not affect behavior and HpC neurogenesis (Figures S3O and S3P). These results reveal that healthy mice develop despair-like behaviors and deficits in HpC neurogenesis after inoculation with GM from UCMS mice.





**Figure 1. Transfer of GM from UCMS Mice Induces Despair-like States and Decreases Neurogenesis in Healthy Recipient Mice**

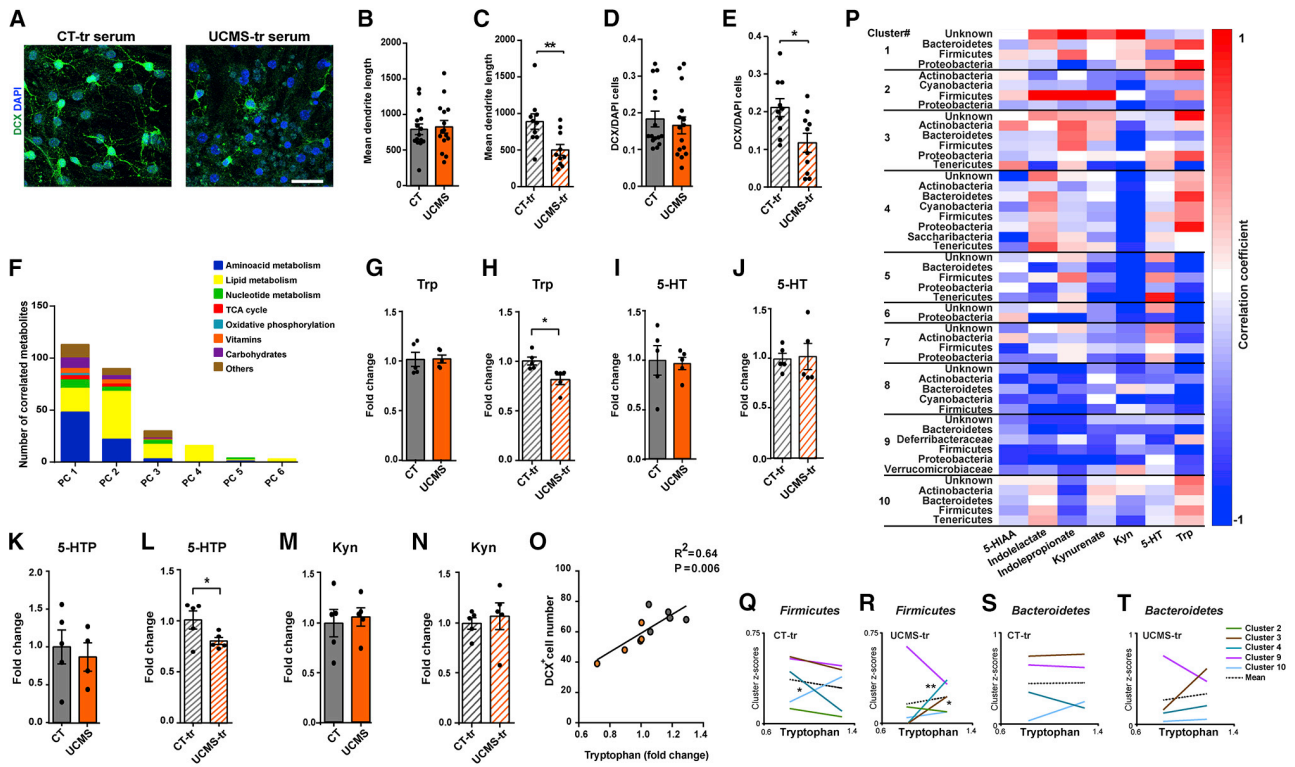
(A) Schematic illustration of the fecal transplantation paradigm and the groups used in the study.  
 (B and C) Experimental designs used for assessing GM changes and behavior in microbiota donor, CT and UCMS (B), and recipient, CT-tr and UCMS-tr (C), mice.  
 (D and E) The total number of DCX<sup>+</sup> neurons was decreased in both UCMS ( $p = 0.001$ ) (D) and UCMS-tr ( $p = 0.002$ ) (E) mice.  
 (F) Representative images of DCX<sup>+</sup> neurons in a section of DG from CT-tr and UCMS-tr mice. Scale bars: 100  $\mu$ m.  
 (G and H) Immobility in the TST was increased in UCMS mice ( $p = 0.03$ ) (G) and in mice that received UCMS microbiota ( $p = 0.03$ ) (H).  
 (I and J) Immobility in the FST was significantly increased in both UCMS ( $p = 0.0002$ ) (I) and UCMS-tr ( $p = 0.0003$ ) (J) mice.  
 (K) Heatmap showing 16S rRNA expression patterns in different experimental conditions. Bacterial families are shown in the right, and heatmap scale is 0–8.  
 (L) PCoA of 16S rRNA expression data showing group differences in principal-coordinate axes (PC1: 65.3% and PC2: 14.1%).  
 (M) Bar plots showing a reduction of the Shannon Index with UCMS compared with CT ( $p = 0.05$ ) and a trend of reduction with UCMS-tr compared with CT-tr.  
 (N) Isolation and annotation of the bacterial phyla and families identified by clustering methods, followed by representation of the clusters as a heatmap. Scale of cluster Z scores is 0–1. (Right) Autocorrelation showing the identical expression of variability of each cluster across the groups. Autocorrelation matrix scale: –1 to 1. Data are shown as mean  $\pm$  SEM.

abx, antibiotics; DCX, doublecortin; DG, dentate gyrus; FST, forced swim test; TST, tail suspension test. See also Figures S1 and S3.

### Stress-Induced Changes in GM Are Transferrable to Host Mice

In light of these results, we examined the GM profile by 16S rRNA sequencing. We first confirmed that abx significantly decreased microbial diversity as expected (Figures S3A–S3D). Taxonomic analysis of bacterial families revealed several UCMS-related changes in GM composition, most of which were transferred to UCMS-tr mice (Figures 2K–2N; Figure S1). Principal-coordinate analysis (PCoA) showed that the different groups clustered differently (Figure 1L). It is to be noted that the relative dispersion between the points corresponding to UCMS and UCMS-tr was

not significant (Bray-Curtis index; data not shown). These changes were also detectable in phylum level, namely, in Firmicutes, Tenericutes, and candidate division TM7 (Figures S1B, S1G, and S1H). Moreover, microbial diversity, as depicted by the Shannon Index, was decreased in UCMS and UCMS-tr mice (Figure 1M). Furthermore, we categorized the most variable bacterial families and phyla in between groups using a correlation network methodology (Figure 1N; Figures S1N and S1O). This type of cluster analysis further helped us to account for the effects of abx on different groups of bacteria (Figures S3B and S3C).



**Figure 2. Inoculation with UCMS Microbiota Impairs Trp Metabolism**

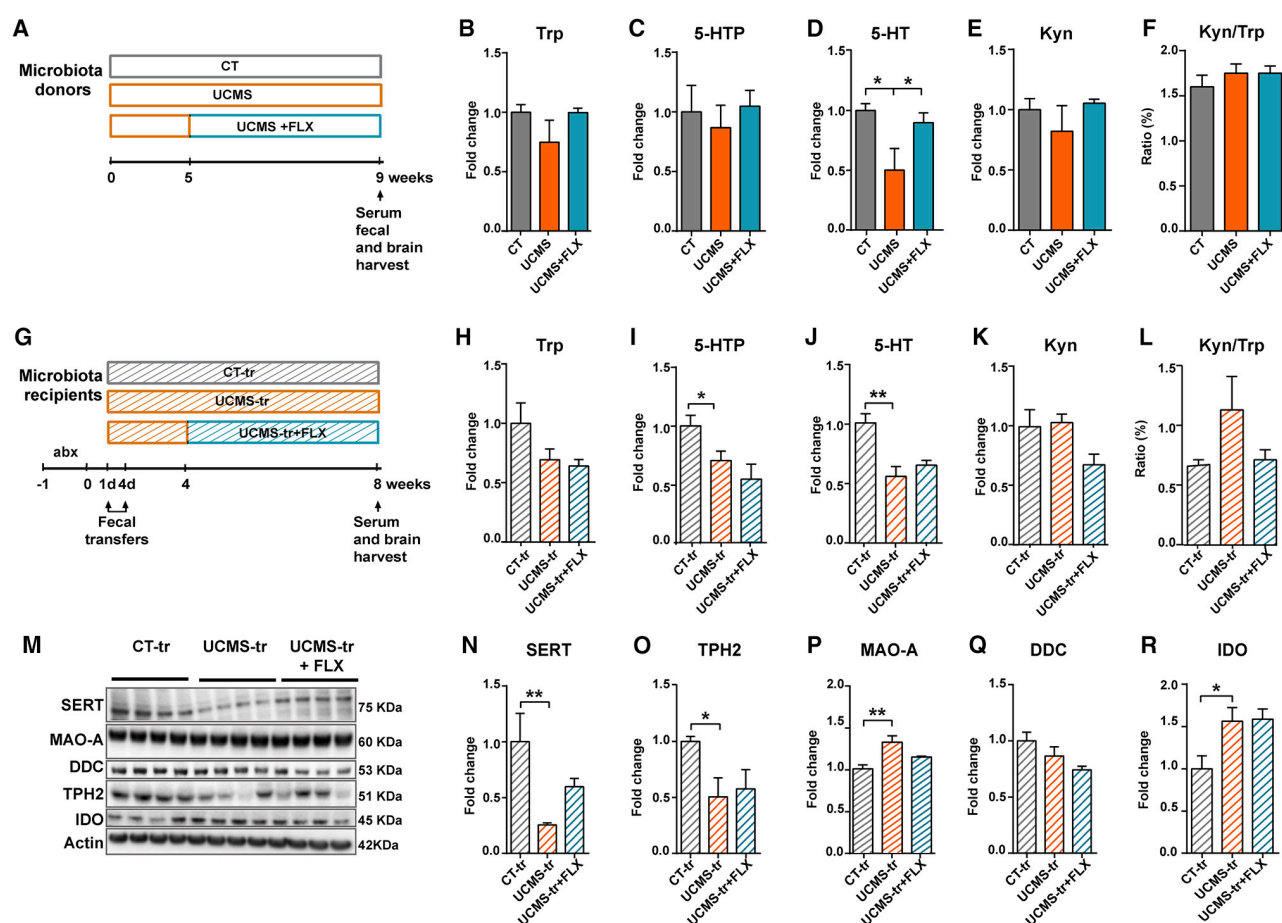
(A) Representative images of DCX<sup>+</sup> neurons in neurosphere cultures treated with serum extracted from either CT-tr or UCMS-tr recipient mice. Scale bar: 30  $\mu$ m. (B–E) Serum from UCMS-tr mice significantly decreased the number of DCX<sup>+</sup> cells ( $p = 0.02$ ) and mean dendrite length ( $p = 0.003$ ) in neurosphere cultures. (F) PCA of serum metabolite levels revealed significant changes in amino acid levels. (G–N) Bar plots depicting serum levels of Trp, 5-HT, 5-HTP, and Kyn in GM donor (CT, UCMS) and recipient (CT-tr, UCMS-tr) mice. UCMS-tr mice had significantly lower levels of Trp ( $p = 0.03$ ) and 5-HTP ( $p = 0.05$ ). (O) Scatterplot demonstrating the positive correlation between the number of DCX<sup>+</sup> neurons and Trp levels in recipient mice ( $R^2 = 0.64$ ,  $p = 0.006$ ). (P) Correlation heatmap of the clusters of bacteria identified by Weighted Gene Correlation Network Analysis (WGCNA) (Figures S2N and S2O) against the major serum Trp metabolites. Heatmap scale is 0–1. (Q–T) Individual trends of Trp variation with clusters corresponding to Firmicutes and Bacteroidetes. Data are shown as mean  $\pm$  SEM. See also Figures S2–S4.

### Serum from UCMS-tr Mice Decreases Neural Stem Cell Differentiation

Given the key role of GM in metabolism, we asked whether GM transfer could impact HpC neurogenesis by changing the levels of serum metabolites. To answer this question, we isolated serum from donor and recipient mice, and applied the different sera on an *in vitro* heterogeneous population of neural stem and progenitor cells. We measured the total number of DCX<sup>+</sup> cells, as well as mean dendrite length, as a proxy of neurite outgrowth. Our results showed that sera derived from UCMS-tr mice significantly decreased dendrite length (Figures 2A and 2C) and DCX<sup>+</sup> cell number (Figures 2A and 2E), suggesting changes in serum composition that affect neural stem cell differentiation. Sera from UCMS mice had no impact on neurospheres (Figures 2A, 2B, and 2D), suggesting that other pathways are preferentially involved in UCMS-induced neurogenesis deficits. Treatment of neural stem cells with sera harvested from ab-treated mice did not affect neuronal differentiation (Figures S3J and S3K).

### Inoculation with UCMS-Microbiota Impairs Trp Metabolism

We then performed untargeted metabolomic analysis to identify the molecular signature in the serum that could explain these results. Principal-component analysis (PCA) revealed that amino acid metabolism was the most affected pathway (Figure 2F; Figure S2). UCMS-tr mice had significantly lower levels of 5-HT's precursor, Trp (Figure 2H), and its immediate precursor, 5-hydroxytryptophan (5-HTP) (Figure 2L), although 5-HT and kynurenine (Kyn) levels were not changed (Figures 2J and 2N). Trp and its metabolites were unchanged in UCMS mice (Figures 2G, 2I, 2K, and 2M). We also observed that the level of 5-hydroxyindoleacetic acid (5-HIAA), a product of 5-HT catabolism, was increased in UCMS-tr mice (Figures S4A, S4B, and S4K), whereas metabolites belonging to the Kyn or indole derivative pathway did not seem significantly affected by either UCMS or the transfer of UCMS microbiota (Figures S4C–S4G). Interestingly, we found a significant correlation between Trp levels and the number of DCX<sup>+</sup> neurons in the DG (Figure 2O). Concerning



**Figure 3. Selective 5-HT Reuptake Inhibitor FLX Does Not Restore 5-HT Availability in the HpC of UCMS-tr Mice**

(A) Experimental design used for assessing the effect of FLX in mice that sustained UCMS. (B–F) Bar plots depicting the levels of (B) Trp, (C) 5-HTP, (D) 5-HT, (E) Kyn, and the (F) Kyn/Trp ratio in the HpC. 5-HT levels were significantly decreased in UCMS mice ( $p = 0.015$ ) and restored by FLX ( $p = 0.04$ ). (G) Experimental design used for assessing the effect of FLX in UCMS-tr mice. (H–L) Bar plots showing levels of (H) Trp, (I) 5-HTP, (J) 5-HT, (K) Kyn, and the (L) Kyn/Trp ratio in the HpC. UCMS-tr mice had significantly lower levels of 5-HTP ( $p = 0.03$ ) and 5-HT ( $p = 0.001$ ) that were not restored by FLX. (M) Representative western blots of SERT, MAO-A, DDC, TPH2, and IDO in the HpC. (N–R) Bar plots depicting HpC levels of (N) SERT, (O) TPH2, (P) MAO-A, (Q) DDC, and (R) IDO relative to CT-tr. UCMS-tr mice had significantly lower levels of SERT ( $p = 0.01$ ) and TPH2 ( $p = 0.05$ ), and higher levels of MAO-A ( $p = 0.005$ ) and IDO ( $p = 0.04$ ). Data are shown as mean  $\pm$  SEM.

the gut, UCMS-tr mice had lower levels of Trp and 5-HT, and a decrease in mean Kyn levels (Figures S4M–S4O). Finally, we found that abx did not change 5-HT and Kyn serum levels, but affected 5-HT levels in the gut (Figures S3E–S3I).

### Changes in Serum Trp Levels Correlate with GM Changes

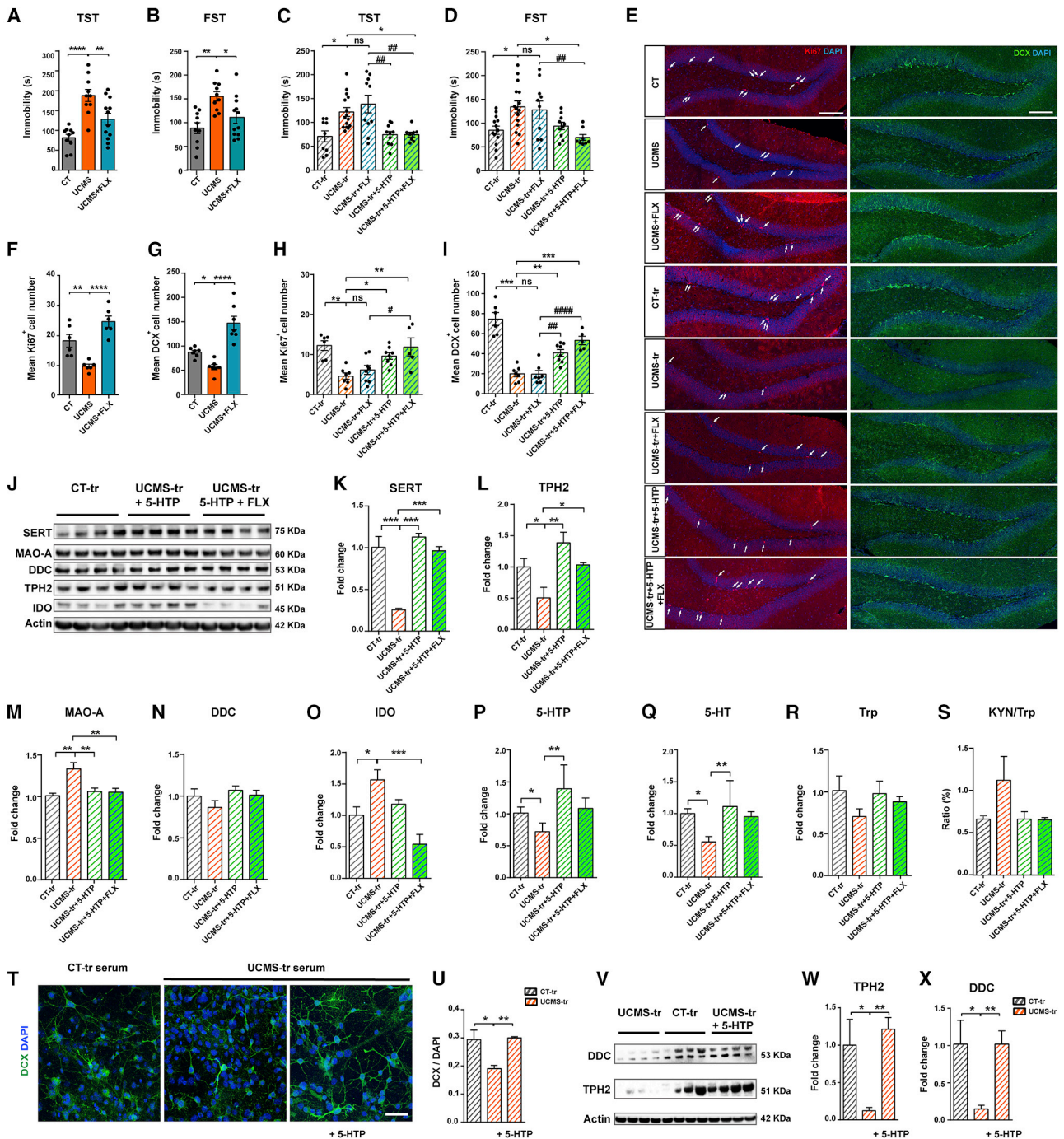
Using a correlation matrix, we found correlations between identified clusters of GM and Trp metabolites, namely Trp (Figure 2P). We isolated the most prominent bacteria phyla in each cluster, the Firmicutes and Bacteroidetes, and correlated them to the Trp expression profile. We found that changes in Trp levels with respect to bacterial variations in control (CT)/CT-tr and UCMS/UCMS-tr were positive in the case of clusters 4 and 10 (Figure 2P). By applying the same method, we found that abx

eradicate the specific microbial clusters that were identified as highly correlated to Trp, i.e., clusters 4 and 10 (Figures S3B and S3C).

### GM from UCMS Mice Decrease 5-HT Biosynthesis in the HpC

Taking into account that serum Trp levels predict brain 5-HT synthesis (Fernstrom et al., 2013), potentially impacting response to serotonergic treatment, we checked brain levels of Trp, 5-HTP, and 5-HT. Based on our results showing deficits in HpC neurogenesis, we chose to focus on the HpC for our analysis. We found that UCMS did not affect the levels of Trp and 5-HTP (Figures 3B and 3C), but decreased 5-HT levels (Figure 3D). We then checked whether this decrease in 5-HT was due to a preferential catabolism of Trp toward the Kyn pathway





**Figure 4. Restoration of 5-HT Biosynthesis Alleviates Depressive Symptoms and Restores Neurogenesis in UCMS-tr Mice**

(A and B) Graphs depicting immobility in the (A) TST and (B) FST. FLX restored immobility in the TST ( $p = 0.009$ ) and FST ( $p = 0.02$ ). (C and D) FLX did not restore despair-like behavior, but 5-HTP decreased immobility in both the (C) TST ( $p = 0.02$  versus UCMS-tr;  $p = 0.002$  versus UCMS-tr+FLX) and (D) FST ( $p = 0.03$  versus UCMS-tr;  $p = 0.006$  versus UCMS-tr+FLX). (E) Representative images of Ki67<sup>+</sup> and DCX<sup>+</sup> cells in the DG. White arrows indicate Ki67<sup>+</sup> cells. Scale bars: 100  $\mu$ m. (F and G) Bar plots of the number of (F) Ki67<sup>+</sup> and (G) DCX<sup>+</sup> cells in the DG. UCMS decreased both Ki67<sup>+</sup> and DCX<sup>+</sup> cell numbers, and FLX attenuated this decrease ( $p < 0.0001$ ). (H and I) 5-HTP restored the number of Ki67<sup>+</sup> ( $p = 0.02$ ) cells and DCX<sup>+</sup> neurons ( $p = 0.01$ ) in UCMS-tr mice. Data are shown as mean  $\pm$  SEM. (J) Representative western blots of SERT, MAO-A, DDC, TPH2, and IDO in the HpC. (legend continued on next page)

and found that Kyn levels were unchanged (Figures 3E and 3F). Interestingly, we observed that UCMS-tr mice had significantly lower levels of both 5-HTP and 5-HT (Figures 3I and 3J). Kyn levels were not significantly higher in UCMS-tr mice, although the mean Kyn/Trp ratio was increased (Figures 3K and 3L). Next, we investigated the expression levels of the enzymes participating in these metabolic pathways. We found that the levels of the 5-HT transporter (SERT), which mediates 5-HT reuptake from the synaptic cleft, and Trp hydroxylase 2 (TPH2), the enzyme catalyzing the conversion of Trp to 5-HTP, were significantly decreased in UCMS-tr mice, whereas the levels of the 5-HT degradation enzyme, monoamine oxidase inhibitor A (MAO-A), were increased (Figures 3M–3P). Dopamine decarboxylase (DDC) levels were not changed, whereas the levels of indoleamine oxygenase (IDO), the enzyme that catalyzes Trp conversion to Kyn, were increased (Figures 3M, 3Q, and 3R).

#### FLX Does Not Restore Brain 5-HT Levels in UCMS-tr Mice

In view of these results, we asked whether these changes in 5-HT bioavailability could impact SSRI efficacy. To answer this question, we added cohorts of donor and recipient mice that were treated with FLX for 4 weeks (Figures 3A and 3G). We found that FLX successfully elevated 5-HT levels in the HpC of UCMS mice (Figure 3D), but not in UCMS-tr mice (Figure 3J). 5-HTP levels also remained unchanged by FLX (Figures 3C and 3I). We then studied the impact of FLX on the expression of enzymes and proteins related to 5-HT biosynthesis, degradation, and reuptake. We found that FLX did not restore the levels of SERT, which constitutes the target of SSRI action, in UCMS-tr mice (Figure 3N). Moreover, FLX did not change TPH2 or MAO-A levels (Figures 3O and 3P). Finally, we found that FLX did not restore IDO levels (Figure 3R).

#### GM from UCMS Mice Impede the Neurogenic and AD Effects of FLX

Next, we tested the behavioral and neurogenic effects of FLX on donor and recipient mice. As expected, FLX decreased immobility in UCMS donor mice both in the TST and the FST (Figures 4A and 4B). Moreover, FLX largely increased the number of Ki67<sup>+</sup> cells and DCX-expressing neuroblasts in UCMS mice (Figures 4E–4G). However, we found that FLX did not improve immobility (Figures 4C and 4D) and did not restore the levels of either Ki67<sup>+</sup> or DCX<sup>+</sup> cells in UCMS-tr mice (Figures 4E, 4H, and 4I).

#### 5-HTP Restores HpC Neurogenesis and Alleviates Depression-like Symptoms

To investigate the implication of 5-HT biosynthesis in the lack of FLX efficacy, we tested whether treatment with the immediate 5-HT precursor, 5-HTP, alleviates depression-like symptoms in UCMS-tr mice. Both behavioral and neurogenesis readouts were analyzed. We found that 5-HTP completely rescued despair-like behavior in the TST and FST (Figures 4C and 4D), and restored the number of Ki67<sup>+</sup> and DCX<sup>+</sup> cells (Figures 4E, 4H, and 4I).

#### 5-HTP Increases 5-HT Levels and Availability in the HpC

To further determine whether 5-HT biosynthesis is implicated in the restoration of the behavioral and neurogenesis deficits by 5-HTP, we measured the effect of 5-HTP on 5-HT, Trp, and Kyn in the HpC, as well as on the levels of key enzymes implicated in these metabolic pathways. As expected, 5-HTP increased the levels of 5-HTP in the HpC (Figure 4P). This effect was associated with a significant increase in 5-HT (Figure 4Q) and a decrease in the mean levels of Kyn (Figure 4S). Interestingly, 5-HTP also significantly increased the HpC levels of SERT and TPH2 (Figures 4J–4L), whereas it decreased MAO-A (Figures 4J and 4M) and IDO levels (Figures 4J and 4O).

#### 5-HTP Restores Neural Stem Cell Differentiation

In light of these results, we tested the effect of 5-HTP on neural stem cell differentiation. We applied sera from either CT-tr or UCMS-tr mice on neurosphere cultures and added 5-HTP in one subset of UCMS-tr-treated neurospheres. We confirmed that UCMS-tr sera impair neuronal differentiation, depicted by a decrease in DCX<sup>+</sup> cell number, and then observed that 5-HTP completely attenuated this impairment (Figures 4T and 4U). Moreover, we observed a significant decrease of TPH2 and DDC in the lysate of UCMS-tr-treated neurospheres and found that 5-HTP supplementation restored the levels of both enzymes (Figures 4V–4X).

#### DISCUSSION

We report here that inoculation with GM derived from chronically stressed mice induces despair-like behavior, decreases adult HpC neurogenesis, and impairs the serotonergic pathway of Trp metabolism in healthy recipient mice that become poorly responsive to SSRI treatment. Supplementation with 5-HTP, the immediate 5-HT precursor, alleviates despair symptoms and restores HpC neurogenesis. These effects are paralleled

(K–O) Bar plots depicting HpC levels of (K) SERT, (L) TPH2, (M) MAO-A, (N) DDC, and (O) IDO relative to CT-tr. 5-HTP restored HpC levels of SERT ( $p < 0.001$ ), TPH2 ( $p = 0.002$ ), and IDO (UCMS-tr+5-HTP+FLX versus UCMS-tr:  $p = 0.0005$ ).

(P–R) Bar plots showing relative levels of (P) 5-HTP, (Q) 5-HT, and (R) Trp in the HpC. 5-HTP treatment increased 5-HTP ( $p = 0.004$ ) and 5-HT ( $p = 0.009$ ) levels in UCMS-tr mice.

(S) Bar plot displaying the Kyn/Trp ratio in the HpC.

(T) Representative images of DCX<sup>+</sup> neurons in neurosphere cultures treated with serum extracted from either CT-tr or UCMS-tr mice. A subset of NSC was treated with UCMS-tr serum supplemented with 5-HTP. Scale bar: 30  $\mu$ m.

(U) 5-HTP restored the number of DCX<sup>+</sup> neurons in UCMS-tr-treated NSC.

(V) Images of western blots for DDC and TPH2 lysate from neurosphere cultures treated with either CT-tr or UCMS-tr serum, or with UCMS-tr serum supplemented with 5-HTP.

(W and X) UCMS-tr serum decreased (W) TPH2 ( $p = 0.03$ ) and (X) DDC ( $p = 0.03$ ) expression, and 5-HTP restored these effects (TPH2:  $p = 0.01$ ; DDC:  $p = 0.03$ ). Data are shown as mean  $\pm$  SEM.

See also Figure S4.

by restoration of 5-HT biosynthesis. Understanding the role GM play in affective behaviors and response to treatments holds substantial potential for a successful therapeutic management of depressive disorders.

Mounting evidence shows that the GM act as a virtual endocrine organ, producing signals that trigger responses at both local and distant scales (Zhang and Davies, 2016). Given the key role of GM in host metabolism, perturbations of this complex ecosystem by chronic stress could impair the efficacy of AD treatments. The neurogenic hypothesis for MDDs points to impairments in adult HpC neurogenesis, and human studies also report reduced HpC volume in MDDs (Campbell et al., 2004; Sahay and Hen, 2007; Boldrini et al., 2013). Importantly, HpC neurogenesis is increased by ADs and confers resilience to chronic stress (Santarelli et al., 2003; Anacker et al., 2018). The contribution of 5-HT in adult HpC neurogenesis is widely accepted (Gould, 1999). Interestingly, intestinal dysbiosis has also been shown to produce deficits in HpC neurogenesis in mice (Möhle et al., 2016).

Our results support recent animal (De Palma et al., 2015; Marin et al., 2017) and human (Naseribafrouei et al., 2014; Jiang et al., 2015; Zheng et al., 2016) studies showing that GM composition is modified by chronic stress and depression. In our experimental conditions, although UCMS-tr mice adopted the bacterial profile of their donor counterparts, the transmitted perturbations were exacerbated. Our serum metabolomic analysis then revealed that UCMS-tr sera were characterized by a significant decrease in amino acids, whereas fewer changes were observed in UCMS sera. This discrepancy could be explained by the dramatic decrease of a specific cluster of GM, which we found to be positively correlated to Trp levels, specifically in UCMS-tr mice.

Our results suggest that the decrease in serum Trp and 5-HTP levels could be either GM and/or host dependent. The use of Trp by some gut bacterial strains for their own metabolism (Bouknight and Sadoff, 1975) could limit its availability for the host. Moreover, given that other essential amino acids were also decreased, the decrease in Trp could result from general dysbiosis-induced perturbations in nutrient absorption and bioavailability. Although our study did not identify a specific microbe responsible for the phenotype observed, our analysis revealed a correlation between the Firmicutes and Trp levels, suggesting that perturbations in microbes belonging to this phylum could be responsible for these changes.

Because brain 5-HT availability is strictly dependent on Trp released from the gut into the circulation (Agus et al., 2018), we assumed that lower serum Trp would reflect lower levels of brain 5-HT (Fernstrom et al., 2013). We found that although 5-HT was decreased in both UCMS and UCMS-tr hippocampi, its precursor 5-HTP was specifically decreased in the UCMS-tr condition, suggesting alterations in both 5-HT availability and biosynthesis. Interestingly, we found that TPH2 and SERT were drastically decreased, strongly indicating a deficit in 5-HTP production and 5-HT reuptake, and that MAO-A levels were increased, indicating an increase in 5-HT degradation. Collectively, these experiments reveal alterations in the biosynthesis, reuptake, and degradation of 5-HT.

Stimulation of adult HpC neurogenesis seems to be a prerequisite for AD efficacy, and it is achieved with different classes of pharmacological ADs (Perera et al., 2007; Surget et al., 2008, 2011; Schmuckermair et al., 2013). Our dysbiosis-induced depression model prevented a subset of the signature cellular and behavioral effects of FLX, a standard AD. FLX efficacy was accompanied by deficits in the serotonergic pathway of Trp metabolism. Our results are reminiscent of previous studies on *hydroxylase 2* knockin mice, which are resistant to FLX treatment due to congenital 5-HT deficiency unless treated with 5-HTP (Jacobsen et al., 2012; Sachs et al., 2013; Siesser et al., 2013). The key role of 5-HT deficiency was further confirmed when we showed that 5-HTP administration restored HpC neurogenesis and despair behavior, as well as the levels of SERT, TPH2, and MAO-A. These findings indicate that 5-HT biosynthesis is responsible, at least in part, for the resistance to FLX. To further confirm the role of 5-HT biosynthesis in neuronal differentiation, we showed that 5-HTP supplementation restored the number of DCX<sup>+</sup> neurons in UCMS-tr-treated neuronal stem cells, as well as the levels of TPH2 and DDC. Altogether, these results point to a role of Trp metabolism in neuronal differentiation and show that a decrease in 5-HT bioavailability, combined with deficits in 5-HT biosynthesis and reuptake, could explain the lack of efficacy of FLX, as well as the therapeutic gain of 5-HTP.

The fact that humans rely mostly on dietary Trp intake, whose metabolism is exclusively governed by microbiota (Agus et al., 2018), makes Trp an actionable actor for new therapeutic strategies in MDD. Exploiting microorganisms specifically involved in Trp catabolism should be considered as a potentially powerful tool to treat MDDs. Our study also suggests that plasma levels of Trp should be considered as a biomarker to orientate therapeutic choices in depressive disorders. Taken together, this study provides evidence that stress-induced dysbiosis can impair FLX efficacy via changes in Trp metabolism. Other non-mutually exclusive pathways could be also implicated, including vagal nerve innervation, neuroendocrine signaling, or neuroimmune regulation. Hence, as Agus et al. (2018) stated, combining metabolomics with metagenomics and metatranscriptomics approaches seems a promising strategy to identify the microbes and microbial genes involved in Trp metabolism. Once identified, novel therapies aiming at using genetically engineered bacteria to modulate Trp levels should be considered to further address the complex issue of treatment-resistant depression.

## STAR★METHODS

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### SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.celrep.2020.02.099>.

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### AUTHOR CONTRIBUTIONS

Conceptualization, E.S.; Methodology, E.S., G.C., and L.K.; Investigation, E.S., G.C., L.K., M.B., and C.M.; Software, S.S.; Writing – Original Draft, E.S.; Writing – Review & Editing, E.S. and P.-M.L.; Funding Acquisition, P.-M.L., G.E., and E.S.; Resources, P.-M.L. and G.E.; Supervision, E.S. and P.-M.L.

### DECLARATION OF INTERESTS

The authors declare no competing interests.

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Rabbit polyclonal anti-DCX	Abcam	Cat#ab18723; RRID: AB-732011
Chicken polyclonal anti-DCX	Abcam	Cat# Ab153668; RRID: AB_2728759
Rabbit polyclonal anti-Ki67	Abcam	Cat# ab15580; RRID: AB_443209
Rabbit polyclonal anti-IDO	Abcam	Cat# ab106134; RRID: AB_10860804
Rabbit polyclonal anti-TPH	Abcam	Cat# ab30574; RRID: AB_778658
Goat polyclonal anti-SERT	Abcam	Cat# ab130130; RRID: AB_11157503
Rabbit monoclonal anti-MAOA	Abcam	Cat# ab126751; RRID: AB_11129867
Mouse monoclonal anti-DDC	Abcam	Cat# ab49916; RRID: AB_2088940
Goat polyclonal anti-IL1beta/IL-1F2	R and D systems	Cat# AF-401-NA; RRID: AB_416684
Rabbit polyclonal anti-Iba1	Wako	Cat# 019-19741; RRID: AB_839504
Rabbit anti-Mouse secondary Alexa Fluor 488	Thermo Fischer scientific	Cat# A-11041; RRID: AB_2534098
Goat anti-Chicken secondary Alexa Fluor 568	Thermo Fischer scientific	Cat# A-11041; RRID: AB_2534098
Goat anti-rabbit IgG (H + L)-HRP conjugate antibody	Bio-Rad	Cat# 1706515; RRID: AB_2617112
Rabbit anti-goat IgG (H+L)-HRP	Thermo Fischer scientific	Cat# 81-1620; RRID: AB_2534006
Goat anti-mouse IgG1 – heavy chain (HRP)	Abcam	Cat# ab97240; RRID: AB_10695944
<b>Chemicals, Peptides and Recombinant Proteins</b>		
Vancomycin	Merck	Cat#V2002; CAS Number: 1404-93-9
Ampicillin	Merck	Cat#A9518; CAS Number: 69-52-3
Colistin	Merck	Cat#C4461; CAS Number: 1264-72-8
Amphotericin B	Merck	Cat#A9528; CAS Number: 1397-89-3
Fluoxetine hydrochloride	Anawa	Cat#A2436; Cat# BG0197
5-hydroxy tryptophan	Merck	Cat#H9772; CAS Number: 4350-09-8
Normal goat serum	Sigma-Aldrich	Cat#G9023; CAS Number: N/A
Fluoromount aqueous mounting medium	Sigma-Aldrich	Cat#F4680; CAS Number: N/A
4',6'-diamidino-2-phenylindole (DAPI)	Sigma-Aldrich	Cat#10236276001; CAS Number: 0028718903
Pierce BCA Protein Assay Kit	Thermo Fischer Scientific	Cat#23227; CAS Number: 7758-99-8
NuPAGE LDS Sample Buffer (4X)	Thermo Fischer Scientific	Cat#NP0007; CAS Number: N/A
NuPAGE 4-12% Bis-Tris Protein Gels, 1.0 mm, 17-well	Thermo Fischer Scientific	Cat#NP0329PK2; CAS Number: N/A
NuPAGE 4-12% Bis-Tris Protein Gels, 1.0 mm, 10-well	Thermo Fischer Scientific	Cat# NP0321BOX; CAS Number: N/A
Trans-Blot Turbo RTA Mini PVDF Transfer Kit	Biorad	Cat #1704272; CAS Number: N/A
Immobilon Crescendo Western HRP Substrate	Millipore	Cat #WBLUR0100; CAS Number: N/A
RIPA lysis and extraction buffer	Thermo Fischer Scientific	Cat#89901; CAS Number: N/A
cOmplete Protease Inhibitor Cocktail	Roche	Cat#11697498001; CAS Number: N/A
cOmplete ULTRA Tablets, Mini, EASYpack Protease Inhibitor Cocktail	Roche	Cat#5892970001; CAS Number: N/A
Triton X-100 solution	Sigma-Aldrich	Cat# 9002-93-1; CAS Number 9002-93-1
<b>Critical Commercial Assays</b>		
Mouse TNF-alpha platinum ELISA 2 x 96 tests Kit	Thermo Fisher Scientific	Cat# BMS607/3TWO; RRID: AB_2575664
Mouse IL-6 platinum ELISA 2 x 96 tests Kit	Thermo Fisher Scientific	Cat# BMS603/2TWO; RRID: AB_2575650
FastDNA Spin kit	MP Biomedicals	Cat#MP116540600; RRID: N/A
<b>Software and Algorithms</b>		
Fiji	ImageJ	<a href="https://imagej.nih.gov/ij">https://imagej.nih.gov/ij</a>

(Continued on next page)

### Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Icy open source software	Icy	<a href="http://icy.bioimageanalysis.org">http://icy.bioimageanalysis.org</a>
R software FactoMineR package	R Core Team 2017	<a href="https://cran.r-project.org/web/packages/FactoMineR/index.html">https://cran.r-project.org/web/packages/FactoMineR/index.html</a>
R software gplots package	R Core Team 2017	<a href="https://cran.r-project.org/web/packages/gplots/index.html">https://cran.r-project.org/web/packages/gplots/index.html</a>
MATLAB	Mathworks Inc. 2015 release	RRID: SCR_001622
ZEN Digital Imaging for Light Microscopy	Zeiss	RRID: SCR_013672
Graphpad	Prism version 6	RRID: SCR_002798
EthoVision XT	Noldus	RRID: SCR_000441
Codes and scripts in R and MATLAB		Codes available upon request: <a href="https://github.com/SohamSahaNeuroscience/Microbiota-analysis">https://github.com/SohamSahaNeuroscience/Microbiota-analysis</a>

### LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Eleni Siopi ([eleni.siopi@inserm.fr](mailto:eleni.siopi@inserm.fr)). This study did not generate new unique reagents.

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

#### Animals

Experiments were performed using adult (8 week old) male C57BL/6J mice purchased from Janvier labs (St Berthevin, France). They were housed in groups of five and maintained in controlled room temperature (22–24°C) and humidity, 12h/12h light /dark cycle, with lights on at 7:00 AM, *ad libitum* access to dry food pellets and water, at the Pasteur Institute animal care facility, officially registered for experimental studies on rodents. All animal experiments were designed according to the 3R's rules and approved by the local ethical committee on animal experimentation of the Institut Pasteur (project CETEA #2013-0062 and #2016-0023) and supervised by the French Ministry of Research.

#### Unpredictable chronic mild stress (UCMS) procedure

Mice were chronically exposed to interchanging unpredictable mild stressors, administered daily in random order for 8 weeks, using a modified version of a previous protocol ([Ibarguen-Vargas et al., 2008](#)). Two stressors were applied daily, one in the morning and one in the evening, and none of the stressors involved food or water deprivation. The different stressors are listed below: (1) cage shaking (1 time, 5 min), (2) cage tilting 45°C (2 h), (3) moist bedding (2 h), (4) overnight illumination (12 h), (5) reversing day and night (24 h), (6) restraint (30min), (7) recurrent cage change (2 h), (8) predator odor (rat urine). Behavioral experiments were performed over a period of 1 week while the animals were still undergoing UCMS, starting at week 8 of the UCMS protocol. At week 9, mice were euthanized and randomly allocated to histological or molecular endpoints using a counter-balanced design. The control animals were socially housed (5 animals/cage) and left undisturbed unless necessary procedures including routine cleaning.

#### Treatments

Microbiota recipient mice (8 weeks of age) were treated with an antibiotic (abx) cocktail, consisting of a mixture of ampicillin (1 mg/ml), streptomycin (5mg/ml), colistin (1 mg/ml), vancomycin (0.5mg/ml) and amphotericin (0.1 mg/ml). The abx treatment lasted one week and ceased 24h prior to microbiota inoculation. Fresh fecal samples (1 mg in 5 mL sterile PBS) were used for the transfer protocol, harvested from either control (CT) or UCMS mice. Recipient mice received the fecal suspension (300 µL per mouse) by oral gavage at 1 and 4 days following abx discontinuation, and were maintained in isolators from one day prior abx discontinuation and up to the onset of behavioral testing. FLX (18 mg/kg/day) and 5-hydroxytryptophan (5-HTP, 100 mg/kg/day) were administered in the drinking water for 4 weeks as previously described ([Siesser et al., 2013](#); [Jacobsen et al., 2016](#)).

#### Experimental sets

A total of 150 mice were used in this study, allocated in four separate experimental sets, as described below:

##### Experimental set 1

Analysis of GM populations, behavior, neurogenesis and metabolic changes following microbiota transfer from UCMS mice to abx-treated mice. Four groups of mice (n = 10/group) were included in this set: a control (CT) group, a group of mice that sustained an 8-week chronic stress protocol (UCMS group), and two groups of abx-treated mice that were inoculated with fecal microbiota

from either CT mice (CT-tr) or UCMS mice (UCMS-tr). All subjects were subjected to behavioral testing. Six mice per group were randomly assigned for neurogenesis analysis and five mice per group for the GM and serum metabolite analysis.

#### **Experimental set 2**

Study of the effect of FLX on anxiety and depression-like behavior, HpC neurogenesis and Trp metabolites in microbiota donor mice. Three groups of mice were included in this set: a control group (CT) (n = 10), and two groups of mice that sustained an 8-week UCMS protocol and that were treated or not with FLX [UCMS+FLX (n = 12) and UCMS (n = 10) groups respectively]. All subjects were subjected to behavioral testing. Six-seven mice per group were randomly assigned for neurogenesis analysis and five mice per group for molecular and metabolite analysis.

#### **Experimental set 3**

Study of the effect of 5-HTP on behavior, HpC neurogenesis, Trp metabolism (brain, gut, serum) and FLX efficacy, following UCMS-microbiota inoculation. Five groups of microbiota recipient mice were included in this set: a CT-tr (n = 13), a UCMS-tr (n = 15) and a UCMS-tr+FLX (n = 10) group, as described in experimental set 1 above, and two groups of UCMS-tr mice that received either 5-HTP alone (UCMS-tr+5-HTP group, n = 10) or adjunct to FLX (UCMS-tr+5-HTP+FLX group, n = 10). All the subjects were subjected to behavioral testing. Six-eight subjects were randomly assigned for neurogenesis analysis and five mice per group for molecular and metabolic analysis.

#### **Experimental set 4**

Study of the effect of abx on GM composition, anxiety and depression-like behavior, neurogenesis, Trp metabolism (serum and gut) and neural stem cell differentiation. A total of 10 mice/group were used in this study. Ten mice per group were used for the behavioral assessment and five mice per group were randomly assigned for molecular and metabolite analysis.

## **METHOD DETAILS**

### **Behavioral testing**

For all behavioral tests, mice were transferred to the testing room at least 1 h before testing. Animals were tested in random order during each testing period and equipment was thoroughly cleaned with 80% ethanol between trials to minimize scents. Microbiota donor mice were tested over a period of 1 week while the animals sustained the last week of UCMS (Figure 1B). Microbiota recipient mice were tested upon exit from the isolators (Figure 1C). All analyses were performed with the experimenter blind to the experimental condition.

### **Tail suspension test**

Mice were suspended at approximately one-third from the end of the tail, using regular tape, to an aluminum bar connected to a strain gauge. The mouse was suspended from a 30 cm high metal rod. The test was recorded during a 5 minute period. Upon viewing of the video recordings, the total time spent in an immobile posture was measured. Mice were considered immobile when they stopped struggling to escape and hung passively, motionless, by the tail. Longer periods of immobility are associated with depressive states.

### **Forced swim test**

Mice were placed into a clear Plexiglas cylinder (25 cm in height and 10 cm in diameter) filled up to two thirds with water (24°C), for a 5-minute session. The sessions were video-recorded and the duration of immobility was measured upon viewing of the video recordings. Immobility was defined as the lack of active movements except from those required for floating.

### **Open field**

Animals were placed in white Plexiglas containers (43 × 43 cm<sup>2</sup>) and behaviors were recorded by a video camera during 30 min. A tracking system (Noldus Ethovision XT 3.0) was used to map center and periphery zones and to calculate the time spent in each zone. The time spent in the center and the total distance traveled were calculated as measures of anxiety behavior and ambulatory activity respectively.

### **Elevated plus maze**

The test was conducted using a plus-cross-shaped apparatus made of black Plexiglas, which was elevated 58 cm above the floor and comprised two open and two closed arms (30 × 6 cm) that extended from a central platform (7 × 7 cm). The Noldus Ethovision 3.0 tracking system was used to record behavior for 5 min. The number of entries and the total time spent in the open arms of the apparatus were calculated as measures of anxiety.

### **Light and dark box**

A two-compartment box containing a dark chamber (black walls with upper lid) and a light chamber (~300 lux, white Plexiglas walls, no upper lid) was used. The chambers were connected by a 10 × 10 cm door in the middle of the wall. Animals were placed in one corner of the light chamber facing the wall and were allowed to freely explore for 10 min. The Noldus Ethovision 3.0 tracking system was used to record behavior. The number of entries and time spent in the light chamber were estimated as measures of anxiety.

### Animal sacrifice and tissue collection

Mice allocated to immunofluorescence studies were deeply anesthetized with a mixture of xylazine-ketamine (10mg/g bw, Sanofi, Bagneux, France) and were perfused transcardially with a solution containing 0.9% NaCl at 37°C, followed by 4% paraformaldehyde (PFA) in phosphate buffer (pH 7.3). All animals were sacrificed between the hours of 10am and 12 noon. Animals were sacrificed in randomized order to minimize experimental bias. The mice were then decapitated, and the brain was carefully removed. Mice allocated to molecular biology analysis, were deeply anesthetized with xylazine-ketamine (10mg/g bw). Approximately 1 mL of blood was taken via cardiac puncture. The whole HpC was carefully dissected, immediately snap-frozen in liquid-nitrogen, and maintained at 80°C until further processing. The blood was put into an ice-cold tube and placed directly into wet ice before centrifugation at 14,000 rpm for 10 minutes for serum collection. The serum was used for metabolic measurements and for the neurosphere assays. Small intestines were collected upon sacrifice and the stool was pushed out. The samples were immediately snap-frozen in liquid nitrogen and moved to 80°C until further processing. Fecal samples were harvested at the end of the UCMS protocol in donor mice and upon exit from the isolators in recipient mice.

### Neural stem cell cultures

Neurosphere cultures were prepared from the subventricular zone area of postnatal day 5 C57/BL6J mice, as previously described (Katsimpari et al., 2008). After 7 days in culture, floating neurospheres were trypsin-dissociated and allowed to reform spheres at least three times before further use. Neural stem cell cultures (neurospheres) were maintained in DMEM/F12 medium with EGF and basic FGF (both at 20ng/ml final concentration). For the differentiation assay, neurospheres were plated on poly-lysine/laminin coated coverslips in serum-free, growth-factor-free DMEM/F12 medium to initiate adhesion. To assess the effects of different serum conditions on differentiated neurospheres, 4  $\mu$ L of sera derived from CT, UCMS, CT-tr and UCMS-tr mice were added to 500  $\mu$ L of culture medium for 4 days. At the end of the experiment, neurospheres were fixed with 4% paraformaldehyde for 30 minutes. For each condition 4 different culture wells were used. All incubations took place in 24-well plates.

### Western blots

Neurospheres, brains and gut tissue were lysed in RIPA lysis buffer (25mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS; Pierce Thermo Scientific) supplemented with protease (cOmplete, Sigma) and phosphatase (phosSTOP, Sigma) inhibitors. Protein concentration was measured with Pierce BCA protein Assay Kit (ThermoFischer Scientific) prior to the western blot assay. Tissue lysates were mixed with 4x NuPage LDS loading buffer (Invitrogen) and reducing agent (Invitrogen NP0004), and proteins were separated on a 4%–12% SDS-polyacrylamide gradient gel (Invitrogen NP0329) and subsequently transferred by semi-dry or liquid transfer onto a PVDF membrane (Trans-blot Turbo Mini PVDF, Biorad). The blots were blocked in 3% BSA in Tris-buffered saline with Tween (TBS-T) and incubated with mouse anti-actin (1:6000, A5441, Sigma). To detect protein signal, the following Horseradish peroxidase-conjugated secondary antibodies were used: Goat Anti-Rabbit IgG (H+L)-HRP Conjugate (1:6000, #1706515, Biorad) and Goat Anti-Mouse IgG1 heavy chain (HRP) (1:6000, ab97240, abcam) and rabbit anti-goat IgG (H+L)-HRP (1:6000, # 31402 Invitrogen). Chemiluminescence detection of proteins was performed with Crescendo Western HRP Substrate (Merck Millipore) with a Chemidoc Imaging System (Biorad). Bands were quantified using the Fiji (ImageJ) software.

### Sandwich ELISA immunoassays

The following kits were used for ELISA immunoassays: mouse IL-6 (BMS603/2, Invitrogen), and mouse TNF- $\alpha$  (BMS607/3, Invitrogen). All immunoassays were performed and analyzed according to the manufacturer's instructions. Data were normalized based on protein concentrations, measured by a BCA assay.

### Immunofluorescence

Perfused brains were cut at 40-micron thick coronal sections using a vibrating microtome (VT1000S, Leica). Immunostaining was performed on free-floating sections. Non-specific staining was blocked by 0.2% Triton and 10% goat serum albumin (Sigma-Aldrich). Sections were incubated with the following primary antibodies at 4°C: rabbit anti-DCX (1:400, Abcam ab18723), chicken anti-DCX (1:400, Abcam ab1536668) and rabbit anti-Ki67 (1:200, Abcam ab15580). For neurosphere cultures, cells were fixed on coated coverslips. They were pre-incubated in 10% normal goat serum, 0.1% Triton X-100 in PBS for 1h and were then incubated overnight at 4°C with chicken polyclonal anti-DCX (1:400, Abcam ab153668). Sections or cells were then incubated with secondary antibodies (biotinylated or Alexa-conjugated secondary antibodies at 1:1000, Jackson ImmunoResearch Laboratories) at room temperature. Fluorescent sections or cells were stained with the nuclear dye 4',6'-diamidino-2-phenylindole (DAPI) and then mounted using Fluoromount aqueous mounting medium (Sigma-Aldrich).

### Confocal imaging and quantification

Images were acquired using a confocal laser-scanning microscope (LSM 710, Zeiss, France) with Zen Imaging software (Zeiss). Z stacks of the dentate gyrus were obtained (step size: 1  $\mu$ m) using sequential tile scanning. Cell counting was performed using the Icy open source platform (<http://icy.bioimageanalysis.org>). Values are expressed as the mean number of Ki67<sup>+</sup> or DCX<sup>+</sup> cell counts in 8-10 sections per animal.

### Microbial DNA extraction and 16S sequencing

Fecal samples were collected in autoclaved Eppendorf tubes and were stored at 80°C until further processing. DNA was extracted using the FastDNA Spin kit (MP Biomedicals) according to the manufacturer's instructions. DNA concentrations were determined using a Nanodrop (Thermo Scientific). Microbial profiling was assessed by 16S rRNA metagenomic analysis, performed on an Illumina MiSeq instrument using the v3 reagent kit. PCR amplicons targeted the V3-V4 region of the 16 s rDNA (Biomics platform, Institut Pasteur). Following purification, a second PCR amplification was performed to barcode samples with Nextera XT Index Primers. Libraries were loaded onto a MiSeq instrument and sequencing was performed. De-multiplexing of the sequencing samples was performed on the MiSeq and individual FASTQ files were recovered for analysis.

### 16S sequencing data analysis

Sequences were clustered into operational taxonomic units (OTU) and annotated with the MASQUE pipeline (<https://github.com/aghozlane/masque>) as previously described (Cole et al., 2009). The final list of 16S rRNA targeted amplicons were converted into the negative logarithm of the expressions of the bacterial taxa (phylum and family). Final data were expressed as the average (represented in a heatmap) and sum (represented in barplots) of the negative logarithm of OTU expression across experimental conditions. All data indexing, segregation and heatmap plotting were performed using custom-made scripts in MATLAB (Mathworks Inc., 2015 release). Further statistical tests were conducted in Prism software (GraphPad). The microbiota profiles obtained were further characterized using the following methods:

- Principal coordinate analysis (PCoA): We used the Bray-Curtis metric to calculate the sample distance across the four conditions: CT, CT-tr, UCMS and UCMS-tr. The Bray-Curtis metric is given as:  $B_{cij} = 1 - (2C_{ij} / (S_i + S_j))$ , where  $C_{ij}$  is the sum of the minimal relative abundance for only those species in common between experimental protocols;  $S_i$  and  $S_j$  being the total number of OTUs counted at these conditions. The Bray-Curtis distance has a bound value between 0 and 1. PcoA plots with PC1 and PC2 are shown in a scatter-plot to cluster samples with lesser distance metric.
- Weighted Gene Correlation Network Analysis (WGCNA). In order to gain insight into specific behaviors of bacterial composition in different experimental protocols, we performed WGCNA applied to microbial communities, by adapting the methods described in Langfelder and Horvath (2008). Briefly, WGCNA identifies clusters of variables (relative abundance of OTUs) which are correlated to given traits (bacterial phyla or families) using hierarchical clustering approach, weighted adjacency functions, topological overlap measures, and a dynamic tree cutting method. The following parameters were used:

Variables	Values
Minimum module size (minMod)	10
Power of scale free network (ds)	3
Dynamic Cut Height	0.99999
Soft threshold	0.15

Each OTU relative expression was represented by a node in a vast network and their adjacency (a score between 0 and 1). A weighted Pearson's correlation was used as a measurement of co-expression for each bacterial family. This takes into account the actual strength of the connection so that an adjacency matrix could be constructed. The adjacency matrix was determined using a 'soft threshold', which is given by:  $Adjacency = 0.5 \times (1 + r)^{sft}$ , where  $r$  is the Pearson's correlation and  $sft$  is the soft threshold as shown in the table above. A scale-free topology model is generated by raising the co-expression similarity to a power:  $a_{ij} = s_{ij}^\beta$  with  $\beta \geq 1$ .  $A_{ij}$  is a weighted adjacency between two genes is proportional to their similarity on a logarithmic scale,  $\log(a_{ij}) = \beta \times \log(s_{ij})$ . PickSoftThreshold package in R was used (our data had  $\beta = 3$ ). The adjacency matrix is then used to generate a Topological Overlap Measure (TOM), describing the relative co-occurrence between two nodes and placing it in the framework of the entire network. The whole network connectivity distribution is shown as a network heatmap, and the branches in the hierarchical clustering dendrograms correspond to modules. The modules were color-coded and data was segregated from each module to classify them into major bacterial families.

- Autocorrelation among modules: The significant modules (or clusters) were determined using an ANOVA test on the cluster z-scores across the experimental conditions: CT, CT-tr, UCMS and UCMS-tr. We identified 5 modules, cluster 2, 3, 4, 9 and 10, based on the criteria above. In order to account for the degree of similarity between the components in the modules and cross-sectional similarities in the observed data, an autocorrelation of the obtained clusters was performed and shown as a correlation matrix. The mean z-scores of the significant clusters across the experiments were also calculated. The common phyla within all the clusters were the most prominent Firmicutes and Bacteroidetes, although less abundant families like Proteobacteria, Actinobacteria and Tenericutes were also common.



- d. Correlation of GM with metabolites. Metabolites related to Trp metabolism were correlated to the cluster z-scores of each Phylum/family in each condition to check for coincidental variability of bacterial relative expression and metabolite profiling. Pearson's  $r$  was used to generate the correlation matrix across conditions and the identified clusters. We isolated the Firmicutes and Bacteroidetes in each cluster and correlated them to Trp expression profile for the experimental conditions: CT, UCMS, CT-tr and UCMS-tr.

### Serum extraction and metabolite analysis

Blood samples were collected (Microvette 200 Z-Gel, Fischer scientific) and centrifuged at 14,000 rpm for 10 minutes at 4°C. The clot was removed and serum was transferred into a clean polypropylene tube using a Pasteur pipette. Serum samples were then stored at -80°C. Two series of serum samples were analyzed in two different GC/MS, LC/MS and LC/MS/MS platforms (Metabolon Inc, California, USA and metabolic platform at SFR Necker, France). Hippocampus and gut samples were analyzed at the metabolic platform at SFR Necker, France. Protein fractions were removed by serial extractions with organic aqueous solvents, concentrated using a TurboVap system (Zymark) and vacuum dried. For LC/MS and LC/MS/MS, samples were reconstituted in acidic or basic LC-compatible solvents containing > 11 injection standards and run on a Waters ACQUITY UPLC and Thermo-Finnigan LTQ mass spectrometer, with a linear ion-trap front-end and a Fourier transform ion cyclotron resonance mass spectrometer back-end. For GC/MS, samples were derivatized under dried nitrogen using bistrimethyl-silyl-trifluoroacetamide and analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization. Chemical entities were identified by comparison to metabolomic library entries of purified standards. Data were analyzed following log transformation and NA values were replaced with minimum observed values when necessary. Principal component analysis (PCA) was applied on the 710 assessed metabolites. The six first principal components (PC) explained 63.78% of the variance of the data, and PC1 and PC2 accounted for 19.6% and 17.63% of the variance respectively. To get an insight of the metabolic pathways represented within each PC, we looked at the correlation coefficients between the PC and the metabolites. We kept the metabolites that were correlated to a PC with a coefficient superior to 0.65. PCA and heatmap plotting was performed using the R software (R Core Team 2017, FactoMineR and gplots packages).

### QUANTIFICATION AND STATISTICAL ANALYSIS

Data were analyzed with GraphPad Prism (version 6, San Diego, USA), with  $p < 0.05$  considered statistically significant. Statistical parameters and the exact number of animals employed in each study are reported in the Experimental sets part of the Experimental Models and Subject details. Statistical significance is reported in the figure legends. All data were expressed as mean  $\pm$  SEM. Data were analyzed using the non-parametric Mann-Whitney test or one-way analysis of variance (ANOVA) followed by Bonferonni post hoc test when appropriate. Linear regression was used to determine relationships between variables and a Pearson's correlation was used to calculate statistically significant relationships.

### DATA AND CODE AVAILABILITY

The codes generated during this study are available upon request at <https://github.com/SohamSahaNeuroscience/Microbiota-analysis>.

1 **Gut microbiota requires vagus nerve integrity to promote depression**

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22       Recent studies have shown that gut microbiota (GM) can influence hippocampal  
23    plasticity and depression-like behaviors (1,2,3), yet the underlying mechanisms  
24    remain elusive. The gastrointestinal branches of the vagus nerve, which constitute a  
25    direct bidirectional route of communication between the gut and the brain, are well  
26    suited to carry neural messages associated with changes in peripheral states to the  
27    brain (4,5). For instance, the ability of some gut bacterial strains to induce anxiety-  
28    like behaviors and alter the expression of proteins in the hippocampus depends on  
29    vagal afferents (6,7). Similarly, gut vagal sensory signaling affects adult hippocampal  
30    plasticity and neurogenesis (8). Impairment in brain plasticity is known to generate  
31    depressive states in mice (9,10). Yet, it is not known whether GM abnormalities  
32    require a functional, intact vagus nerve to promote depression or if GM borrows  
33    other pathways, such as humoral immune responses. Here we used the fecal  
34    transplantation paradigm to study whether changes in GM necessitate the integrity of  
35    the vagus nerve to impact on adult hippocampal neurogenesis and depressive-like  
36    behavior. Alteration of GM was induced by unpredictable chronic mild stress  
37    (UCMS), a standard model of depression (11).

38       We used mice (8 weeks old, C56BL6/j) that sustained 9 weeks of UCMS (UCMS  
39    group), and their controls (CT group), collectively called “microbiota donor” mice.  
40    Fresh fecal samples were harvested from microbiota donor mice at the end of the 9<sup>th</sup>  
41    week of UCMS and were transferred by oral gavage to “microbiota recipient” mice (8  
42    weeks old, C56BL6/j), which had been treated with broad-spectrum antibiotics (abx)  
43    (12) during one week prior the fecal transfer. Abx was discontinued 24h prior  
44    microbiota transplantation. Microbiota recipient mice received either the CT (CT-tr  
45    group) or the UCMS (UCMS-tr group) fecal suspension at 1 and 4 days following abx  
46    discontinuation (Figure 1A,B). In order to assess whether the microbiota transfer can

47 affect behavior and neurogenesis via the vagus nerve, we distinguished two different  
48 subgroups of microbiota recipient mice: animals that had sustained  
49 subdiaphragmatic vagotomy (Vx) 4 weeks prior fecal transplantation, and a sham  
50 group (Figure 1A,B). Microbiota recipient mice were maintained in isolators for 7  
51 weeks following the fecal transfer.

52 To assess the impact of fecal transfer on GM composition, we performed 16S  
53 rRNA gene V3-V4 region sequencing on the fecal samples. Taxonomic analysis of  
54 bacterial families revealed differences between CT and UCMS GM compositions,  
55 which were transferred to their respective recipient mice, with the most significant  
56 change being observed in Bifidobacteriaceae (Figure 1D). More precisely, both  
57 UCMS and UCMS-tr mice had significantly lower levels of Bifidobacteriaceae  
58 compared to CT and CT-tr mice respectively ( $P=0,0016$  and  $P=0,0039$ , Tukey post-  
59 hoc). This finding supports previous studies showing that Bifidobacteria and  
60 prebiotics exert anti-depressant effects in rats (13, 14).

61 We next sought to determine whether these UCMS-induced GM changes are  
62 sufficient to change behavior. We employed a battery of standard behavioral tests to  
63 assess anxiety- and depression-like behaviors. Our results showed that UCMS  
64 microbiota recipient mice adopted the behavioral phenotype of donor mice,  
65 characterized by depressive-like responses, and that transmission of the depressive-  
66 like phenotype was abrogated by Vx (Figure 1C,E,F). For instance, in the novelty  
67 suppressed feeding (NSF) test (Two-way ANOVA,  $F(1, 28)=6.5$ ;  $P=0.02$ ), Tukey's  
68 post-hoc showed that while the transfer of UCMS microbiota significantly increased  
69 the latency to eat ( $P<0.05$ ), Vx completely reversed this phenotype ( $P=0.005$ )  
70 (Figure 1C). Moreover, in both the tail suspension and forced swim tests [Two-way  
71 ANOVA,  $F(1, 28)=10.99$ ,  $P=0.003$ ; and  $F(1, 26)=4.23$ ;  $P=0.05$  respectively], UCMS-tr

72 mice were more immobile compared to their control counterparts ( $P<0.001$ ;  $P=0.02$ ),  
73 and Vx abolished the phenotype ( $P=0.02$ ;  $P=0.03$ ) (Figure 1E,F). Taken together,  
74 our observations indicate that UCMS-induced changes in GM lead to depressive  
75 states only if the vagus nerve remains functional.

76 To further investigate whether these depressive states are accompanied by  
77 changes in adult hippocampal neurogenesis, we performed immunostaining for Ki67,  
78 a marker of proliferating cells, and doublecortin (DCX), a marker of transient  
79 proliferating neuronal progenitor cells, in the dentate gyrus (DG) of the hippocampus.  
80 The large pool of DCX<sup>+</sup> cells in the DG, besides being a step in the maturation  
81 process of new neurons, is of importance in buffering the negative feedback on the  
82 hypothalamic–pituitary-adrenal axis (15). Counting of Ki67<sup>+</sup> cells in the DG [Two way  
83 ANOVA,  $F(1, 24)=9.96$ ;  $P=0.005$ ], revealed that the transfer of UCMS-derived  
84 microbiota significantly decreased Ki67<sup>+</sup> cell number ( $P<0.001$ ), and that this  
85 decrease was completely abolished by Vx ( $P<0.001$ ) (Figure 1G,H). Our results on  
86 the number of DCX<sup>+</sup> cells went on the same line. UCMS-tr mice had a significant  
87 decrease in DCX<sup>+</sup> cells ( $P=0.001$ ) that was attenuated by Vx ( $P=0.03$ ) (Figure 1G,I).  
88 These data show that vagal nerve activity is essential to mediate the impact of gut  
89 dysbiosis on hippocampal adult neurogenesis.

90 The present study demonstrates that chronic stress induces changes in GM  
91 composition, notably diminution of the Bifidobacteriaceae family. When transferred to  
92 healthy recipient mice, these changes promote depressive states and decrease adult  
93 hippocampal neurogenesis. While the exact underlying cascades of events are not  
94 elucidated, we found that the transmitted dysbiosis requires an intact vagus nerve to  
95 be effective. Our results are in line with recent studies showing altered cecal and  
96 fecal microbiota composition in experimental models of stress (16,17) and in

97 depressed patients (18). These alterations may contribute to the neuroprogression of  
98 stress-related depression by altering physiological and/or metabolism processes that  
99 activate vagal afferents, such as microbial peptides, neurotransmitter release and  
100 immunity/inflammation (19,20). Our results are in line with recent data showing that  
101 the vagus nerve mediates the anxiolytic effects of some probiotic strains in rodents  
102 (6,21,22). The two existing studies pertaining to the effect of the vagus nerve on  
103 hippocampal plasticity show contradictory results, (23, 24). While the reasons  
104 underlying these discrepancies are not clear, they may be due to methodological  
105 differences, such as the Vx procedure itself. Relevant electrophysiological data on  
106 vagal tone should be implemented in future studies to decipher how GM promote  
107 depression. Our data further support the emerging hypothesis that GM can influence  
108 brain function and behavior directly through a nervous pathway mediated by vagal  
109 afferents.

110

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122

123 **Author contributions**

124 ES initiated the project in the P-M.L. laboratory, designed the study, performed the  
125 experiments and wrote the manuscript. S.S. analyzed the 16S data. C.M. contributed  
126 in the subdiaphragmatic vagotomy surgeries. M.B. contributed in some experiments.  
127 P-M.L. supervised the study and edited the manuscript with input from the other  
128 authors.

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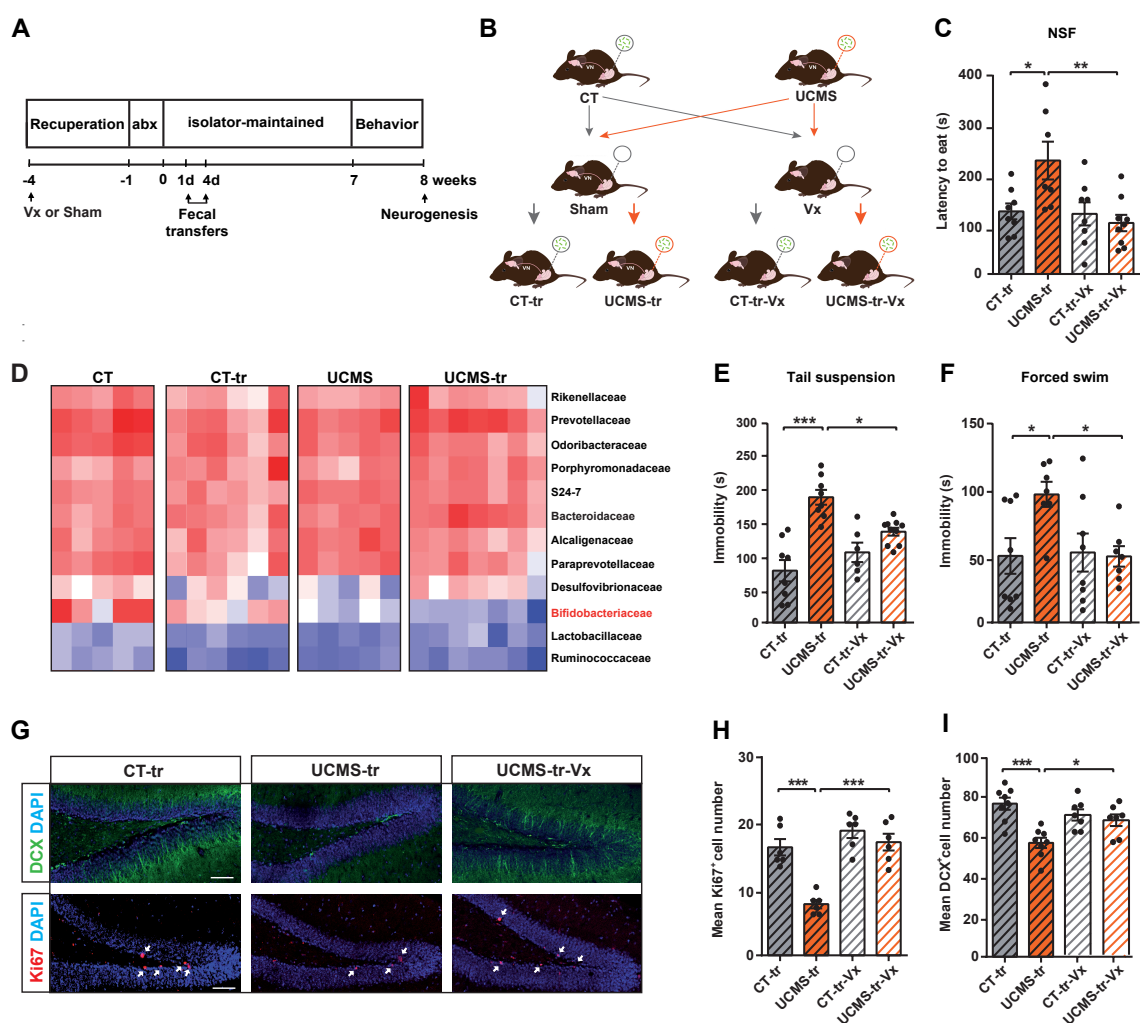
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228 **Figure 1. Gut microbiota from chronically stressed mice generates depression**  
 229 **and decreases hippocampal adult neurogenesis through the vagus nerve.**

230 **(A)** Experimental design used for assessing the microbiota changes, behavioral  
 231 responses and hippocampal neurogenesis in mice that were colonized by microbiota  
 232 from either CT or UCMS mice. Microbiota recipient mice were treated with broad  
 233 spectrum antibiotics (abx) for one week prior fecal transfer and were kept in isolators  
 234 in order to maintain their bacterial profile. Mice sustained Vx at 4 weeks prior fecal  
 235 inoculation. **(B)** Schematic representation of the fecal transplantation paradigm and  
 236 the different groups used in the study. **(C)** In the NSF test, mice that were inoculated  
 237 with UCMS-derived microbiota displayed an increased latency to eat the pellet

238 (\* $P < 0.05$ ) that was completely abolished by Vx (\*\* $P < 0.01$ ). **(D)** Heatmap showing the  
239 mean relative abundances of the different bacterial families in the 16S rRNA  
240 sequencing across all the experimental replicates. **(E, F)** In the tail suspension and  
241 forced swim tests, which are both indicative of despair-like behavior, UCMS-tr mice  
242 displayed increased immobility (\*\* $P < 0.001$  and \* $P < 0.05$ ), and Vx abolished this  
243 behavior (\* $P < 0.05$ ). **(G)** Representative images of Ki67<sup>+</sup> and DCX<sup>+</sup> cells in the  
244 dentate gyrus of CT-tr, UCMS-tr and UCMS-tr-Vx mice. Scale bars: 100  $\mu$ m. **(H)**  
245 UCMS-microbiota transfer decreased the number of Ki67<sup>+</sup> proliferating cells in the  
246 dentate gyrus of recipient mice (\*\* $P < 0.001$ ) but not in mice that sustained Vx. **(I)**  
247 Transfer of UCMS microbiota decreased the number of DCX<sup>+</sup> immature neurons in  
248 the dentate gyrus (\*\* $P < 0.001$ ). Vx abolished this decrease (\* $P < 0.05$ ). All data are  
249 represented as mean  $\pm$  SEM. Statistical significance was calculated using two-way  
250 ANOVA followed by Tukey post-hoc test. abx: antibiotics, NSF: novelty suppressed  
251 feeding, Vx: vagotomy.

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# ABSTRACT

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Mood disorders, including major depressive disorder and bipolar disorders, are heterogeneous psychiatric diseases that affect around 15% and 2% of the general population, respectively. Treatments work with a delayed onset of action and approximately 30% of patients do not respond to any therapy. Depression should become the leading cause of global burden disease by 2030 according to the World Health Organization. Therefore, a better understanding of the pathophysiology of these diseases would be of first help to propose new diagnostic tools and to move towards more effective therapeutic strategies. Validity of our classification system in psychiatry is questioned, because of heterogeneity and great overlap between mental illnesses, impeding preclinical and clinical research. To face these issues, a new research area based on dimensions has emerged. It consists of exploring domains derived from fundamental behavioral components to link them to neurobiological systems. Beyond mood, emotional biases differentiate mood states in patients. Mania episodes are associated with positive biases, *i.e.* emotional stimuli become more rewarding and less aversive, while the opposite characterizes depression.

The objective of this thesis was to assess behavioral responses to innately appetitive and aversive olfactory cues in mice as a proxy for assigned olfactory hedonic valence. Such approach offers the possibility to identify hedonic bias in mouse models of depression and mania, and to study the underlying neural mechanisms.

Recently, the basolateral nucleus of the amygdala (BLA) has been shown to encode valence, namely the value we assign to the perceptions of our external and internal environments, with distinct subpopulations encoding either positive or negative valence. It is well established that the amygdala activity is altered in mood disorders. Then, we hypothesize that modifications in the activity of the BLA and its connections to other cortico-limbic areas could be responsible for the emotional biases observed in mood disorders.

To test this hypothesis, we first assessed hedonic valence assignment in the GBR 12909-induced mouse model of mania. Surprisingly, apart from the classical mania-like phenotype characterized by hyperlocomotion, GBR 12909-treated mice exhibited strong negative olfactory and gustatory hedonic biases. In other words, appetitive odors and tastants were less appetitive and aversive ones were more aversive, as opposed to our expectations. On the other side, we uncovered a negative olfactory hedonic bias in the corticosterone-induced mouse model of depression, as we predicted. This bias was accompanied by specific BLA circuits activity disturbances. Furthermore, manipulating some of these BLA circuits activity thanks to chemogenetics was sufficient to partially restore the negative olfactory hedonic bias induced by chronic corticosterone administration.

Taken together, our results highlight the interest of olfactory hedonic evaluation in mouse models of depression and mania, and demonstrate the causal role of BLA circuits in hedonic biases associated with depressive-like states.