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The effect of age on spatial working memory and topography of dopamine neurons in the ventral tegmental area in a mouse model of healthy aging

Jothini John-Vyani-Rajalingam

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SORBONNE UNIVERSITÉ

ED158 : CERVEAU, COGNITION ET COMPORTEMENT

Doctoral Thesis in Neurosciences

**The effect of age on spatial working memory and
topography of dopamine neurons in the ventral
tegmental area in a mouse model of healthy aging.**

by Jothini JOHN-VYANI-RAJALINGAM

| | |
|--------------------------------|-----------------------|
| Dr. Eleni TZAVARA | President of the jury |
| Dr. Yukiori GOTO | Reviewer |
| Dr. Mathieu WOLFF | Reviewer |
| Dr. Aline MARIGHETTO | Examiner |
| Dr. Annabelle REAUX-LE GOAZIGO | Invited |
| Dr. Denis SHEYNIKHOVICH | Scientific supervisor |
| Dr. Satoru OTANI | Thesis director |
| Pr. José-Alain SAHEL | Co-director |

Aging in Vision and Action team
Institut de la Vision

“I believe that scientific knowledge has fractal properties, that no matter how much we learn, whatever is left, however small it may seem, is just as infinitely complex as the whole was to start with.”

Isaac Asimov

Abstract

Healthy aging is associated with progressive deterioration in cognitive functions participating in the dynamic adaptation of goal-directed behavior in response to internal needs and environmental factors. Such behavior relies crucially on working memory (WM), a short-term mnemonic component of executive function that is defined as the ability to encode, maintain and manipulate relevant information for motivated actions. While primate studies have identified the prefrontal cortex as the key brain area supporting WM, studies in humans have demonstrated age-related decline in the different WM subprocesses, with most pronounced deficits observed primarily in tasks requiring manipulation of information within WM, but not in simple maintenance tasks.

To study healthy aging, rodent, and in particular mouse models, have gained an increasing importance during the last decades because they are amenable to genetic, cellular and biochemical approaches, and thus can contribute significantly to our understanding of the critical neurobiological mechanisms that mediate decline of executive processes across the lifespan. In particular, rodent studies have demonstrated an age-related decline in the overall WM function and suggested that neurodegeneration in the ventral tegmental area (VTA), at the origin of the mesocortical dopaminergic pathway, may be an important neural correlate of age-related WM deficits. However, to be a useful model of human aging, a deeper characterisation of the rodent WM

function is needed both in terms of the subprocess specificity and in terms of its underlying neural correlates.

This thesis thus studied cognitive decline in the healthy aged mouse model by characterizing age-related alterations related to WM subprocesses as well as their underlying dopaminergic network. Specifically, we investigated to what extent WM deficits observed in older mice reflect human findings, and developed advanced methodological tools to study the effect of age on VTA dopamine neurons.

Methodology-wise, we utilized both behavioral and anatomical approaches. Behavioral assessments involved spatial WM tasks that tested the extent of WM maintenance failure by varying the cognitive effort required to encode information and the cognitive load required to maintain and manipulate multiple information online. This allowed us to evaluate age-related changes in WM subprocesses in mice. Anatomically, we investigated the density of VTA DA neuron density by specifically focusing on the density of cellular bodies. We used whole-brain immunostaining and clearing as well as stereotaxic injections combined with slice immunostaining to elucidate the origin of age-related reduced DA release in aging by quantifying the density of DA cell bodies in VTA. We used Bayesian statistical methods to refine our analyses and estimate the effect of age on the obtained data.

Our results revealed that while maintenance of single information within WM remained stable across age groups in mice, dynamic manipulation of multiple information declined with age, mirroring deficits observed in aging humans. We also show that interindividual variability in WM performance, a hallmark of aging across species, is related to the balance between specific WM subprocesses. Furthermore, we found similar densities of VTA DA neuronal cell bodies in young and aged mice. This data goes against the hypothesis of overall DA degeneration as a neural correlate of age-related decline and suggests that alternative mechanisms, such as shifts in molecular profiles can be considered as a possible determinant of the healthy aging process. Additionally, our study puts in evidence the existence of compartmentalized DA and Glu varicosities in VTA mesocortical fibers, suggesting also the potential role of such neurons in WM regulation.

By bridging human and rodent studies, this research enhances our understanding of age-related cognitive decline, particularly in WM function, and further validates the mouse model of healthy aging. The identification of common mechanisms across species provides insights into potential therapeutic targets for preserving cognitive function during aging, with implications for the development of interventions aimed at mitigating age-related cognitive deficits. Furthermore, our findings highlight the importance of refining methodological approaches to accurately characterize age-related changes in both behavior and anatomy, contributing to the advancement of translational research in the field of cognitive aging.

Résumé

Le comportement dirigé par des objectifs repose sur des ajustements dynamiques basés sur les besoins internes et les contextes environnementaux. Ces comportements s'appuient sur des fonctions exécutives telles que la mémoire de travail (MT), cruciale pour la planification et la coordination du comportement. La MT est définie comme la capacité à stocker, maintenir et manipuler des informations pertinentes pour des actions motivées. Au cours du vieillissement sain, il y a un déclin progressif de la MT. Des études ont montré des altérations liées à l'âge dans les sous-processus de la MT, avec des déficits principalement observés dans les tâches nécessitant la manipulation de plusieurs éléments d'information au sein de la MT, et proposent que la neurodégénérescence des neurones dopaminergiques (DA) dans l'aire tegmentale ventrale (VTA) puisse entraîner de tels déficits. Alors que les déficits de la MT liés à l'âge sont bien documentés chez l'homme, leur traduction chez les modèles murins reste difficile, et il est difficile d'évaluer si les altérations liées à l'âge dans le circuit de la MT sont communes à toutes les espèces.

Cette thèse vise à caractériser davantage les altérations liées à l'âge dans les sous-processus de la MT dans le modèle murin, et à mettre en évidence les caractéristiques de déclin cognitif dans ce modèle. Plus spécifiquement, nous avons enquêté dans quelle mesure les déficits de la MT observés chez les souris âgées reflètent les résultats chez l'homme et exploré l'origine à laquelle de tels déficits pourraient être attribués.

Plus précisément, étant donné que le déclin de la MT lié à l'âge est associé à une diminution de la libération de DA, nous avons cherché à découvrir l'origine de cette diminution en examinant la densité des neurones DA dans le VTA.

Pour répondre à ces questions, nous avons utilisé des approches comportementales et anatomiques. Les évaluations comportementales impliquaient des tâches de MT spatiale qui testaient la capacité de maintien de la MT en variant l'effort cognitif requis pour encoder l'information et la charge cognitive nécessaire pour maintenir et manipuler plusieurs informations en ligne. Cela nous a permis d'évaluer les changements liés à l'âge dans les sous-processus de la MT chez les souris. Anatomiquement, nous avons étudié la densité des neurones DA du VTA en nous concentrant spécifiquement sur la densité des corps cellulaires. Nous avons utilisé l'immunohistochimie et la clarification sur cerveaux entiers ainsi que des injections stéréotaxiques combinées à l'immunohistochimie sur coupe pour identifier l'origine de la libération réduite de DA lors du vieillissement en quantifiant la densité des corps cellulaires de DA dans le VTA. Nous avons utilisé des méthodes statistiques bayésiennes pour affiner nos analyses et caractériser les effets de l'âge sur nos données.

Nos résultats ont révélé que, tandis que le maintien d'une seule information dans la MT est resté stable à travers les groupes d'âge chez les souris, la manipulation dynamique de plusieurs éléments d'information a diminué avec l'âge, reflétant les déficits observés chez les humains vieillissants. De plus, contrairement à l'hypothèse de la neurodégénérescence des neurones DA, nous avons trouvé des densités similaires de neurones DA chez les souris jeunes et âgées. Cela suggère que des mécanismes alternatifs, tels que des changements dans les profils moléculaires, impliquant potentiellement une régulation à la hausse de la libération de glutamate comme réponse neuroprotectrice, doivent également être considérés comme une conséquence du processus de vieillissement. De plus, notre étude met en évidence l'existence de varicosités DA et Glu compartimentées dans les fibres mésocorticales du VTA, suggérant également un rôle potentiel de ces neurones dans la régulation de la MT.

En reliant les études humaines et murines, cette thèse contribue à l'amélioration de notre compréhension du déclin cognitif lié à l'âge, en particulier dans la fonction de

la MT. L'identification de mécanismes communs entre les espèces fournit des informations sur les cibles thérapeutiques potentielles pour préserver la fonction cognitive lors du vieillissement, avec des implications pour le développement d'interventions visant à atténuer les déficits cognitifs liés à l'âge. De plus, nos résultats mettent en évidence l'importance de perfectionner les approches méthodologiques pour caractériser avec précision les changements liés à l'âge tant sur le plan comportemental que anatomique, contribuant ainsi à l'avancement de la recherche translationnelle dans le domaine du vieillissement cognitif.

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List of Abbreviations

| | |
|---------------|-----------------------------------|
| 3D | Three-dimension |
| AP | Anteroposterior |
| CI | Credibility interval |
| CTB | Choleratoxin-B |
| DA | Dopamine |
| DNMTP | Delay Non Match To Place |
| DV | Dorsoventral |
| GABA | γ -Aminobutyric acid |
| Glu | Glutamate |
| MCMC | Monte-Carlo Markov Chain |
| ML | Mediolateral |
| LC | Locus Coeruleus |
| LTM | Long-Term Memory |
| PFC | Prefrontal Cortex |
| ROPE | Region Of Practical Equivalence |
| vGluT2 | vesicular Glutamate Transporter 2 |
| vmPFC | Ventromedial Prefrontal Cortex |
| VTA | Ventral Tegmental Area |
| VTE | Vicarious-trial-and-error |
| TH | Tyrosine Hydroxylase |
| WM | Working Memory |

To Amma and Appa

CHAPTER 1

Introduction

The World Health Organization reported that life expectancy has increased by more than 6 years in the last two decades and reached on average 73.4 years in 2019. However, while healthy life expectancy has also increased by 8%, this was mainly due to a decline in mortality rather than an increase of years lived without disabilities. Improving the quality of life of older people is therefore a health issue for which the understanding of the biology of aging is primordial.

Aging is characterized by the progressive accumulations of alterations onto all biological levels, that together affect organ function, including the brain. Brain aging, and specifically cognitive aging, is the main leading cause to the development of age-related neurodegenerative diseases. Yet, though many effort are made in order to identify, and counteract, the critical factors underlying the development of such pathology, a lot still remains to be uncovered.

Amongst individuals, there is a high variability in the rate of cognitive aging and its associated cognitive decline. The origin of such variability is thought to be at the root of the transition from healthy to pathological symptoms of aging, and have been

a subject of multiple investigations on the characterization of hallmarks of cognitive aging.

Working memory is a critical function that participates in the dynamic integration of the environment in relation to internal motivation. Specifically, it acts as a mental buffer allowing the storage, maintenance and manipulation of information for immediate goal-directed behavior. Interestingly, the integrity and performance associated to working memory function follow an inverted U-shape function across the lifespan. Working memory is therefore suggested to be a primordial hallmark of cognitive aging.

Human working memory is characterized by three key subprocesses: encoding, maintenance and manipulation. During healthy aging, WM-dependent manipulation and storage of information are thought to be the most vulnerable to healthy aging, whereas WM maintenance of information remains unchanged. Age-related WM function is thought to be a consequence of an age-related decrease of dopamine levels in the prefrontal cortex. The prefrontal cortex is the main brain region responsible for WM function, where dopamine levels are associated to WM performance. Specifically, the rate of dopamine synthesis in the prefrontal cortex is correlated with WM performance in younger adults, and overall in older people, there is a decrease of dopamine synthesis in the prefrontal cortex. Thus, it is proposed that dopamine levels in the prefrontal cortex is also a hallmark of cognitive aging.

However, what is the origin of such decrease? A common hypothesis for such decline is the neurodegeneration of dopamine neurons, specifically in the ventral tegmental area. However, human studies are limited for the testing of such hypothesis and animal models provide an alternative for such a research. Yet, though there is an extensive field of research on working memory function overall, there is still a lack of consensus on how to define this function across human and animal models, especially in aging studies. Specifically, in rodents, the distinction between WM maintenance and WM manipulation is not clearly addressed, and confounding conclusions are found across the already sparse literature on age-related WM performance in wild-type rodents. Similarly, though multiple reports indicate a decrease of dopamine levels

in the prefrontal cortex across species, investigations on the origin of such decrease remain sparse and limited in their methodological approaches.

Therefore, this thesis aimed at providing a characterization of the effects of age on working memory and its underlying dopaminergic network in the mouse model.

1.1 Aging

Aging is an inherent process associated to life, regardless of how appreciated it is. Yet, a concept so close to us is far from being clearly conceptualized, let alone understood. Intuitively, we would define aging as a natural and very unavoidable process starting at the “beginning” of life and lasting until its end. In more scientific terms, we typically consider aging as a process starting from adulthood and characterized by a progressive decline of biological processes, ranging from the micro to macroscale. However, the latter happens to be not so accurate, and, to this day, no clear consensus was reached as to how to define aging, its implications and, above all, why such process should exist in the first place.

1.1.1 Why do we age ?

Aging is considered as an "evolutionary paradox" (Lidsky et al., 2022). Evolution relies on the conservation of beneficial traits within a population, i.e. natural selection. Yet, aging, a process overall associated to functional decline, is almost unequivocally found across all species ranging from bacteria, plants to mammals (McHugh & Burke, 2022). Therefore, remains the question, why do we age ?

To this day, though many theories have been postulated on the origin and significance of aging, this remains an ongoing debate, with two opposite point of views: non-programmed and programmed evolution of aging (Pamplona et al., 2023). In the former, it is proposed that aging may actually not have any evolutionary purposes (Rose et al., 2012; Cannon et al., 2015; Lipsky & King, 2015): it supposes aging to be "a random side effect without any biological function" (Pamplona et al., 2023). More specifically, non-programmed theories of aging suggest that up until adulthood and maturity, maintenance of optimal functions were primordial for reproduction and thus, transmission of life. However, what happens after this critical point was no longer relevant on an evolutionary perspective. Consequently, there was no associated meaning in selecting individuals based on their ability to age.

On the other hand, many support the idea of aging to be an evolutionary significant process, and thus argue for a programmed evolution of aging. Specifically, they suggest aging to be a predetermined process encoded in the genome of each species and is overall part of an adaptive process (Pamplona et al., 2023). For instance, the homogeneity of lifespan range in each species and the identification of longevity-related biological mechanisms such as telomere shortening (Libertini, 1988) or the accumulation of reactive oxygen species i.e. oxydative stress (Korshunov et al., 1997), would argue against non-programmed theories of aging.

Though it is difficult to identify on the phylogenetic tree, where age-related biological processes first emerged, it is clear that common longevity associated processes exist across species, including in mammals (Lidsky et al., 2022; Kowald & Kirkwood, 2016).

1.1.2 Biological aging

Over years of research, the identification of common processes underlying the biology of aging show that longevity is under the influence of a complex set of factors such as genetic, molecular, and environmental ones (López-Otín et al., 2013; Tartiere et al., 2024). Collectively, these factors are suggested to lead to biological alterations, most of which eventually result in age-related deteriorations of previously optimal functions (Gladyshev & Gladyshev, 2016). López-Otín and colleagues identified and described the hallmarks of aging, that regroup longevity-related mechanisms across all biological scales, which were regrouped under three categories: primary, antagonistic and integrative hallmarks (López-Otín et al., 2013; Tartiere et al., 2024). The primary group relate of "intrinsically negative" hallmarks found at the genomic level such as genomic instability, telomere attrition or epigenetic alterations. The antagonistic group regroups dose dependent effects at a cellular level i.e. protective in low amounts but detrimental when chronic or in high amounts. This includes metabolic dysregulation, mitochondrial dysfunction and cellular senescence. Finally, integrative hallmarks correspond to the alteration in tissue homeostasis such as stem cell exhaustion or altered intercellular communication. Eventually, the combination of all of the above is thought to underlie the biology of aging and its observable functional

repercussions. However, though the long-term accumulation of such alterations is thought to lead to the deterioration of an organism, the rate and intensity of their physiological deterioration varies across individuals: when comparing younger and older adults, the extent of differences can range from very subtle to large.

1.1.3 Healthy versus pathological aging

In aging studies, individuals are often characterized based on their chronological age to set a threshold between “young”, “middle-aged” and/or “older” adults. However, within the same age group, high heterogeneity is found amongst individuals, especially within older groups. It is thought that aging exacerbates interindividual differences that result in subtle physiological alterations (Hultsch et al., 2002; Bielak et al., 2014) part of what is thought to be part of a continuous aging process, or healthy aging (Gladyshev & Gladyshev, 2016).

Healthy aging, also referred to as *successful* aging in human studies, is defined by the absence of strong clinical symptoms that would hinder daily life and alter an individual’s autonomy. Specifically, healthy aging is associated to the absence of any diseases for which age is the prevalent factor (Rowe & Kahn, 1997; Depp & Jeste, 2009). In contrast, the emergence of age-related alterations that go beyond what is characterized as the healthy aging process, and that are overall associated with the development of age-related diseases is termed pathological aging. Pathological aging is therefore characterized by the development of clinical symptoms that strongly affect the quality of life of an older individual (ex: arthritis, Parkinson’s, osteoporosis, etc.). Overall, the line differentiating healthy from pathological aging remains unclear and the distinction between those two concepts are solely based on clinical observations. Thus remains the question: what are the hallmarks of **healthy** aging ?

Though age-related alterations can be found across all biological scales, it is unknown to what extent such alterations ultimately impact organ function, and more specifically, what critical factors contribute to the transition from healthy to pathological aging. In the case of age-related neurodegenerative diseases such as Alzheimer’s or

Parkinson's, diagnosis are made upon the appearance of clinical symptoms. A large effort is made in the development of therapeutics to alleviate clinical symptoms, but no preventive therapeutics were found to this day. As cognitive aging is the main prevalent factor for the development of such diseases, an important area of research is dedicated to the characterization of the biology underlying healthy cognitive aging with the aims of uncovering the critical factors that may underlie the transition from healthy to pathological cognitive aging.

1.1.4 Cognitive aging

Cognitive aging refers to age-related alterations in high-order mental processes that, for example, underlie learning, remembering or making a decision. Such alterations occur throughout the lifespan and may result in positive, or negative consequences on an individual's mental abilities. As mentioned earlier, the rate of healthy aging, including cognitive aging, is heterogeneous amongst individuals and is the subject of many interest: why do some individuals show earlier signs of cognitive decline than others?

In human psychology, cognitive processes are often subdivided in two categories: *crystallized* and *fluid* intelligence. The former underlie experience and knowledge based abilities. Multiple studies show that processes associated to *crystallized intelligence*, such as semantic memory (Jarjat et al., 2021) or general knowledge (Loaiza, 2024) improve with healthy aging. However, cognitive processes associated to *fluid intelligence*, such as processing speed or decision-making, are especially vulnerable to healthy aging (Hedden & Gabrielli, 2004). *Fluid intelligence* relies on cognitive flexibility and underlie rapid adaptation and integration of oneself to both its internal needs and external factors. Healthy cognitive aging is therefore for the most associated to a decline in such adaptive processes requiring the integration and processing of information *de novo*.

At the forefront of such processes lies executive functions, also referred to as cognitive control. Associated to *fluid intelligence*, executive functions participate in the

orchestration of goal-directed actions and regroup a collection of high-order processes such as attention, working memory, decision-making, lateral inhibition and cognitive flexibility. These collectively act as a mental buffer that allow the representation of an individual's present state that allow predictions of future actions in order to reach a specific target. In older individuals, performance in tasks requiring executive functioning is often predictive of the performance in other cognitive demanding tasks testing learning abilities or associative memory (Loaiza, 2024; Hedden & Gabrielli, 2004). Executive functions is therefore thought to represent a good indicator of the rate of cognitive aging and extent of age-related cognitive decline.

Amongst executive functions, working memory is the best candidate in the characterization of cognitive age across the lifespan and is considered as a hallmark of healthy cognitive aging. Working memory allows the maintenance and manipulation of a relevant information for immediate motivated behavior. It allows the maintenance of short-term associations and is at the basis of many cognitive functions such as learning. Working memory capacity is thought to evolve following an inverted U-shape function across the lifespan. Optimal working memory function peaks during early adulthood, then declines over the later years (Loaiza, 2004). Because of its central role in many cognitive processes, it is suggested that the detection and characterization of WM deterioration is a key candidate for the evaluation of the extent of age-related cognitive decline in individuals. Understanding what constitutes working memory, its underlying network and how such a system may be vulnerable to the aging process will therefore provide valuable insights on the critical factors involved in the transition from healthy to pathological aging (Loaiza, 2004; Hedden & Gabrielli, 2004).

1.2 Goal-directed behavior & executive functions

Executive functions and WM operate within the realm of a particular set of behavior called goal-directed behavior. They are at the forefront of adapting one's self to its environment and represent a particular process distinct from other types of behavior such as habits or reflexes.

1.2.1 What are goal-directed behaviors?

Goal-directed behavior represent the recruitment of a set of fundamental processes that allow for an individual to set and adapt itself to reach a specific purpose. This allows us to, for example, keep in mind our shopping list for tonight's dinner, or reach a water bottle when we are thirsty. Based on both internal states and environmental stimuli, goal-directed behaviors are driven by specific objectives or desired outcomes and rely on the ability to set, plan, execute and monitor actions towards a pre-defined target (Allen et al., 2017). According to the associated dual-process theory, goal-directed actions are distinct from habits as the latter are triggered by an external stimuli and not sensitive to outcomes, unlike goal-directed actions (Bouton, 2021). The planning and execution of goal-directed actions are therefore not only dependent on executive functions but also on motivation for the attribution of values to both strategies and outcomes (predicted as well as real).

1.2.1.1 Setting a goal: the role of motivation

Motivation is the main driver of goal-directed behavior as it allows the setting of an objective. It is dependent of both external (context) and internal factors (valence and salience) (Kim, 2013; Mannella et al., 2013). Valence represents the value of a goal, which can either be positive or negative, and respectively lead to an approach or aversive behavior. Salience, on the other hand, represents the importance and relevance of the goal. It implies a ranking of different goals on a "priority spectrum" ranging from neutral to urgent that are based on the individual's internal state. Internal and external stimuli, goal-directed actions and their predicted outcomes are all attached to salience and valence factors (Pezzulo et al., 2014).

To illustrate these concepts, let's imagine a squared box in which a mouse is placed. In one corner of the box, a nutritious and mouse-friendly bowl of food is placed (positive valence). When the mouse is in the box, its behavior will depend on both its inner state and needs along with the environment. If the mouse is hungry, its behavior will more likely be directed towards the food's location (positive salience) to fulfill its priority objective: satisfying its hunger (approach motivation). However, if the mouse is in a satiated state, though the food will still be associated to a positive valence, the need to satisfy its hunger is no longer present (neutral salience). Accordingly, the mouse's behavior is unlikely to be specifically directed towards the food's location (no motivation).

Now, let's imagine that the mouse receives an electrical shock each time it touches the foodbowl. In this case, though the food is still attached with positive valence, the food bowl on the other hand is associated to pain (negative valence + positive salience). When satiated, the mouse is therefore more likely to adapt its behavior in order to actively avoid the foodbowl (aversive motivation). However, if the mouse is hungry (positive salience), then the positive valence of the food relative to the negative valence of the food bowl will *in fine* be stronger. This will entail the mouse to still adapt its behavior to direct itself towards the food's location, despite the associated pain (salience of positive valence > salience of negative valence: approach motivation).

Motivation therefore relies on the dynamic integration of internal needs and states (for example, hunger) and external inputs (for example, food) to drive and adjust behavior.

1.2.1.2 Planning & monitoring: the role of executive functions

Planning goal-directed actions underlies the ability to project oneself in the future. This is thought to be dependent on (1) the maintenance of associations between a set of actions and their predicted outcomes and (2) the choosing of a relevant set of actions to reach the goal (Mannella et al., 2013). Executive functions play a critical role in such aspect of goal-directed behavior (Ott & Nieder, 2019) and allow to direct

behavior based on the simultaneous and dynamic integration of the environment and the present internal state (Zanto & Gazzaley, 2019). Roughly, attention and inhibitory control are thought to allow focus on relevant external or internal stimuli by enhancing their signaling. WM allows the online maintenance of such information and their processing. Decision-making processes enable the selection of imminent action to be orchestrated and executed. Finally, cognitive flexibility is the adaptability and dynamic switching of decision-making processes based on the context (Logue & Gould, 2014; Zanto & Gazzaley, 2019).

Executive functions also oversee the execution of planned actions. They are involved in the detection of errors and conflict between the planned behavior, its execution and its outcomes, so that the system constantly evaluates the ongoing behavior against the intended goals (Botvinick et al., 2001). This enables, when needed, the optimization of performance and the maintenance of an adapted strategy to reach the set goal.

1.2.1.3 Towards habit formation: Optimization and automaticity

Due to the uncertainty of the initial outcome prediction, often, the initial strategy selected to reach a goal may not always be the most effective (Monosov, 2020; Hennig et al., 2021). To counteract this situation, traces of actions and their outcomes are thought to be recorded in long-term memory, and to be later retrieved when a similar context arises again (Kesner et al., 2022; O’Doherty et al., 2017). This retrieval allows the fine tuning and strategic adaptation of behavior while considering their history. The repetition-based tuning of such action sequences enable the optimization of behavior to reach a goal and underlies a transfer of causal sequences from short to long-term memory. The involvement of long-term memory is therefore thought to participate in the strengthening or devaluation of a sequence of actions based on its recorded efficiency and enable the fine tuning of behavior to reach the desired outcome (Kesner et al., 2022).

If individuals repeatedly execute a sequence of goal-directed actions and the uncertainty of the predicted outcomes decreases (outcome devaluation), the recorded efficiency of the selected strategy increases which will allow the system to establish more

accurate and reliable predictions of the action-outcome-response sequences (Ashby et al., 2010). This phenomenon is called automaticity (McCloskey & Johnson, 2019). The resulting consolidation of motor patterns participate in the promotion of the efficiency in action execution, along with the increased predictability of the outcome, outcome sensitive goal-directed action are thought to shift into to outcome independent habit (Balleine & Doherty, 2010; Gremel & Costa, 2014).

1.2.2 Circuitry of goal-directed behavior

Goal-directed actions rely on a complex neural circuit allowing a dynamic integration of internal and external information. They are most dependent on the integrity of frontal cortices and the striatum in the basal ganglia. Along with others, these two regions are interconnected through the mesocorticolimbic pathway involving the midbrain (Ventral tegmental area (VTA)), basal ganglia (striatum), limbic structures (amygdala) and cortex (hippocampus, prefrontal cortex (PFC)) (Fig.1.1) (Krashia et

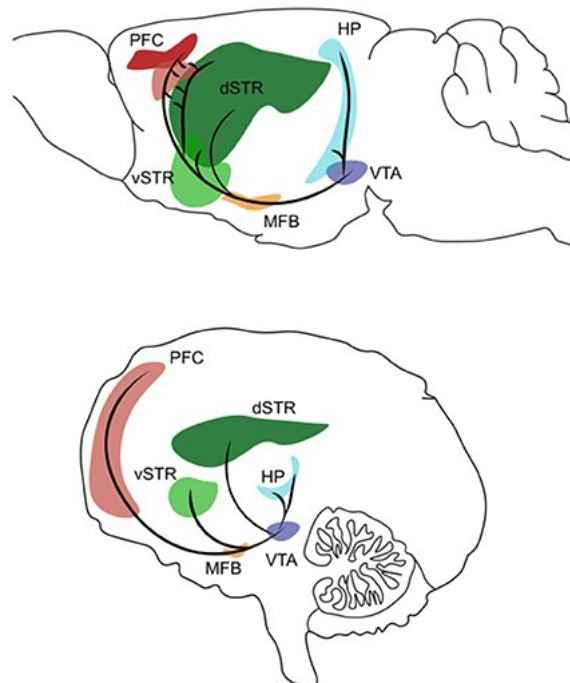


Figure 1.1: Mesocorticolimbic networks in mice (top) and humans (bottom). PFC: Prefrontal Cortex, dSTR: Dorsal Striatum, vSTR: Ventral Striatum, HP: Hippocampus, VTA: Ventral Tegmental Area, MFB: Medial Forebrain Bundle.

Adpated from Krashia et al., 2022

al., 2022). Mesolimbic connectivity is thought to be involved in the processing and encoding of motivational factors while cortical structures are thought to integrate such factors for the planning and monitoring of goal-directed actions (Mannella et al., 2013; Kesner et al., 2022).

Specifically, the amygdala is thought to participate in the encoding of the valence of an external stimulus along with the striatum, where outcome valence are also encoded (Smith & Torregrossa, 2021; Ray et al., 2022; Balleine et al., 2007; Kang et al., 2021). On the other hand, frontal cortices such as the prefrontal cortex and the anterior cingulate cortex, are thought to participate in the encoding of salience, where the possible strategies are elicited along with both their predicted and updated outcomes in real time (Miller & Cohen, 2001; Howland et al., 2022; Kesner et al., 2022; Manella et al., 2013; Andersen & Cui, 2009). Finally the hippocampus is thought to participate in the encoding and retrieval of goal-related memories by providing contextual information to salient stimuli (Kesner et al., 2022; Wikenheiser et al., 2016).

1.3 Working memory

Let's imagine a bird fishing. When hungry, the bird hovers over the sea in the hopes of catching a fish. When it catches the shadow of a fish swimming close to the surface, then it will most likely adapt its trajectory in order to reach the fish's location. Such adaptation relies on the ability of the bird to maintain the location of the fish when it was first seen, but also to manipulate this information to compute where it will be by the time it reaches the sea. This simple, yet not so simple process, is one of the many example in which working memory function is vital as it allows individuals to temporally store relevant information to process and adapt behavior towards a specific goal.

Both because of its importance and its underlying complexity, working memory (WM) has for long been a subject of interest. In humans, WM is considered to actually be at the base of *fluid intelligence* as it is a necessary function that underlie the abilities to solve new problems and think flexibly without depending on previously acquired knowledge (Carruthers, 2013). Overall, working memory occupies a fundamental role in the planning, organization, initiation and monitoring of goal-directed actions, and its progressive deterioration in elder populations therefore proves to be not only a strong inconvenience in their daily lives, but also precursory of the deterioration of other functions such as learning or reasoning.

1.3.1 Conceptual models in human cognition

The term 'working memory' first emerged in computer science, where scientists used this term to refer to structures within their programs that temporarily held information (Newell & Simon, 1956). Few years later, the same term was applied to describe, in human cognition, the storage of relevant information needed to execute planned actions (Miller et al., 1960). From the 60's, pioneer works allowed the emergence of multiple conceptual theories of WM function.

The earliest model was Atkinson & Shiffrin's "modal model" (Atkinson & Shiffrin, 1968) (Fig. 1.2). Here, WM is considered as an aspect of short-term memory where

short-term memory is represented as a single mechanism that allows the temporary holding of information until its use for further processing. In this model, the authors suggested that most of the incoming sensory information were ‘forgotten’ and only a small portion of them remained in short-term memory *i.e.* WM. Such information was suggested to be emphasized on through coordination with long-term memory (LTM) which allowed their retention. Therefore, WM was proposed to participate in the initiation of new LTM formation thanks to a control process that favored strategic information recirculation, and proposed as a transfer mechanism from short-term to LTM.

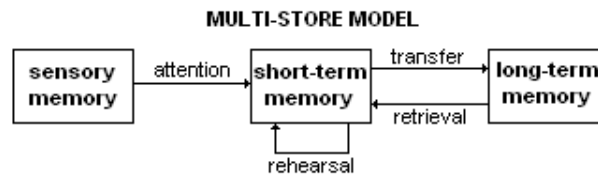


Figure 1.2: The Modal (or multistore) model. Atkinson and Shiffrin’s (1968) original model of memory, consisting of the sensory register, short-term store, and long-term store.

However, soon WM was characterized as a particular and distinct form of short-term memory, and based on the ‘modal’ model, Baddeley and Hitch proposed the ‘multicomponent model’ (Baddeley and Hitch, 1974; Baddeley, 2000; Repovš & Baddeley, 2006) (Fig. 1.3). Here, WM was divided into three specific stores that allowed both the storage and processing of relevant information: the phonological store (for verbal information), the visuospatial store (for visual and spatial information) and the central executive store (for information transfer from one store to the other). A LTM dependent episodic buffer was also proposed to monitor the aforementioned stores which was thought to enable complex representations of associations, including semantic information.

As in the ‘modal model’, the episodic buffer is suggested to interpret sensory information thanks to LTM. This allows the scheduling and prioritizing of information transfers for dynamic behavioral adaptation, as well as prevent from information decay through a rehearsal process. Therefore, short-term memory was considered as distinct from working memory, as the former implied the storing and repetition of

unprocessed and *unmanipulated* information whereas the latter was characterized as the *manipulation* of stored information (Adams et al., 2018; Chai et al., 2018).

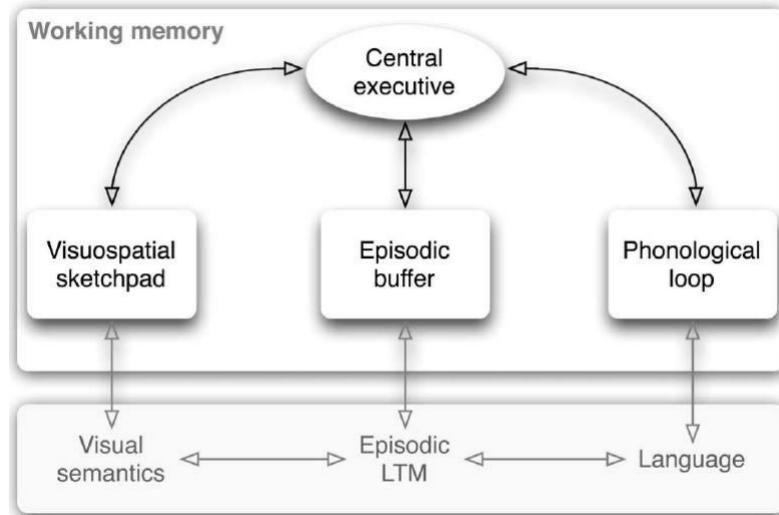


Figure 1.3: The multicomponent model. Baddeley’s (2006) current model of WM, representing the three stores and the central executive. WM components (top) are proposed to interact with LTM components (bottom) for the representation of experience-based (episodic LTM) associations and semantic information (visual semantics, language). LTM: Long-Term Memory
From Repovš & Baddeley, 2006.

As opposed to the specific and distinct stores suggested in Baddeley’s model, Cowan proposed another influential model: the embedded-process WM model (Cowan, 1988; Cowan, 1999; Cowan, 2001) (Fig. 1.4). Here, information is thought to first go through a brief sensory store that will activate features stored in LTM, that are more

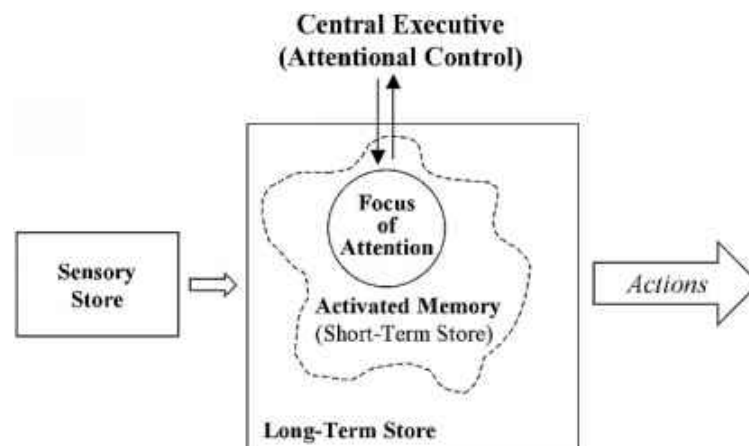


Figure 1.4: The embedded-process model. Simplified diagram of Cowan’s embedded-process model.
From Morrison, 2005.

or less complex *e.g.* phonological, orthographic, visual, etc. In spite of having ‘only’ a phonological and a visuospatial store as in the multicomponent model, here it is assumed that each feature represents a store, from which the limits are blurry *e.g.* some are basic and niche while other may be overlapping. Thus, instead of separate and distinct stores, here it is suggested that new inputs can either overwrite or interfere with previously activated information based on the extent of shared features. Similar to Baddeley’s model, such information is thought to decay when not rehearsed. To prevent information decay, attentional processes play a key role in this model, where they are thought to drive WM function by selecting and shifting focus on the information considered as relevant, to be stored within WM and therefore necessitating refreshing (Adams et al., 2018; Chai et al., 2018).

Nowadays, Baddeley & Hitch’s and Cowan’s models are the most referred to in the understanding of how WM functions, especially in human cognition (Morrison, 2005; Adams et al., 2018; Chai et al., 2018). Though the specifics of these models are still under debate, they share similar grounds on the definition of WM. More specifically, they rely on the subdivision of WM based on three subprocesses : the encoding of information through the selective integration of sensory inputs that are tuned through LTM (*i.e.* selective encoding), the temporary holding of such information in a capacity limited short-term store (*i.e.* maintenance) and their updating (*i.e.* manipulation) that will allow the processing of such information upon which behavior will be adapted. Overall, this dynamic system relies on a fine balance between stability and flexibility of the encoded information, since they are sensitive to interference and temporal decay: the less an information is “associated” (*i.e.* used) and the less it is integrated or maintained (*i.e.* ultimately forgotten) (Buschman, 2021).

However, these models fail to converge on how sensitive this process is to the type of information being stored. The time scales on which these associations and manipulations operate also remain to be clearly defined. Though WM is thought to be engaged on a short time scale for ‘quick on the spot decisions’, the actual span in which such is the case *e.g.* a few seconds, minutes or hours, remains to be clearly defined. More importantly, these conceptual models are also based on the characteristics of human

cognition: for example, it is not possible to refer to a phonological loop (as in the multimodal model) when referring to animal models such as rodents.

Human WM capacity is thought to be the most developed across species. Still, the shared homology between humans and non-humans primates allowed the upbringing of pioneer works that, among others, contributed to the identification of the role of the prefrontal cortex in WM and its implication in WM maintenance (Goldman-Rakic, 1995). Yet, though other animal models, such as rodents, possess a more limited WM capacity, they can also provide valuable information on the understanding the functional network underlying WM. To do so, it is essential to characterize and provide a clear definition of what is considered as WM in rodent studies.

1.3.2 Working memory in rodent cognition

Outside of primates, the term ‘working memory’ was first used in the context of animal cognition in the 70’s by Olton & Honig (Olton & Samuelson, 1976; Honig, 1978). In Olton’s study, rats were tested in an 8-arm radial arm maze (Fig. 1.5). To motivate rats, they were food-restricted and placed in the maze where all arms were baited with a food reward. Olton observed that all rats entered on average 7 arms out of 8 in one go before a revisit. Based on this above chance-level performance, Olton concluded on the existence of a memory that allowed rats to remember their visits along the maze in order to optimally retrieve a maximum of baits within a session. He defined this temporary holding of spatial representations as ‘spatial memory’ (Olton & Samuelson, 1976).

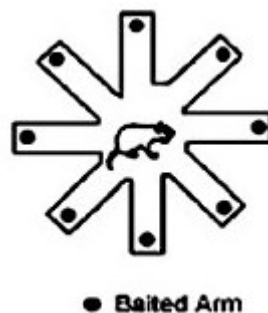


Figure 1.5: Olton’s radial arm maze task.
Adapted from Espina-Marchant et al., 2006

Honig, on the other hand, tested pigeons in an operant delayed-matching task. Pigeons were faced with a flickering light and rewarded only when they pecked on the correct light, after a short delay (seconds). Honig observed that with longer delays, the performance of animals decreased. He therefore concluded that animals were able to represent a cue over a delay period even though the cue is no longer present. He characterized this ability as WM. Based on his studies, Honig made a clear distinction between WM and another type of memory called reference memory (Honig, 1978; Dudchenko, 2004).

Reference memory is defined as the long-term and stable association between stimuli or between a stimulus and a response (Dudchenko, 2004). For example, it is the type of memory that allows the memorization of a rule based on which a stimuli will be associated to a response (*e.g.* "I press a doorbell so it will ring"). WM on the other hand is defined by the transient associations of stimuli or stimulus-response (*e.g.* "I have to temporarily remember this sequence of numbers to be able to note it"), which makes it particularly sensitive to interference. To minimize reference memory like biases, in most animal studies, the time span in which WM is referred to was considered to be based on the ability to solve a task, based only on the information gathered within the scope of a single trial, that will no longer be relevant in subsequent trials (Dudchenko, 2004). Based on this definition, delay-dependent memory testing tasks were, and still are, the most popular paradigms to test WM in animal studies.

Still, the limits bounding working from reference memory are not so clear and difficult to avoid. It was suggested that animals, within a session, were able to discriminate between information gained on a recent trial as opposed to information gained on other trials (Morris, 2002). The former may then be used to guide behavior, as opposed to only information gained from the present trial. This point of view puts into perspective that the time span in which WM is effective may not qualitatively differ from LTM, and especially reference memory (Dudchenko, 2004). Thus, the span in which WM is suggested to be actually effective was considered not to be time dependent *per se* but dependent on how long the gathered information was useful *i.e.* based on the salience of the to-be-remembered stimuli and their underlying

associations (Dudchenko, 2004). The initial WM storage of information was therefore considered to be based on both the importance (*i.e.* saliency) of the information and the repetition of the stimulus, that conditioned the transfer to LTM processes, as suggested in the previously described WM models (Fig. 1.3; Fig. 1.4).

The degree in which WM use can be dissociated from reference memory is essential in the specific study of WM function in animal models and requires to be thoroughly controlled and taken into account. Indeed, it is difficult to control the extent to which LTM storage is involved, especially when encoding takes place over multiple consecutive trials and retention intervals are long (*i.e.* more than a minute). With an increased length of the delay or number of items to be remembered, some suggest that temporary forms of consolidation outside of the predefined WM range may become involved to sustain performance (Castner, Goldman-Rakic & Williams, 2004). In this thesis, we will consider as WM, short-term computations that are based only on information available within the scope of one single trial that ranges over a couple of minutes.

1.4 A central hub for working memory: the prefrontal cortex

The prefrontal cortex is the main brain region responsible for WM function. Though it is the most developed in region in primates, the existence of this region in rodent models was for long debated. Nowadays, there is a consensus on the existence of such a region in rodents, which is considered to be functionally homologous to the one found in primate brains.

1.4.1 Anatomo-functional subdivisions

Since the 19th century, the importance of frontal lobes in cognition was well-known and the word ‘prefrontal’ was recorded for the first time in the later decades of that century (Ferrier & Yeo, 1884). In the following years, Brodmann’s pioneer work on the cytoarchitecture of mammal brains lead to a first anatomical delimitation of the PFC in primates. Overall, the cortex is characterized by its organization in layers ranging from I to VI. In the PFC, the presence of a well-defined granular layer IV was characteristic only to humans and non-human primates, as opposed to other mammals, including rodents (Carlèn, 2017). Therefore, it was considered that the prefrontal region was specific to primates, as frontal cortices in other mammals lacked of the presence of a layer IV thus being agranular.

In the late 40’s, Rose & Woolsey proposed an additional connectivity-based characteristic of PFC: its interconnectivity with medio-dorsal thalamic nuclei (Rose & Woolsey, 1948). Based on this extended definition of PFC, the existence of a structural homology across species was put forth. Specifically, in rodents, though agranular, frontal regions were proved to also be interconnected with the mediodorsal thalamus. This prompted the use of the term ‘prefrontal’ to characterize, also in rodents, the most anterior region of frontal cortices (Carlèn, 2017).

Still, what constitutes the PFC remains under vivid debate (Carlèn, 2017; Le Merre et al., 2021; Laubach et al., 2018). In humans and non-human primates, PFC is

often subdivided in dorsolateral, dorsomedial, ventromedial and orbital regions (Fig. 1.6). The dorsolateral region is thought to be an evolutionary specificity of primates because of its interconnectivity with the mediodorsal thalamus, which is not found in other species, including rodents (Carl en, 2017; Ott & Nieder, 2019). These delimitations are, for the most, based on cytoarchitectural proprieties. However, in the last decades, studies on rodents suggest that such anatomical parcellation may not be relevant on a biological level (Carl en, 2017; Le Merre et al., 2021). Specifically, overall cytoarchitecture, connectivity, function and topography are proposed to be the main criteria to be used for an accurate and relevant subdivision of PFC.

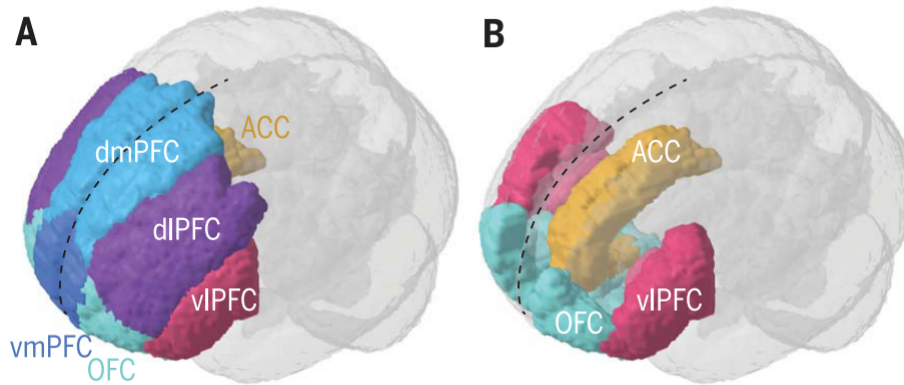


Figure 1.6: Functional division of the human prefrontal cortex. Dashed black line indicates sagittal midline. dmPFC: dorsomedial prefrontal cortex; vmPFC: ventromedial prefrontal cortex; vIPFC: ventrolateral prefrontal cortex; OFC: orbital frontal cortex.
From Carl en, 2017

Indeed, pure anatomical or functional cortical divisions are limited and may *in fine* be too artificial to portray any accurate biological significance (Le Merre et al., 2021). Recently, a connectivity analysis conducted on rodent cortices, proposed a simplified division of the cortex in ‘modules’ (Harris et al., 2019). A module was characterized based on (i) the density of the corticocortical interconnectivity and (ii) their participation in similar functions. In this study, six modules were defined, including a prefrontal module. The latter regroups all historical PFC subdivisions *i.e.* the pre- limbic area, infralimbic area, anterior cingulate areas and orbital areas (Le Merre et al., 2021).

Within the prefrontal module, Le Merre and colleagues suggest a parcellation of the mouse prefrontal module based on anatomy, functionality, connectivity and topography criteria: a dorsomedial PFC that encompasses the orbital area and anterior cingulate area; a ventromedial PFC (vmPFC) that comprises the prelimbic, infralimbic, and medial orbital area; and a ventrolateral PFC that comprises the ventrolateral orbital area and lateral orbital area (Fig. 1.7) (Le Merre et al., 2021).

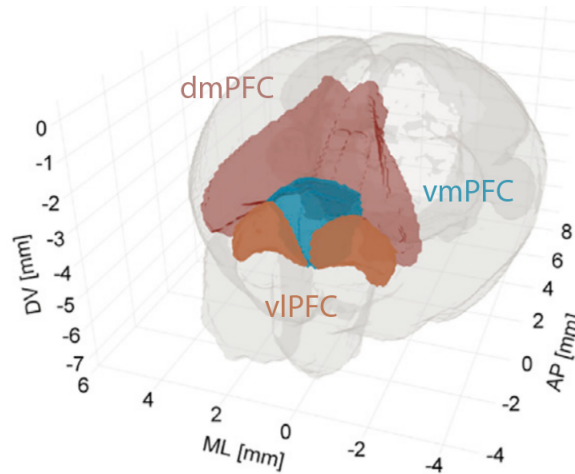


Figure 1.7: Mouse prefrontal subdivisions. dmPFC: dorsomedial prefrontal cortex; vmPFC: ventromedial prefrontal cortex; vlPFC: ventrolateral prefrontal cortex.

Adapted from Le Merre et al., 2021

1.4.2 Importance of prefrontal integrity for working memory

PFC integrity is thought to be primordial in the accurate depiction of an individual's present state and its predictions of future states. Prefrontal activity reflects motive, timescales and plans which are thought to collectively enable the emergence of complex representations that allow the coordination of goal-directed actions (Le Merre et al., 2021; Carlèn, 2017; Fuster, 2015; Miller and Cohen, 2001). Overall, the core function of the PFC is thought to be its contribution, and more specifically, its facilitation of dynamic behavioral adaptations in response to the environment.

vmPFC is thought to be especially involved, among others, in tasks involving delays and contextual representations, including WM. The vmPFC is characterized by its dense connectivity with the limbic structures, the striatum and the VTA, and is

thought to have a key role in the assessment of internally generated information (*e.g.* emotions and memories) (Euston et al., 2012; Le Merre et al., 2021). Therefore, this region is thought to be especially important in executive functions, and goal-directed behavior overall, as it is thought to guide an animal's current and future choices through the generation of internal goals and action sequences.

The integrity of vmPFC is especially important for WM. Pioneer work found the existence of prefrontal neurons encoding for delay in non-human primate dorsolateral PFC, which were also later found in rodent vmPFC (Fig. 2.2) (Goldman-Rakic, 1995; Fuster, 1973). vmPFC is therefore essential for the stable maintenance of an encoded information within WM (Le Merre et al., 2021; Laubach et al., 2018; Abbas et al., 2018). Though historically the relationship between delay WM and vmPFC were the most focused on, recent studies also show that overall PFC participate in both the encoding and retrieval of information during WM use (Vogel et al., 2022).

1.4.3 Dopamine modulation of prefrontal networks for working memory

The dynamic coding of WM within the PFC is thought to be regulated by prefrontal dopamine release. DA is a catecholamine synthesized in DAergic cells located in the midbrain, namely in VTA, the substantia nigra and the locus coeruleus (LC) (Morales & Margolis, 2017; Takeuchi et al., 2016). In the mesostriatal pathway (reward system) and nigrostriatal pathway (motor), DA acts both as a neurotransmitter and a neuromodulator. However, in the cortex, DA acts only as a neuromodulator and participates in the tuning of cortical networks (Ott & Nieder, 2019), including prefrontal ones.

Two families of DA receptors are found in the cortex: D1-like and D2-like receptors (Seamans & Yang, 2004; Beaulieu & Gainetdinov, 2011). The D1-like receptor (D1R) family regroups D1 and D5 receptors, that are abundant on post-synaptic membranes. On the other hand, D2-like receptors (D2R), namely D2, D3 and D4, are very sparse and found both on pre- and post-synaptic membranes.

High densities of DA D1 receptors are found in high-order cognitive regions including the PFC (Froudust-Walsh et al., 2021). D1R is known to participate in WM and allow the filtering of irrelevant stimuli (Gallo et al., 2022). Specifically, prefrontal DA transients are thought to provide a gating signal that allow the updating and storing of relevant mnesic traces within WM and participate in the reduction of variability in neuronal responses (Ott & Nieder, 2019). DA release is also thought to participate in the maintenance and manipulation of stored mnesic traces within WM as D1R activation follows an inverted-U shape function where sub- or supra-optimal activation is associated to increased WM failures in delay-dependent tasks (Fig. 1.8) (Seamans & Yang, 2004; Vijayraghavan et al., 2007).

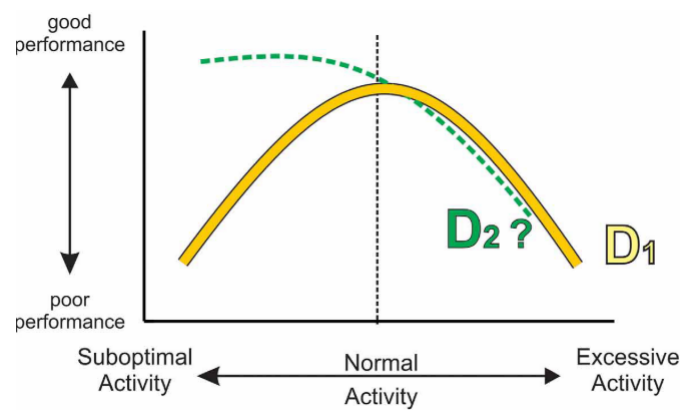


Figure 1.8: Inverted U-shape activation of D1R.

Adapted from Floresco, 2013

Prefrontal DA therefore contributes to cognitive flexibility and enable the short- and long-term modulation of synaptic plasticity within the PFC (Young & Yang, 2005; Otani et al., 2015). Its controlled local release participates in the fine tuning of WM-related networks and is specifically proposed to condition the filtering, holding or processing of an information within WM (Ott & Nieder, 2019).

1.5 Working memory, dopamine and healthy aging

In humans, age-related WM decline is associated to a decreased WM capacity and flexibility. Specifically, though the ability to hold an information during simple delays is not affected by aging, the flexible manipulation of complex information is thought to be the most vulnerable to healthy aging. The age-related impairment of this particular subprocess is thought to be main driver of age-related decrease of WM capacity in humans. Neuroimaging studies suggest that age-related WM decline is associated to reduced frontoparietal activity in older individuals (Zanto & Gazzaley, 2019). The frontoparietal activity includes activity from the prefrontal cortex, which suggests that its activity decreases with age and underlie the decrease of WM capacity. Similarly, heterogeneity in older individuals WM capacity is also associated to alterations in the prefrontal dopaminergic system (Bäckman et al., 2006; Salami et al., 2018). Altogether, it is commonly hypothesized that alterations of the prefrontal DAergic system underlies age-related WM decline.

Animal studies provide further evidence for the existence of age-related alterations of prefrontal connectivity and its underlying dopaminergic system. Studies on both non-human primates and rodents suggest a decrease in prefrontal connectivity and reduced prefrontal dopamine (DA) levels (Mizoguchi et al., 2009; Ota et al., 2006; Segovia et al., 2007; Bäckman et al., 2006) such as alterations of the intrinsic electrophysiological properties of neurons in the PFC and the midbrain (Rizzo et al., 2015; Kabaso et al., 2009; Branch et al., 2014), alterations in the morphology of prefrontal pyramidal neurons such as a reduced dendritic arborization (Bloss et al., 2013) and alterations in spine density (Dickstein, et al., 2013; Bories et al., 2013; Duan et al., 2003), alterations of the distribution of receptors in synaptic terminals (Bañuelos, et al., 2014), reduced prefrontal DA levels (Mizoguchi et al., 2009), and reduced prefrontal DA-related fibers (Chisholm et al., 2013). A decrease of local prefrontal DA release may therefore be at the origin of alteration in prefrontal connectivity and its resulting age-related WM decline. Yet the origin of such age-related decrease of prefrontal DA release remains unclear. The most popular hypothesis for such a decrease is the neurodegeneration of DA releasing neurons in the VTA, also referred to as mesocortical neurons. Such

hypothesis is supported by the increased high vulnerability to neurodegeneration of dopamine neurons, as dopamine is related to oxidative stress (Bäckman et al., 2006), which has been proposed to be one of the driver of aging (Pamplona, 2024). However, whether reduced DA release is due to increased age-related neurodegeneration of DA neurons remains difficult to test in humans.

Furthermore, a clear parallel between human and animal studies, in particular rodent studies, remains to be clarified. In older humans, it is clear that age-related WM decline is driven by the lack of cognitive flexibility rather than an inability to hold an information online. This is further evident as task complexity is increased. Yet, whether such is the case in rodents remains difficult to assess as the distinction between WM subprocesses is often overlooked and therefore rarely stated in rodent studies.

1.6 Rationale of thesis

Due to the discrepancies on how WM is defined in human and rodent models, especially in studies on healthy aging, we observed a gap in the translation and characterization of WM function in the mouse model. We therefore aimed at characterizing the effects of healthy aging on WM subprocesses in the mouse model, as described in human studies. Specifically, we hypothesized that age-related alterations of WM subprocesses are homologous between human and mice where, flexibility-related WM subprocesses are the main driver of age-related WM deficit. Specifically, we expected that the maintenance of information within WM was not vulnerable to aging whereas manipulation within WM is the most affected in older individuals.

If such an homology is observed, then, as DA is an essential component of optimal WM function and proposed to be hallmark of age-related WM decline, we further investigated the underlying effect of healthy aging on the DAergic system. Specifically, if aging is associated to a decrease in prefrontal dopamine levels, then at what level is this diminution rooted? As mentioned earlier, it is often assumed that such decline is related to neurodegeneration of the DA cells in the ventral tegmental area, yet very few studies attempted to provide evidence for or against this hypothesis. Two studies conducted in mice imply that there may be an age-related decrease of DA neurons in VTA, however, the methodological tools used are not sensible enough to provide clear arguments for or against the neurodegeneration hypothesis (Gür et al., 2020; Noda et al., 2020).

Indeed, these rely on the overall quantification of tyrosine-hydroxylase (TH) staining, a common dopaminergic marker. Yet, though the expression of TH is reduced within the VTA of older adults, it is impossible to conclude from these studies whether such decrease is due to a reduction of dopamine neurons, reduced DA fibers issued from VTA neurons or outside of VTA (such as the substantia nigra or locus coeruleus), or both. Additionally, TH is not a marker specific to DA only and is also known to label noradrenaline, which is known to be released in VTA from the locus coeruleus. More importantly, recent studies suggest that DA neurons are able to shift in their molecular

identity towards a co-expression of glutamate (Glu), as a form of a neuroprotective mechanism, following toxic lesions. Given the hypothesized toxicity of DA within DA cells and its relation to oxidative stress, another possibility is that a decrease of TH expression is not solely due to neuronal degeneration, but also symptomatic of a shift in molecular expression of VTA DA neurons in older individuals. Therefore, we aimed at characterizing the density of dopaminergic neurons in the VTA of aged mice and hypothesized that the origin of reduced TH expression in VTA was not due to a neurodegeneration of DA cells *per se*, but due to a shift in the molecular expression of DA neurons towards co-expression of Glu.

Therefore, in this thesis, we aimed at confirming in the mouse model the extent of age-related cognitive decline in WM subprocesses, and investigate the origin of decreased DA release in the prefrontal cortex, by characterizing the age-related density of DA neurons in the VTA of aged mice.

1.7 Methodological approach

1.7.1 Study 1: Behavioral characterization of WM subprocesses in the aged mouse model

To investigate whether aging equally affects all WM subprocesses in the mouse model, or whether the mechanisms underlying the maintenance of an information remains unimpaired during healthy aging, we tested mice in a battery of behavioral tasks challenging the maintenance aspect of WM with increased task difficulty. Specifically, we tested how the encoding effort (*i.e.* how precisely an information needs to be encoded) and the cognitive load (*i.e.* how much information needs to be encoded) required to maintain information within WM is impacted in adult and aged mice. As the lifespan of mice is on average two years, we compared the performance of adult (2 months) and aged (18-20months old) mice, which could be considered as the equivalent of young adult (≈ 20 years) and "young old" (≈ 60 years) humans.

In addition to cognitive performances, we also used a pose estimation based tracking system to detect vicarious-trial-and-error (VTE)-like behavior. VTE is a behavioral correlate of decision making and described as a horizontal scanning behavior associated to deliberation (Redish, 2016). It is an important behavioral marker of cognition, however, manual recording of such marker can be tedious and time consuming. With the emergence of deep learning tools, such as Deeplabcut (Mathis et al., 2018) that allow more reproducible and efficient recognition of behavioral signatures, we used to our advantage this method to detect VTE-like behavior, and test its relation to the observed cognitive performances.

1.7.2 Study 2: Anatomical characterization of WM-related dopaminergic neurons in the mouse model

In previous studies, the quantification of VTA DA neurons was based on the overall density of pixels associated to the label of interest in predefined sample of VTA (whole VTA slice, or region of interest). The product would then be averaged over mice and used as data for statistical testing. Though useful, the obtained data can

be limited in its interpretation, especially in the context of aging as no studies distinguished soma from fiber labelling. Therefore, the quantification takes into account labelling related to DA soma within VTA, input or output DA fibers within VTA and input noradrenergic fibers from locus coeruleus. To solve this issue, here we isolated and quantified only the labelling related to the density of DA neurons in VTA by segmenting only DA soma from 3D reconstructed images issued from whole-brain immunostaining and clearing approaches. To do so, we developed a semi-automatic approach to segment DA soma within the VTA of adult and aged mice which allowed us to quantify the density of DA cell bodies within VTA. Then, to test the existence of an age-related shift in the molecular profile of DA neurons within VTA, namely with an upregulation of the Glu component, we coupled stereotaxic injections of a retrograde tracer within PFC to specifically label mesocortical neurons in VTA, with triple immunostainings to label DA, Glu and DA/Glu co-releasing neurons in adult and aged mice.

1.7.3 Statistical approach

In light of the replication crisis, many advocate for a better and more adequate use of statistical tools in research. Indeed, amongst others, the analysis of variance, or ANOVA, is one of the most popular tools used for testing experimental variables. Yet though valuable, the conditions necessary for ANOVA testing are often not met, therefore the interpretability of the underlying results such statistical method is limited.

1.7.3.1 Limitations of ANOVA

ANOVA is a popular statistical test used for the predicting an outcome variable based on one or two predictive categorical variables. It relies on the comparison between means and test their difference. To test data with ANOVA, the following conditions need to be met : normality (gaussian distribution), homogeneity of variance (equal variance within each group), independence and random sampling. A continuous distribution underlies that the tested data can take on any value within a specified range, such as time, temperature, length etc. However, in both behavioral and anatomical

studies, the extracted data are rarely in the form of a continuous gaussian distribution, and more than often follow a discrete distribution where the data can take only take specific values in a specific range, such as counts (*e.g.* number of cells) or proportions (*e.g.* percentage of correct answers). Such distributions therefore fall under non-normal distributions, and violate the core principles of ANOVA: normality and continuous distribution. Therefore, ANOVA testing on such data is not reliable and therefore not recommended.

1.7.3.2 Type of data distribution

Overall, an understanding of data distribution is necessary for the appropriate use of statistical methods to analyze experimental results. Data distribution refers to the nature of the data, how it is spread out and takes into account whether it is clustered around certain values or ranges. By examining such distribution, we can gain insights into the characteristics and patterns of the data and make informed decisions about which statistical method is the most appropriate given the context of the analysis.

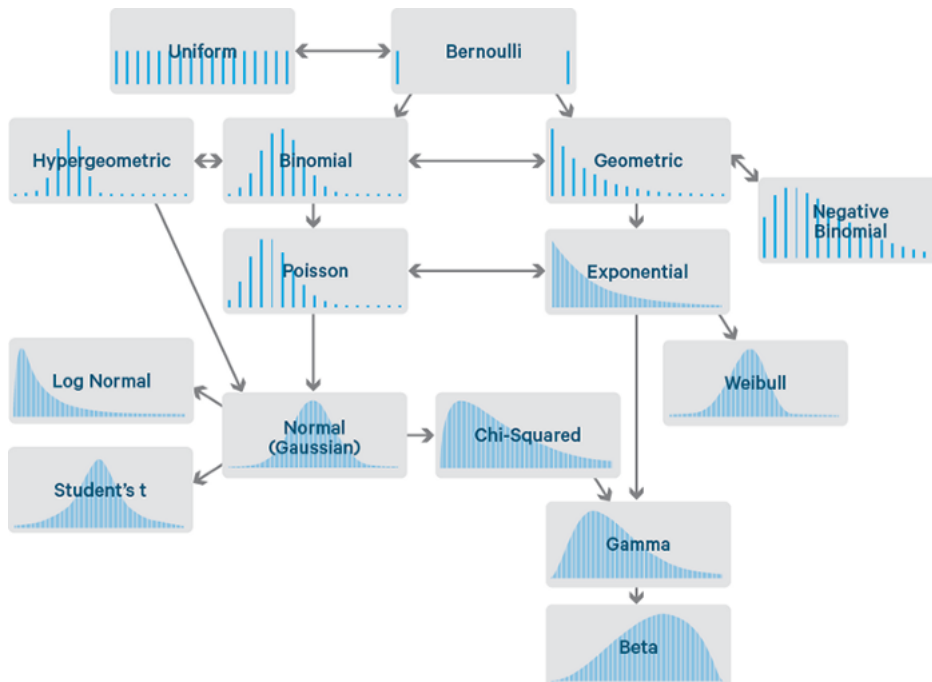


Figure 1.9: Types of data distribution.

From Jagan Singh

In this thesis, the obtained data followed either a Bernoulli distribution (binary data: correct/error, cf. Chapter 2), binomial distribution (proportion of correct over a total,

cf. Chapter 2) or negative binomial distribution (number of segmented neurons, cf Chapter 3).

1.7.3.3 The p-value

Since the data distribution obtained in this thesis are all discrete and ANOVA assumptions are violated, a better fit would be the use of logistic regressions. Logistic regressions are models specific to the testing of non-normal distributions. In our case, a mixed model logistic regression allows the inclusion of one or multiple error terms (also called random factor) that would allow to take into account the interindividual variability of tested animals, along with the fitting of discrete distributions.

ANOVA and logistic regressions are part of the frequentist statistical family. Frequentist statistics rely on the assumption that a null hypothesis is true **before** the data is collected. Here, the question asked is: “*How surprising is my result if there is actually no effect?*”. The data would be surprising if there was a low probability by chance alone of obtaining another data set at least as extreme (*i.e.*, far away from the null hypothesis and unlikely to occur by chance) than the one collected. If this is the case, then the collected data is considered as unlikely under the null hypothesis and rejected, which infers that the null hypothesis does not adequately explain the obtained data and that something else must account for such results. This probability is known as the *p-value*, the probability of obtaining another data set at least as extreme as the one collected by chance alone. The p-value is therefore not a measure of how correct a hypothesis is, nor one that sizes the importance of an effect. Confidence intervals give information on the size and direction of an effect: a 95% confidence interval tells us that if we were to repeat the experiment over and over, 95% of the computed confidence interval would contain the true mean.

Though the p-value is a widely used tool, it is nowadays highly criticized (Halsey, 2019; Skrepnek, 2007; Makowski et al., 2019). Because the p-value is based on the assumption that the null hypothesis is true, it does not give any information about the alternative hypothesis, which is the usually the research hypothesis: a high p-value does not reject the null hypothesis neither can it be interpreted as the null being

true on its own, nor the alternative hypothesis to be false. Based on a frequentist approach, it is therefore not possible to infer on the probability of the null hypothesis and the alternative hypothesis to be true.

1.7.3.4 A case for bayesian inferences

As opposed to frequentist approaches, bayesian approaches offer the possibility to gather information on the probability of both the null and alternative hypothesis. Bayesian inference is based on the Bayes's theorem (1.1) that describes a method to update probabilities of variable of interest to have an influence on the data based on the data and past knowledge (Fig. 1.11).

$$P(H|E) = P(H) \frac{P(E|H)}{P(E)} \quad (1.1)$$

Here, H stands for any hypothesis whose probability may be affected by data (*i.e.* evidence), $P(H)$ is the prior probability which is the estimate of the probability of the hypothesis H before the data E is observed, E is the evidence which corresponds to new data that was not initially used in the computing of the prior probability, $P(H|E)$ is the posterior probability which is the probability of the hypothesis H given the data E , *i.e.*, once E is observed, *i.e.* the probability of a hypothesis given the observed evidence, $P(E|H)$ is called the likelihood which is the probability of observing the data E given the hypothesis H and indicates the compatibility of the evidence with the given hypothesis, and therefore based on the data distribution, $P(E)$ is the marginal likelihood and represents the probability of generating the observed sample for all possible values of the parameters.

As opposed to frequentist approach, where the hypothesis is fixed and data variable, in bayesian inference, we test variable hypothesis based on fixed data. In bayesian approaches, for each data point, the question asked is: "*What is the probability of my effect, given this new data?*". This probability is known as the posterior distribution. It summarizes what is known about the outcome using both the prior information and the new data. The prior is one of the key differences between frequentist and Bayesian approaches: frequentist analyses base results only on the collected data whereas the

bayesian prior could be formulated by expert beliefs and prior knowledge. The posterior distribution captures an “updated” estimate of the probability of the outcome after incorporating new data, including our uncertainties, and can be analyzed to give various statistics, such as the mean and 95% credible interval. The 95% credible interval is different from a frequentist 95% confidence interval; here, it signifies that the parameter range has a 95% probability of including the true parameter value, whereas in the frequentist confidence interval, the 95% confidence interval assumes that the experiment is hypothetically repeated over and over and that 95% of the computed confidence intervals would contain the true mean.

The biggest advantages of Bayesian inference, is that the posterior distribution can also be used to directly calculate the probability of different hypotheses. Bayesian inference is therefore more intuitive at its core and in closer alignment with our natural mode of probabilistic reasoning what is actually looked for when testing data. Additionally, as bayesian inference are less sensitive to outliers and provide more stable estimation of paramater value in smaller samples sizes when compared to frequentist approaches (van de Schoot et al., 2015).

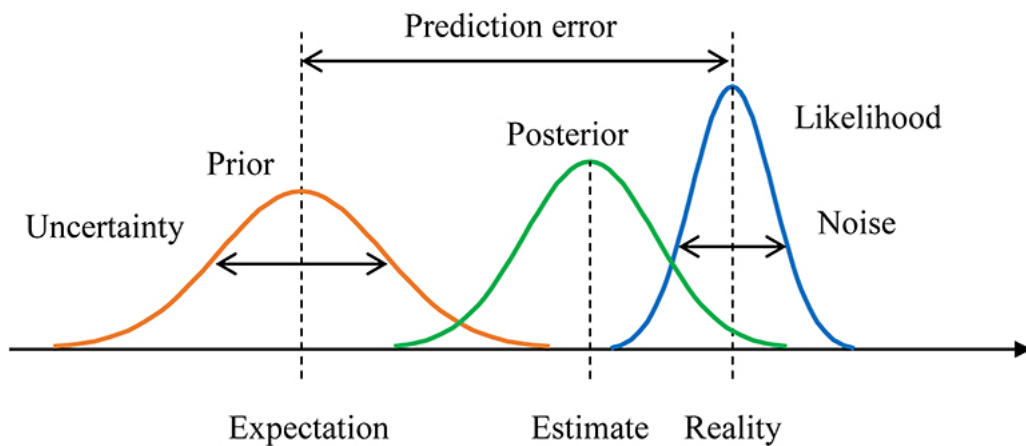


Figure 1.10: Bayesian inference. Example of Bayesian inference with a prior distribution (orange), a posterior distribution (green), and a likelihood function (blue). The prediction error is the difference between the prior expectation and the peak of the likelihood function (i.e., reality). Uncertainty is the variance of the prior. Noise is the variance of the likelihood function.

From Yanagisawa et al., 2019

1.7.3.5 Interpretation of bayesian analysis

We used the Sequential Effect eXistence and sIgnificance Testing (SEXIT) framework to describe Bayesian effects (Makowski et al., 2019). It includes the following indices: the median of the posterior distribution where there is a 50% of probability that the effect is higher and lower, the 95% Highest Density Interval (HDI) implying that there is 95% of probability that the observed effect is within this credible interval, the probability of direction which allows to quantify the certainty by which an effect is positive or negative and assess the direction of a link, and finally whether the effect is of sufficient size to be considered as significant (*i.e.*, not negligible), to determine which effects are important and considerable. Finally, the probability of an effect being large indicate the strength of the effect.

For example, if the probability of existence of an effect (based on the HDI and probability of direction) and its significance are high, but the probability of being large is very small then it suggests that the effect of the tested variable is, with high confidence, not large. If the posterior is closely centred around 0, and that the 2 indices are very low, then this suggests that the effect is null with high confidence.

The significance range threshold is based on the Region Of Practical Equivalence (ROPE) values (Kruschke, 2018). ROPE represents all values that are around the null-value and considered to be similar to the null-value. ROPE testing allows to

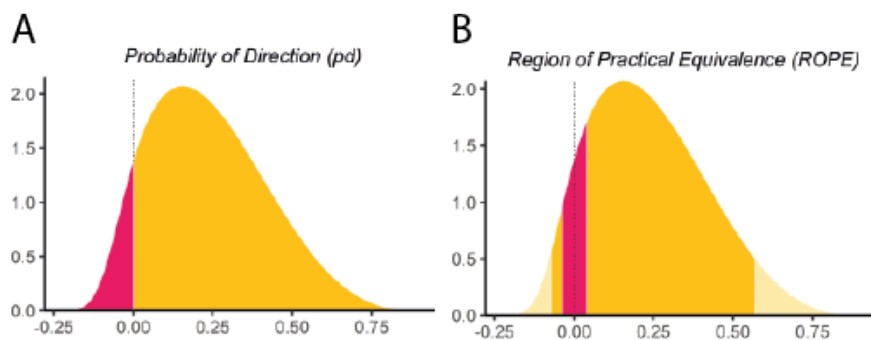


Figure 1.11: Visual explanation of Bayesian results. Here are examples of posterior density. In (A) we see represented the probability of direction which indicate the direction of the effect. Here we see that most of the posterior density is located above 0 (yellow), so the effect is mostly positive. In (B) we see in pink the region of practical equivalence, where values around the null value are considered to be equivalent to the null value.

Adapted from Makowski et al., 2019

gather evidence on the likelihood of both the null and alternative hypothesis. If the estimated values within the Credibility interval encompasses the null and ROPE values as plausible, then it is assumed that there is not enough evidence to reject the null hypothesis. Still, to assess the probability of the null hypothesis (0 and values in ROPE) and alternative hypothesis to estimate how strong the gathered evidence is i.e. how large the effect is, we use the Bayes Factor (Fig. 1.12). The Bayes Factor calculates the ratio of the likelihood of the data under the null hypothesis and the likelihood of the data under the alternative hypothesis.

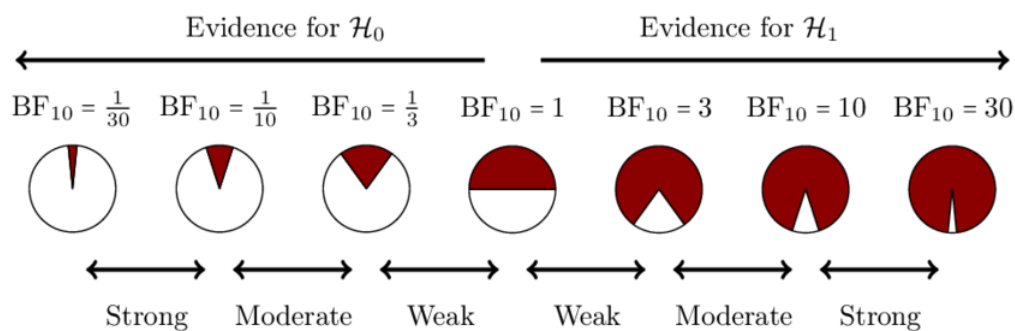


Figure 1.12: A graphical representation of a Bayes factor classification table. As the Bayes factor deviates from 1, which indicates equal support for H_0 and H_1 , more support is gained for either H_0 or H_1 . Bayes factors between 1 and 3 are considered to be weak (i.e., not worth more than a bare mention), Bayes factors between 3 and 10 are considered moderate, and Bayes factors greater than 10 are considered strong evidence. The Bayes factors are also represented as probability wheels, where the ratio of white (i.e., support for H_0) to red (i.e., support for H_1) surface is a function of the Bayes factor. The probability wheels further underscore the continuous scale of evidence that Bayes factors represent.

From Van Doorn et al., 2019

What are the hallmarks of age-related cognitive decline in the mouse model ?

Does age-related WM function in the mouse model present similar characteristics as those observed in human studies ?

At a tissue level, is there an age-related neurodegeneration of DA neurons in VTA ?

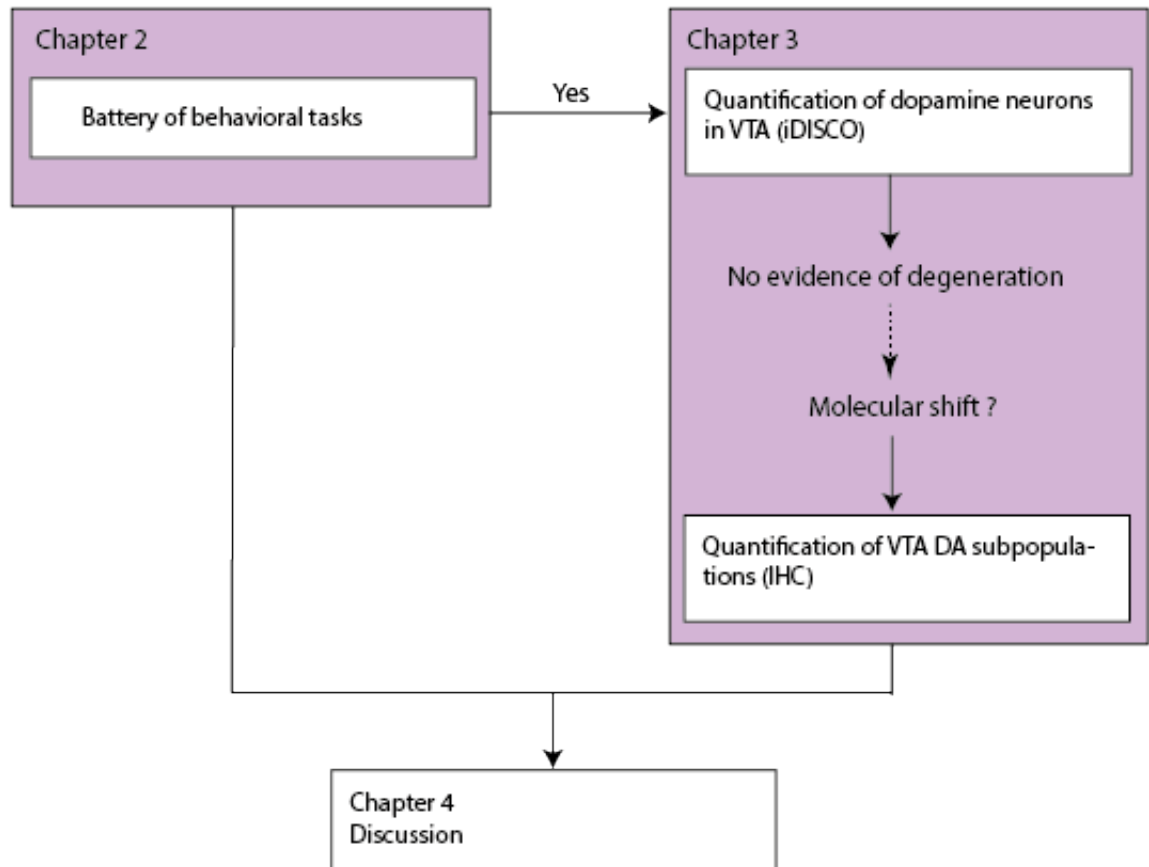


Figure 1.13: Overview of thesis.

CHAPTER 2

*Study 1: The effects of healthy aging on spatial
working memory*

In goal-directed behavior, WM allows the maintenance of salient set of actions (planning) and plays an essential role in their coordination and processing to optimally reach said goal (execution). WM function is defined as the ability to store, maintain and manipulate relevant information for motivated actions. While it is widely accepted that healthy aging affects WM function, it remains unclear whether its subprocesses are equally vulnerable to healthy aging, especially in the mouse model.

In this chapter, I will demonstrate that in PFC dependent spatial WM tasks, the temporal maintenance of relevant information in WM does not depend on the cognitive effort required during encoding, but on the flexible encoding and updating of multiple information i.e. cognitive load. Interestingly our results indicate that the extent of WM failure in such settings are dependent on the inter-individual balance between stability and flexibility of available mnemonic traces, especially in older individuals.

2.1 Introduction

2.1.1 What are the subprocesses involved in working memory?

Previously, we described the cognitive models used to conceptualize WM. On a neurophysiological level, WM is known to be dependent on three key subprocesses: encoding, maintenance and processing of relevant mnemonic traces.

2.1.1.1 Encoding within working memory: How is information stored?

When integrated, incoming sensory inputs are thought to be filtered and stored within WM based on their importance and relevance for current behavior (*i.e.* their saliency) (D'Esposito & Postle, 2015). Across species, imaging and electrophysiological studies show that stimulus encoding in WM tasks depend on the coordination between frontal and sensory cortices and that their degree of coordination was dependent on the number of items to be maintained, *i.e.* based on WM load (Violante et al., 2017; Andersen & Cui, 2009; Kim, 2019; Li & Curtis, 2023; Zhang et al., 2019). Relevant sensory information are thought to be encoded within their respective sensory networks while activity in the frontal cortices, especially the PFC, select and orchestrate sensory encodings for goal-directed behavior. Such selection is thought to rely on attentional processes that allow the focusing on relevant sensory information to be maintained and processed within WM (D'Esposito & Postle, 2015; Buschman, 2021).

Encoded WM mnemonic traces should be both stable enough to be maintained yet also flexible enough to be disrupted and manipulated for higher levels of integration and processing. WM dependent mnemonic traces are therefore characterized by their versatility, and their inherent instability that inevitably result in voluntary or involuntary losses of information. This intricate balance between stability and flexibility bounds WM to a limited storage capacity, in opposition to the unlimited storage capacity within LTM (Bond et al., 1982; Carruthers, 2013; Cowan, 2008; Lewis-Peacock et al., 2018).

WM can be considered not only as a type of memory, but also as a type of forgetting (Lewis-Peacock et al., 2018; Miller, 2021). Because of the amount of resource required in the stabilization of unstable traces, which we define here as cognitive *effort*, voluntary information removal are thought to allow the system to free up space by removing no longer relevant information stored within WM. On the other hand, involuntary losses of information are thought to be due to either temporal decay or interference. Decay is defined as a time-dependent loss of information whereas interference is characterized by the simultaneous short-term encoding of information. Both the cognitive effort required to encode distinct overlapping encodings and the number of item to be stored within WM, which we define here as the cognitive *load*, participate in WM information loss (Buschman, 2021).

Though there is a consensus on the fact that WM storage capacity is limited, the precise nature of this limit remains unclear. More specifically, does this limitation apply only to one unique WM system or, on the contrary, are there multiple independent feature-based “working memories”, that are each limited in their own storage? Some suggest that concomitant encoding of different types of information, for their respective purposes, do not affect WM performance nor task resolution (Bratch et al., 2016; Doherty & Loggie, 2016). Others, on the contrary, imply that multiple encodings affect WM performance by either facilitating or impairing task resolution (Doherty et al., 2019). Interestingly, Buschman proposed that these opposing conclusions may be due to two types of interferences within WM: (i) representational interference and (ii) competitive interference (Fig. 2.1) (Buschman, 2021).

Representational interference characterizes overlapping feature proprieties and their underlying neuronal signatures (Buschman, 2021). More specifically, one could imagine that features are scaled over a spectrum where the more distinct they are from each other, and the less they are to interfere with one another. A common goal might imply that multiple sensory encodings could collectively allow for optimal context representation and facilitate their processing to reach said goal. On the other hand, the more features share similarities and the most likely they are to interfere with

one another, which overall lead to conflicting context representations, and ultimately result in uncertain predictions and behavioral outputs.

Competitive interference characterizes the recruitment of the same neuronal population for the competitive encoding of non-overlapping representations (Buschman, 2021). With increased cognitive load, it is suggested that the amount of information integrated in the same neuronal population may decrease and therefore limit the decodability of information because of loss of accuracy. Similar to representational interference, this would eventually lead to uncertain predictions that impact behavioral outputs.

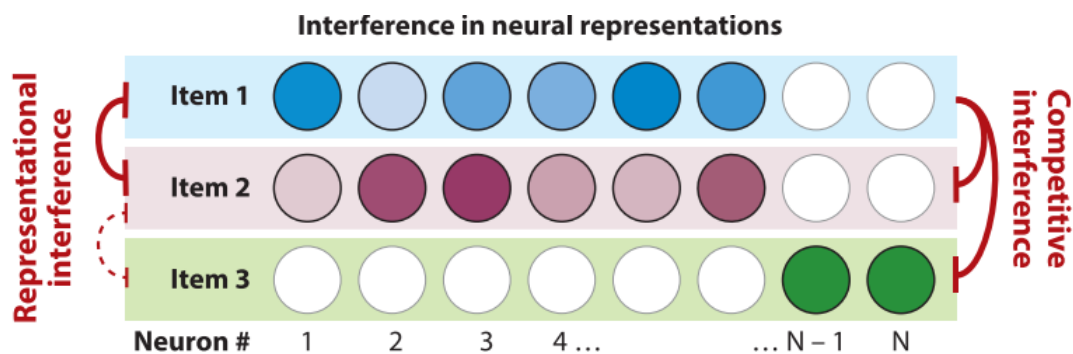


Figure 2.1: Type of interferences in working memory. Schematic of representational and competitive interference. Representational interference occurs when two representations overlap in neural space, as between items 1 and 2 but not items 2 and 3. Competitive interference occurs between any representations in the same region, regardless of their overlap, as between item 1 and items 2 and 3.
From Buschman, 2021

2.1.1.2 Stability within working memory: How is information maintained?

Preserving the integrity of mnemonic traces all the while sustaining them is a vital aspect of WM. Delay-dependent tasks emphasize on this aspect of WM and are one of the most widely used paradigms in animal models (Funahashi, 2017; Dudchenko, 2004). In the 90's, Goldman-Rakic tested non-human primates while they were executing the oculomotor delayed reaction (ODR) task and recorded the activity of their neurons in the PFC, the main brain region implicated in WM (Jacobsen, 1936; Fuster & Alexander, 1971).

In this visuospatial WM task, the animal was trained to fixate the center of a panel before the presentation of a visual cue. After a short delay period, when cued, the animal had to make a saccade towards the location of the previously showed cue to be rewarded. In this pioneer work, the authors showed that neurons within the PFC were active during the delay period and showed a specific neuronal signature. The latter, termed as “Delay activity” was characterized by a persistent and above baseline firing rate of prefrontal neurons during encoding and delay (Goldman-Rakic, 1995) (Fig.2.2). Based on these conclusions, sustained activity within PFC neurons were thought to be the principle mechanism responsible for the maintenance of relevant information within WM.

However, nowadays, it is under vivid debate whether persistent firing is the sole mechanism underlying maintenance in WM (Miller et al., 2018; Wang, 2021; Stokes, 2015; Beukers et al., 2021; Barbosa et al., 2020; Masse et al., 2020). Indeed, Miller and colleagues suggest that such a continual state of activation may consume high amounts of energy that consequently are too difficult to sustain on a biological level (Miller et al., 2018). They propose that delay activity may in fact not be persistent *per se*, but rather briefly and sparsely reactivated with the purpose of “refreshing” stored information, instead of continuously maintaining it. This underlies that though neurons are not explicitly firing, they still conserve a ‘silent memory’ of the maintained information, and that ‘reactivation activity’ allows the refreshing of such memory until its use.

This mechanism is thought to depend on short-term synaptic plasticity (Mongillo et al., 2008; Stokes, 2015; Masse et al., 2020). Specifically, the increased strength in neuronal connectivity, *i.e.* short-term facilitation, is proposed to enable the storage and maintenance of relevant information in their corresponding neuronal groups whilst minimizing losses of information and preserving energy. Some also propose that such modulation of synaptic weights (which is the strength of neuronal connectivity) within WM functional networks are in part tuned by LTM, in particular episodic memory networks (Fig.2.3). Overall, the maintenance of an information within WM

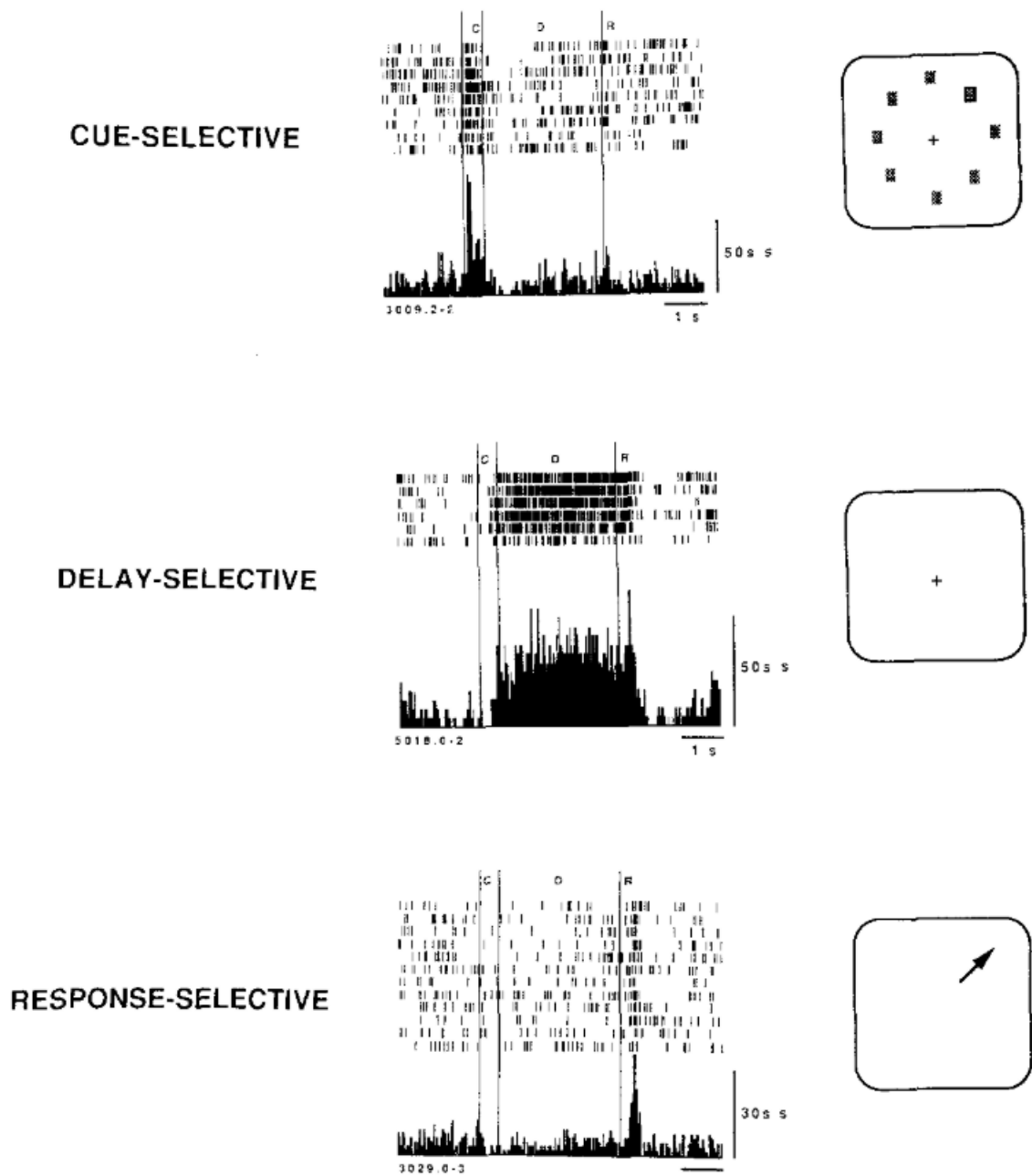


Figure 2.2: Prefrontal activity during the oculomotor delayed reaction task. In PFC, some neurons respond at the onset of the target (top), some respond in relation to the delay (middle), and some are activated in relation to the onset of a response (bottom). In all cases, neuronal activity is time locked to the events of the task and spatially tuned.

From Goldman-Rakic et al., 1995

is thought to rely on the complementarity of active and activity-silent states within the PFC (Mi et al., 2016; Miller et al., 2018; Mongillo et al., 2008; Stokes, 2015).

| Phenomenon | ASWM explanation | EM explanation |
|--|---|---|
| Activity silent maintenance | Short-term plasticity, likely in PFC | Long-term plasticity, likely in HPC |
| Network perturbation increases decodability | Different activity flows due to different network weights | Promotes EM reinstatement by recruiting HPC |
| WM maintenance is accompanied by activity bursts | Activity bursts refresh short-term traces by activating representations | Activity bursts are a signature of retrieval from EM into WM |
| Increased gamma burst rate towards end of delay period | Recoding from silent to active WM trace | Increasing probability that memory is retrieved from EM into WM |
| Flexible forgetting | Traces are transient | Decreased accessibility by context shift |

Figure 2.3: Observed phenomena along with activity-silent working memory and episodic memory interpretation.

ASWM: Activity-Silent Working Memory; EM: Episodic Memory.

From Beukers et al., 2021

2.1.1.3 Processing within working memory : How is information manipulated?

Information storage within WM implies manipulation and integration of the encoded mnemonic traces to create complex neuronal representations, that will be used for decision-making and action planning during goal-directed behavior (Pezzulo et al., 2018). As already theorized in WM cognitive models (see Chapter 1), a dialogue between short- and long-term functional connectivity are thought to shape neuronal representations based on past experiences (Atkinson & Shiffrin, 1968; Baddeley, 2006; Cowan, 2001; Biderman et al., 2020).

As proposed in the activity-silent model, some further suggest that items initially encoded within WM could also be temporarily stored in LTM networks, and be actively and flexibly switched in and out of these networks depending on the information required for the current targeted goal (Burgess & Hitch, 2005; Beukers et al., 2021; Miller et al., 2018). This point of view implies that only information that are relevant and manipulated for current behavior are maintained ‘online’ in WM whereas others, that are not immediately relevant for processing, are ‘silenced’ and stored within LTM networks, and recruited back when necessary.

The malleability of short-term plasticity is also thought to be a backbone of such flexible recruitment. Short-term potentiation of WM functional connectivity is thought to participate in the hierarchical integration of sensory features along with their associated temporality and context (Stokes, 2015). Collectively, these are thought to elicit predictive computations for goal-directed behavior, referred to as *active inferences* (Pezzulo et al., 2018). The latter are thought to be based on probabilistic Bayesian inferences, where predictions arise from the likelihood of an event based on prior information (Kononov & Krajbich, 2018). In this context, information collected within WM are thought to allow an accumulation of evidence (*e.g.* present state) based on prior knowledge (*e.g.* past experiences) for the prediction of future outcomes, that, together, will rule decision-making processes and enable action planning and the resulting behavioral outputs to reach the targeted goal (Fig. 2.4) (Pezzulo et al., 2018; Vertechi et al., 2020).

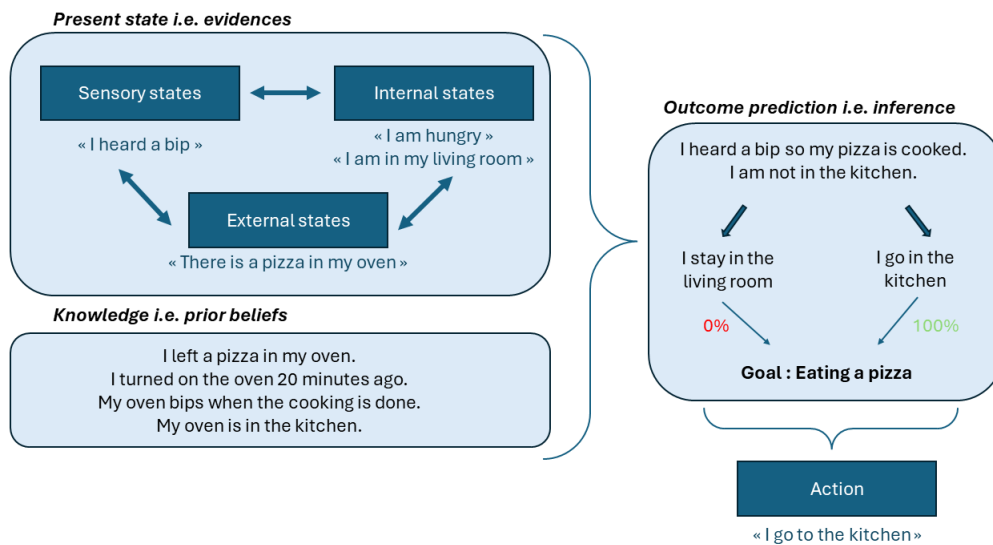


Figure 2.4: An Example of Active Inference.

Interestingly, a behavioral output associated to the prediction of future outcomes was characterized in the rodent model (Redish, 2016). Specific to a spatial setting, this behavior is named the vicarious-trial-and-error (VTE) and is proposed to reflect a deliberation process, where the animal horizontally scans between options, before making a decision. This behavior relies on interactions between the hippocampus and PFC, which are thought to underlie the neural representations of future outcomes

and their evaluation. More importantly, this behavior is thought to emerge in tasks requiring cognitive processes underlying goal-directed behavior such as WM, but not when procedural behavior is involved. Therefore, VTE is considered as a behavioral marker of decision-making in during spatial goal-directed behavior.

2.1.2 How are working memory subprocesses affected by healthy aging?

Optimal WM function relies on a balance between the selection of relevant information (filtering), their maintenance (stability) and their processing (flexibility). Such balance is thought to be based on a fine tuning mediated through short-term synaptic facilitation, especially in the PFC (Mongillo et al., 2008; Mi et al., 2016). Short-term plasticity implies that synaptic weights are particularly dynamic, adaptive and therefore sensitive to the signal to noise ratio within their functional networks (Magee & Grienberger).

During healthy aging, numerous structural alterations that affect neuronal excitability are found, especially in the PFC (Wang et al., 2011; Dickstein et al., 2013; McQuail et al., 2015; Legon et al., 2016). To name a few, reports indicate: reduction of dendritic arborization (Dickstein et al., 2013; Duan et al., 2003; Kabaso et al., 2009), reduced spine density (Bloss et al., 2013; Dickstein et al., 2013), altered receptor distribution (Dickstein et al., 2013; Hernandez et al., 2018; McQuail et al., 2015) and altered intrinsic properties (Legon et al., 2016; Wang et al., 2011; Howell et al., 2019). On top of those alterations, the excitation/inhibition balance in aged individuals is thought to be shifted towards increased inhibition and resistance to excitatory inputs (Liguz-Leczmar et al., 2015; Bories et al., 2013; Bañuelos et al., 2014; McQuail et al., 2015; Tobinaga et al., 2019; Legon et al., 2016; Hernandez et al., 2018).

On a behavioral level, healthy aging is associated with WM decline across species (Zanto & Gazzaley, 2019; Bizon et al., 2009). This decline is for the most part put in evidence in tasks challenging WM flexibility and WM load, such as the *n-back* task in humans (Fig. 2.5). In this example, participants are taught to apply a rule (here,

“click when a letter is repeated”) when faced with an information (*e.g.* visual stimuli, here a sequence of letters). This paradigm necessitates the simultaneous encoding of the presented information (here, each letters), their temporal holding (progressive storage of each letter seen), their processing (“have I seen this letter”) for behavioral adaptation (if yes: “I click and forget this letter”, if no: “I don’t click and maintain this letter”). This paradigm therefore allows to challenge WM load by modulating the cognitive load (number of letters to store until repetition) in a context requiring high cognitive flexibility (dynamic storage, maintenance and processing) and high cognitive effort (all items presented share similar features, here the items are all letters presented in the same font, etc.). Often, older adults perform worse than their younger counterparts, and the gap deepens with increased cognitive load (*i.e.* when there are multiple information to encode and manipulate) (Sala-Llonch et al., 2012; Bopp & Verhaeghen, 2020).

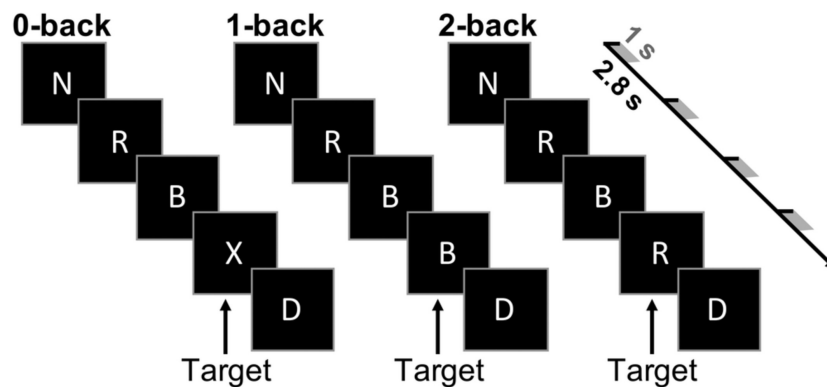


Figure 2.5: Example of a visual verbal n-back paradigm. 0-back condition: The participant only has to recognize a predefined stimulus (here, "X"). 1-back: The participant has to recognize a the consecutive repetition of a stimulus (here, second "B"). 2-back condition: The participant has to recognize the consecutive repetition of a stimulus, that will be presented at $n+2$ trial (here, second "R").

From Costers et al., 2020

In human studies, the distinction of task contingencies and their effect on WM subprocesses are well-defined. However, in rodent studies, though each paradigm test different aspect of WM, the differences in task contingencies and their influence on WM subprocesses are not clearly defined, especially in the context of aging studies. For example, in delay-dependent studies, variation among and across paradigms are observed. Rodents tested in a delayed-non-match-to-place (DNMTP) paradigm

collectively show age-related WM impairments, however the extent of such impairment varies. For example, some observe impairments around a 10s delay (Roy et al., 2022; Dunnett et al., 1988; Chisholm et al., 2013) while others at a 30s delay (Mizoguchi et al., 2009; Beas et al., 2013). On the other hand, rodents tested in the delayed-alternation paradigm do not show any impairment at a 30s delay (Bisaz et al., 2013).

Additionally, the characterization of WM failure can vary from one study to another. In some tasks, WM failure is assimilated to an inability to improve performance over time because of the cognitive load (Marighetto et al., 2008; Bretin et al., 2018) or imposed delays (Dunnett et al., 1988) while others characterize WM failure in a single trial following initial learning (Mizoguchi et al., 2009; Roy et al., 2022; Chisholm et al., 2013), or group session performances (Beas et al., 2013; Bisaz et al., 2013) (Table 2.1).

| Encoding | Maintenance | Processing |
|----------------------------------|--|------------------------------|
| Forced vs Free choice | Delay length | Rule learning vs No learning |
| Independent vs Continuous trials | Same versus Changed environment across sessions (Interference) | |
| Feature discrimination | Encoding versus No encoding during delay (Interference) | |

Table 2.1: Example of WM task contingencies in rodents.

2.1.3 Study 1: Does age affect the temporal maintenance of an information within WM in the mouse model?

2.1.3.1 Rationale & Working hypothesis

WM is thought to rely on specific neuronal codings. For example, a mnesic trace is encoded and stored within its respective sensory cortices and its maintenance is thought to be dependent on waves of persistent firing within the PFC (Lara & Wallis, 2015). The updating of a mnesic trace for the correct goal is thought to be dependent on the interaction with LTM components such as the hippocampus (Numan,

2015). Overall, WM subprocesses are characterized by specific neuronal signatures that together allow WM function. In older humans, it is thought that the age-related decrease in WM capacity is related to alterations in the ability to update and process information encoded within WM, but not in the ability to hold such information (McNab et al., 2015; Pinal et al., 2015). Therefore, in human aging, it is suggested that the critical processes related to WM flexibility are the most vulnerable to healthy aging.

Yet, in rodent models, whether such is also the case remains to be elucidated. Though it is reported that healthy aging leads to WM decline in aged rodents, it is not clear whether such deficits are driven by a deterioration of cognitive flexibility or whether the ability to maintain an information within WM is also affected by healthy aging in the rodent model. Specifically, the variability in task contingencies also affects the extent to which WM subprocesses are interrogated, and it remains to be clearly stated whether healthy aging in humans and rodents can be paralleled. In tasks challenging the maintenance aspect of WM, namely delay-dependent paradigms, the resulting conclusions are heterogeneous, even contradicting (Mizoguchi et al., 2009; Bisaz et al., 2013). Interestingly, such results also seem to be related on the paradigms being used to test WM and its underlying subprocesses.

In this first study, we hypothesized that the maintenance of an information (i.e. how long information needs to be stored) within WM is dependent on both the encoding effort (i.e. how precisely an information needs to be encoded) and cognitive load (i.e. how much information needs to be encoded). Specifically, we hypothesized that the more an information requires encoding effort, the less an animal is able to reliably maintain an information within WM. Similarly, an increased cognitive load would result in a decrease of WM performance. To test our hypothesis, we tested adult and aged mice in their ability to hold and process relevant information within WM, depending on the accuracy required to encode the information (cognitive effort), and the dynamic storage of information (cognitive load). To do so, we evaluated both the cognitive performance and VTE-like behavior of young (2-3months) and healthy aged (18-19months) mice in a battery of spatial WM tasks.

2.1.3.2 Experimental approach

In the first task, mice were tested on the delayed-alternation paradigm, set in a Y-maze. In this task, mice are presented with a pair of adjacent arms in-between which they are required to alternate to retrieve a reward. In-between consecutive alternations, mice are imposed a short delay period of 5s during learning, then tested in increased delays (10s, 20s, 30s, 45s & 60s). Under these conditions, we assume mice will require the encoding of the location of their choice, maintain this information within WM, and make a prediction, based on the acquired rule, on the localization of the reward to adapt their behavior. In this paradigm, since (1) only two locations need to be encoded and (2) these locations remain constant across sessions, we consider the delayed-alternation task to be a paradigm emphasizing on the temporal maintenance and updating of a single relevant information within WM in a context of minimum cognitive effort (low-encoding effort) and cognitive load (low-encoding interference).

Then, we tested mice in the delayed-alternation task, this time set in a 8-arm radial arm maze. Similarly, mice learned to alternate in-between two arms of the maze, then were tested on longer delays (30s, 60s). We consider that the increased spatial complexity of this maze compared to the Y-maze entail an increase in the cognitive effort required to encode spatial locations (high-encoding effort) without affecting the cognitive load as it still relies on the updating of one relevant information (low-encoding interference).

Finally, in the third task, mice were tested on Olton's previously described radial arm maze task, that we will refer to here as the All-baited task (Fig.1.5). In this task, motivated mice will be left to explore an 8-arm radial arm maze where all arms are baited. We assume mice will explore the maze in such a way to optimally retrieve all the baits placed in the maze. Under these conditions, we presume that mice will require not only the encoding and maintenance of a single choice location but also the constant updating of spatial information which will result in a progressive overload of information throughout their exploration. Therefore, we consider the all-baited task to be a paradigm requiring dynamic encoding, maintenance and processing of multiple

information within WM that result in both increased cognitive effort (high-encoding effort) and cognitive load (high-encoding interference).

We collected data based on both task performances and behavioral outputs of mice in the described paradigms. To analyze the obtained data, we took advantage of a Bayesian statistical approach that allowed to account for both the likelihood to observe an effect of age on performance but also, to test the likelihood of our initial hypothesis to be correct according to the collected data (cf. Box 1 in [2.2.6](#)).

2.2 Material & Methods

2.2.1 Ethics

All experimental procedures were approved by French institutional and government animal care committees. Animals were all provided by Janvier Labs at either the age of 2 months or 18 months. Upon arrival and until sacrifice, animals were cared for by the staff of the animal facilities within the Vision Institute and the principles of the 3 R's, as stated in the European Directive 2010/63/EU were rigorously applied.

2.2.2 Animals

For all behavioral experiments, a total of $n=30$ male C57BL6J mice of either 2-3 (adult) or 18-20 (aged) months old were used. Upon arrival and prior to any manipulations, animals were left 5 days of acclimation. They were housed by groups of 1-3 per cage with enrichment (tunnel, cotton, wood stick) to minimize stress. However, when aggressive dominant behavior was observed, the dominant animal was isolated and extra enrichment was added to the cage. Prior to any experiments, all mice were first habituated to handling by the experimenter for a week, during which their normal bodyweight and food intake was also recorded. Food was then progressively restricted over the course of 5-7 days in order to attain 85% of each animals' bodyweight (approximately 70% of normal intake). The weight of each mice was monitored daily. When necessary, daily food allowance was adapted to the need of each animal in order to maintain 85% of their bodyweight over multiple weeks (1-5 weeks). For the duration of whole experimental procedure, mice were always placed for at least 30min in the experimental room for habituation.

2.2.3 Apparatus

We used either a Y-maze or a 8-arm radial arm maze to test mice in the designed spatial WM tasks. The Y-maze was placed in a dim lit room and manually operated. Food rewards were crumbles of chocapics that were placed in an opaque white bowl at the end of the two top arms. In the 8-arm radial arm maze tasks, the maze was

placed in a bright room that equally lit all the arms of the maze. A camera was placed on top of the maze to record all sessions. Similar to the Y-maze, crumbles of chocapics were used as rewards and placed at the end of each arm in a white opaque bowl.

2.2.4 Behavioral paradigms

2.2.4.1 Delayed-Alternation Task in the Y-maze

A total of 10 mice (n=5 young, n=5 aged) were tested in the delayed-alternation task set in the Y-maze (Fig. 2.6). Mice were habituated to the food reward (pieces of chocapic) in their home cages to prevent any form of neophobia. Mice were first habituated to the Y-maze for two days where they were left to freely explore the maze for 5 min. Once mice reached 85% of their bodyweight, training started. First, mice were trained to learn the alternation rule. To do so, mice were placed at the end of the center arm and left to explore until they choose to explore one of the adjacent top arms. A trial was considered as completed when the mouse travelled all the way to the end of the arm, where the reward was located.

Mice were trained for 3 sessions per day, where each session consisted of 10 trials. In the first trial, both arms of the maze were baited thus mice were allowed to freely choose the arm to explore. Then, and for 9 consecutive trials, mice had to alternate in-between arms in order to be rewarded. In each trial, once a choice was made, entry to the unexplored arm was manually closed. When needed, mice were conducted back to the starting position for the next trial. The mouse was blocked in the starting position for a couple of seconds before the start of the next trial.

Mice were trained until they reached a criterion of 80% correct answers in 2 consecutive sessions. Once criterion was reached, mice began the delay testing phase where they were blocked in the starting position for a delay of 10s, 20s, 30s, 45s or 60s. Each delay were repeatedly tested for 2 consecutive sessions and progressively incremented. In-between mice, the maze was cleaned with 70% ethanol until dry. Performance in this task was manually scored where 1 was considered as a correct alternation and 0 an incorrect re-entry.

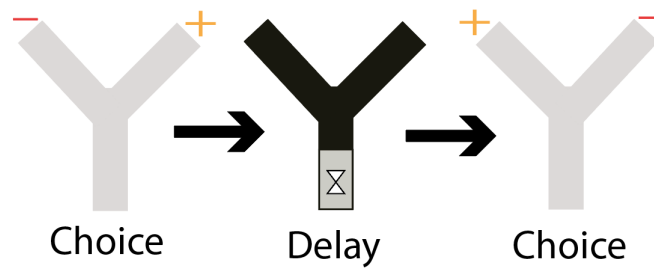


Figure 2.6: Delayed-alternation in the Y-maze.

2.2.4.2 Delayed-Alternation Task in the radial arm maze

A total of $n=17$ mice were tested in this task ($n=9$ young and $n=8$ healthy aged male mice; 1 young mouse died following 30s delay testing). Mice were habituated to the food reward (pieces of chocapic) in their home cages to prevent any form of neophobia. Mice were first habituated to the 8-arm radial arm maze for 10 min over three days where mice were left to freely explore. Mice were then tested in the All-baited task prior to training for the delayed-alternation task in the radial arm maze (see 2.2.4.3). Mice were all food restricted and monitored to maintain 85% of their initial bodyweight.

The radial arm maze was divided into 4 pairs of adjacent arms (pair A, B, C and D). Each pair was opened consecutively for 11 trials, which was considered as one session. Mice were trained in the delayed-alternation task in all pairs each day, which sums up to 4 session per day, separated by a 1 minute inter-session interval where mice were blocked in the center of the maze. Similarly to the protocol in the Y-maze, the same pair of adjacent arms was opened of 11 consecutive trials where in the first trial mice were allowed to freely choose which arm to explore. Then, they had to alternate in between trials in order to be rewarded. Mice were considered as making a choice when their full body was inside the chosen arm. Once a choice was made, entry to the unexplored arm was manually closed and, when needed, mice were conducted back to the starting position in the center of the maze for the next trial (Fig. 2.7).

During training, mice were kept for 5s in the center of the maze and the sequence of pairs presented was pseudo-randomized. Mice were trained until they reached

criterion, which was defined as either 80% of correct answers in each daily sessions for 2 consecutive days (i.e. 80% of correct performance in 8 consecutive sessions) or 80% of correct answers overall for 3 consecutive days (i.e. 80% of correct performance in averaged daily 4 sessions for 3 consecutive days). During the testing phase, mice were isolated in-between trials for a delay of either 5s, 30s or 60s. The sequence of delay was fixed as an alternation of 5s and long delay (30s or 60s) *i.e.*: 5s – 30/60s – 5s – 30/60s – etc. for 10 consecutive trials. This allowed us to verify that performance of mice was maintained in a short delay, and reliably consider that an error on longer delays was most likely due to a WM error (and not because the rule was forgotten or perturbed because of external factors for example). Each delay was tested for 2 consecutive days and progressively incremented. The order of the presented pairs in this phase were also pseudo-randomized. In-between mice, the maze was cleaned with 70% ethanol until dry. Performance in this task was manually scored with 0 being an incorrect re-entry and 1 being a correct alternation. All sessions were also video recorded for pose estimation and tracking.

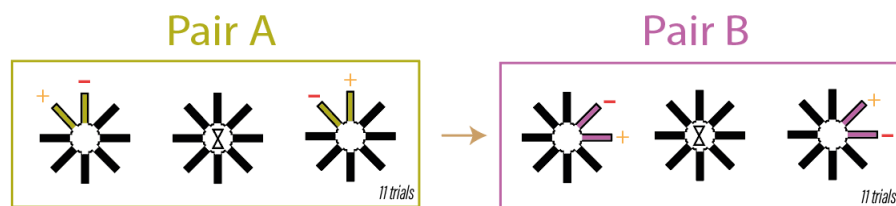


Figure 2.7: Delayed-alternation in the 8-arm radial arm maze.

2.2.4.3 All-baited task in the radial arm maze

A total of $n=20$ mice were tested in this task ($n=10$ young, $n=10$ aged). Once habituated to the radial arm maze, mice began testing in the All-baited task. All arms of the maze were baited with a food reward. Food restricted mice were left to explore the maze for 10min or until all rewards were retrieved. Mice were considered as motivated only when they consumed the food reward as soon as the first run. An arm was considered as explored when the full body of the mouse was inside the arm. Mice were tested in one daily session for seven days. We considered only the performance of mice in the last three days of testing, when performance of mice was

stable and they were habituated to the presence of rewards at the end of each arm. Performance in this task was manually scored over the first 8 runs where mouse were expected to optimally retrieve food rewards. In-between mice, the maze was cleaned with 70% ethanol until dry. All sessions were also video recorded for pose estimation and tracking.

2.2.5 Pose estimation using DeepLabCut and SimBA

All behavioral tasks set in the radial arm maze were recorded from a camera positioned above of the maze. For body part tracking we used DeepLabCut (version 2.3.5) (Mathis et al, 2018, Nath et al, 2019). Specifically, we labeled 1256 number of frames taken from at least 1 video/animal, then 95% was used for training. We used a ResNet-50 based neural network with default parameters for 5 training iterations. We then used a p-cutoff of 0.9 to condition the X,Y coordinates for future analysis. This network was then used to analyze videos from similar experimental settings. This allowed us to track the position of the mouse in the maze, as well as its behavior. We tracked three bodyparts : nose, neck and tailbase (Fig. 2.8).

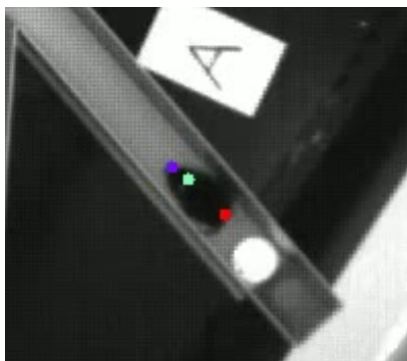


Figure 2.8: Pose estimation using DeepLabCut. Example of tracked bodyparts : in purple the nose, in green the nape of the neck and in red the base of the tail.

We were interested in decision-making related behavioral correlates such as vicarious trial and error (VTE) (scanning behavior before final decision) (Redish, 2016) and exploratory wall sniffing. To approximate detection of such behavior, we used the data gathered from pose estimation of the nose and manually delimited region of interest along the centre of the maze, the entrance of arms and the walls using SimBA version 1.3 (Fig. 2.9) (Nilsson et al., 2020). This allowed us to detect entries of the mouse

in and out of those regions, which are highly susceptible to be correlated with the aforementioned behavioral correlates.

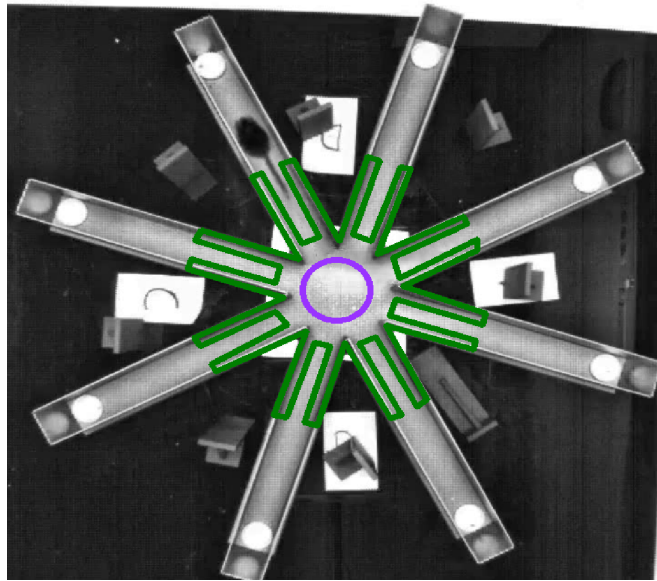


Figure 2.9: Region of interest definition in SimBA. The area between the green and purple delimitation were considered as region of interests where behaviorally significant decision-making behavior occur such as wall sniffing and vicarious trial-and-error.

2.2.6 Statistical analysis

We used Bayesian inferences to analyze the data as it allowed to gather information about the hypothesis being tested based on the data. For the delayed-alternation tasks, our data structure consisted of repeated measures of a binary variable (0 or 1) for the performance. We used the variables Group and Delay as fixed effects, and Mouse as random effect. For the all-baited task, our data structure consisted of repeated measure of a binomial variable (0 to 8) for the performance. We used the variable Group as fixed effect and Mouse as random effect. For all analysis, we used the R software. We used the STAN-based package brms for Bayesian inferences (Burkner, 2017). To test our data, we used uninformative priors.

Following the Sequential Effect eXistence and sIgnificance Testing (SEXIT) framework (2.2.6), we reported the median of the posterior distribution and its 95% Credibility Interval (Highest Density Interval), along the probability of direction (pd), the probability of significance and the probability of being large. The thresholds beyond

which the effect is considered as significant (i.e., non-negligible) and large are $|0.09|$ and $|0.54|$. Convergence and stability of the Bayesian sampling has been assessed using R-hat, which should be below 1.01 (Vehtari et al., 2019), and Effective Sample Size (ESS), which should be greater than 1000 (Burkner, 2017).

2.3 Results

2.3.1 Spatial delayed-alternation task in the Y-maze

First, we compared the performance of young and healthy aged mice in the spatial delayed-alternation task. We considered this paradigm to emphasize on the ability to maintain and update a single information within WM, here a spatial location, in a context of minimum encoding interference (2.1.3.2). In the literature, this paradigm was, for the most, tested in a two-armed maze where the animal had to alternate in-between two locations. Thus, we first tested mice in a Y-maze. Based on our criterion of 80% correct performance over 2 consecutive sessions, all mice reached criterion within 9 sessions over 3 days (Fig. 2.10). There was no difference in learning abilities between age groups.

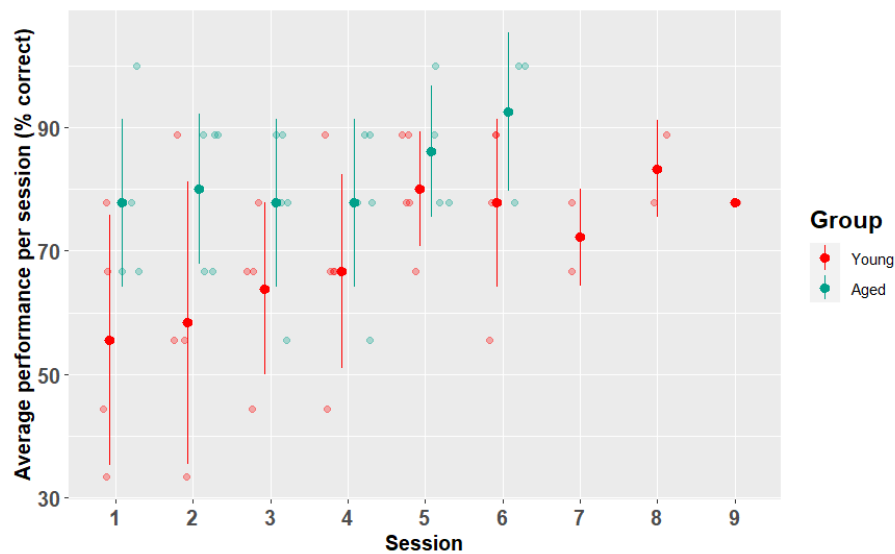


Figure 2.10: Learning in the Y-maze. We plotted the average performance of each mice in a session until criterion was reached. There was three consecutive sessions/day.

Once criterion was reached, mice were tested in increasing delays from 10s to 60s (i.e. 10s, 20s, 30s, 45s, 60s) (Fig. 2.11). We will consider here the 10s delay as the baseline performance.

In order to analyse the results on Fig. 2.11, we first applied classical analysis methods such as a repeated measure ANOVA (Performance by Age x Delay with mice as

error term); there was a significant main effect of delay ($p=0.005$), specifically at 30s ($p=0.021$), 45s ($p=0.014$) and 60s ($p=0.03$) when compared to performance at 10s, but no significant main effect of age ($p=0.544$). The problem with this approach is that the data recorded in this experiment are not continuous because we count the number of successful trials and hence the quantitative variable (bernoulli data distribution with only two possible values) is not supposed to be normally distributed. Therefore, a more correct approach would be to use a mixed model logistic regression using the same model. The results of this analysis vary from the one obtained from the ANOVA: here, though no effect of age is observed ($p=0.25$), the effect of delay is not significant (30s: $p=0.20$; 45s: $p=0.07$, 60s: $p=0.09$). Therefore, the conclusions reached based on ANOVA and logistic regression testing are inherently different.

Still, both of the above methods are based on a frequentist approach to parameter estimation and so they (i) can give unreliable results when the sample is small and (ii) they can not assess how probable it is that the variable does not have an effect at all (see 2.2.6). Indeed, though we cannot reject the null hypothesis based on the results from the logistic regression, it is not possible to assess how certain we are that the null hypothesis is true, especially at a 45s and 60s delay performance.

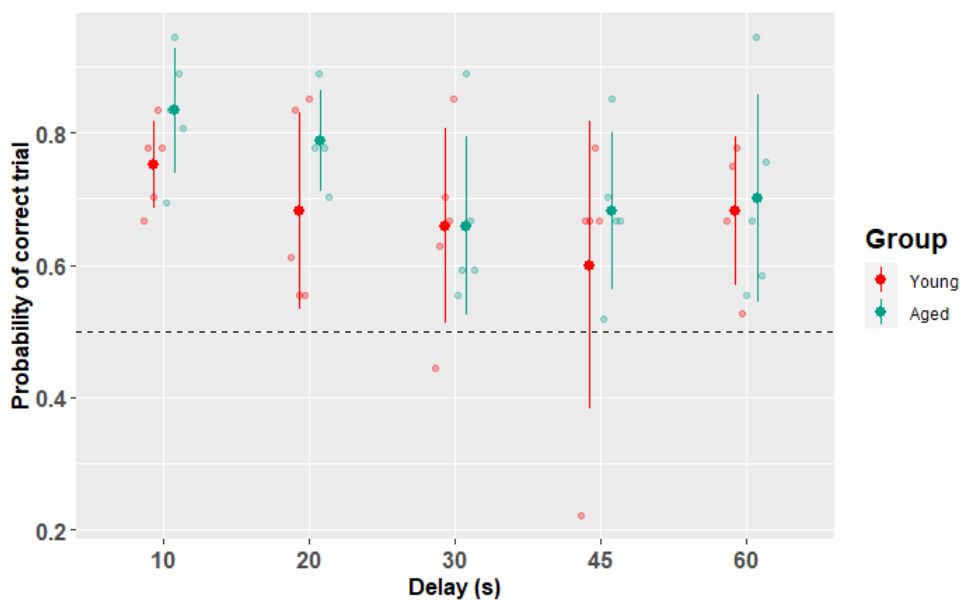


Figure 2.11: Average delay performance in the Y-maze. On the y-axis is represented the probability of each mouse to have a correct answer in a trial for each tested delay.

Therefore, in this thesis, we used Bayesian inferences, that, on top of being more adapted to smaller datasets, they allow a thorough characterization of the effect of the variable of interest on the tested dataset. For the remaining of this chapter, additional statistics using frequentist approaches can be found in Annex B.

For this first task, we therefore applied a Bayesian logistic mixed model, as illustrated in Fig. 2.12, where different rows show the posterior distribution of model parameters. We fitted a **Bayesian binomial logistic mixed model**. Estimation were made using Markov-Chain Monte Carlo (MCMC) sampling with 4 chains of 5000 iterations and a warmup of 1000. We fitted the performance of mice in the task and tested for the effects of age, delay and interaction of age depending on delays. To minimize bias, we included mice as a random effect (formula: $\text{Performance} \sim \text{Group} \times \text{Delay} + (1|\text{Mouse})$). A weakly informative prior as student-t distributions (location = 0.00, scale = 2.50) was set over parameters, meaning that the generated probability distribution were modeled uniquely based on the data distribution. Our model's explanatory power was of $R^2 = 0.05$ (95% CI [0.03, 0.07]) and the part related to the fixed effects alone (marginal R^2) is of 0.03 (95% CI [5.38e-03, 0.07]). All our parameter estimations successfully converged and, using the ESS method (2.2.6), all the indices were considered as reliable. We set our threshold at 95% to consider the probability of an effect to be credible.

In figure 2.12, the GroupAged parameter (1st row) represents the overall difference between young and aged mice . We observe that this distribution is slightly shifted to the right suggesting a positive difference, but we also see that the shaded area near zero (values included in the ROPE, *i.e.* considered as similar to the null value) is close to the center distribution (*i.e.* it is included in the 95% credibility interval), which means that within the estimated parameters the null value and ROPE values are included within the credible interval. In this approach no p-values are provided (see a critique of using p-values here: Halsey, 2019; Skrepnek, 2007; Makowski et al., 2019), instead we report the median of our posterior distribution (Median = 0.53, 95% CI [-0.56, 1.64]), and characterize it based on three criteria: the probability of direction, here 84.09% probability of being positive (> 0), the probability for the effect to be

significant based on the probability for the null variable and the associated ROPE values to be within the credible interval, here 79.42% of being significant (> 0.09), and the strength of the effect, i.e. the probability of the effect to be large, here 48.51% of being large (> 0.54). Here, since the probability of direction and the probability of significance doesn't not reach above 95%, then we consider that, based on our data, there is not sufficient evidence to conclude on an effect of age on the overall delay performance in this task.

For the overall effect of delays on performance, regardless of age, our model reports the following: the effect of the 20s delay (Median = -0.18, 95% CI [-0.84, 0.48]) had a 70.22% probability of being negative (< 0), 60.14% of being significant (< -0.09), and 13.31% of being large (< -0.54); the effect of the 30s delay (Median = -0.39, 95% CI [-1.00, 0.19]) had a 90.66% probability of being negative (< 0), 84.55% of being

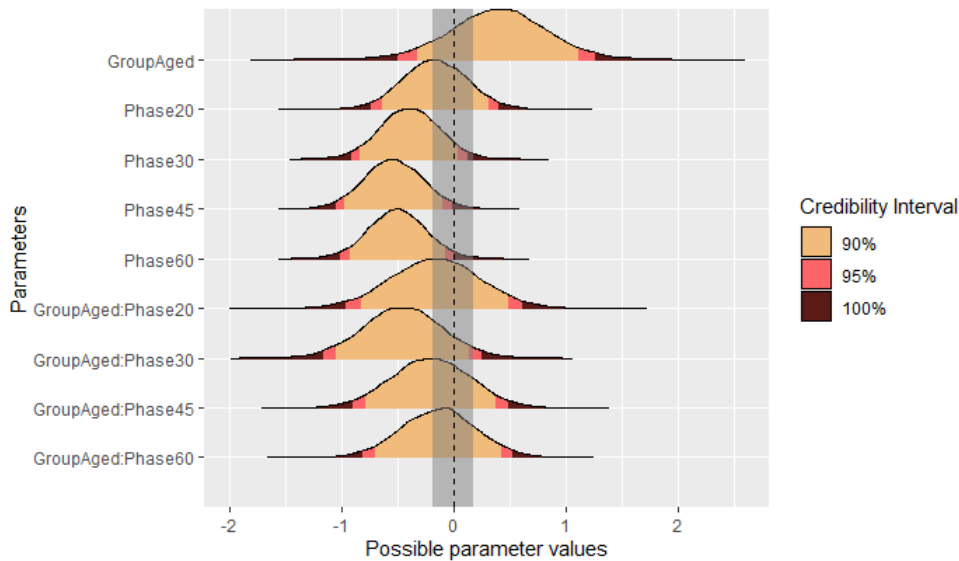


Figure 2.12: Bayesian estimation of parameters in the Y-maze. Here, the posterior distribution of each tested effect is represented (age, delay, age x delay). Colors represent Credibility Interval (CI), which is the interval most likely to include the real parameter of the tested effect. The dashed line represents a parameter value of 0 (i.e. no influence of the effect on the data distribution). The region shaded in grey represents the region of practical evidence (ROPE) that represents the area around the null value (i.e. 0) enclosing values that are equivalent to 0. Therefore, when the posterior distribution encloses within a 95% CI these values, then the tested effect has no effect on the data. Here, only the 45s and 60s delay do not enclose the null value, however they also encompass the ROPE: the effect of longer delays is marginal.

significant (< -0.09), and 31.72% of being large (< -0.54); the effect of the 45s delay (Median = -0.56 , 95% CI [$-1.15, 0.04$]) had a 96.56% probability of being negative (< 0), 93.61% of being significant (< -0.09), and 51.50% of being large (< -0.54); the effect of the 60s delay (Median = -0.53 , 95% CI [$-1.12, 0.06$]) had a 96.31% probability of being negative (< 0), 92.98% of being significant (< -0.09), and 47.53% of being large (< -0.54). These indicate a plausibility that longer delays (45s and 60s) have an effect on the performance of mice. However, since the threshold for significance was not reached, we consider this effect is most likely to be marginal.

When testing an interaction effect between the performance in delays based on the age group, our model reports the following : the effect of the interaction between age and a 20s delay (Median = -0.23 , 95% CI [$-1.18, 0.69$]) had a 68.84% probability of being negative (< 0), 61.81% of being significant (< -0.09), and 26.50% of being large (< -0.54); the effect of the interaction between age and a 30s delay (Median = -0.54 , 95% CI [$-1.40, 0.30$]) had a 89.66% probability of being negative (< 0), 85.04% of being significant (< -0.09), and 49.66% of being large (< -0.54); the effect of the interaction between age and a 45s delay (Median = -0.27 , 95% CI [$-1.10, 0.57$]) had a 73.08% probability of being negative (< 0), 65.96% of being significant (< -0.09), and 26.18% of being large (< -0.54); the effect of the interaction between age and a 60s delay (Median = -0.18 , 95% CI [$-1.01, 0.61$]) had a 67.77% probability of being negative (< 0), 59.58% of being significant (< -0.09), and 19.07% of being large (< -0.54). These indicate that healthy aged mice do not perform differently from adult ones in any of the tested delays.

Result summary

Overall, in the delayed-alternation task set in a Y-maze, our results suggest that age does not affect the performance of mice in during delays. To our surprise, longer delays (>30s) also had little influence on performances.

We concluded that the delayed-alternation in the Y-maze, in fact, does not represent a significant enough WM challenge when set in the Y-maze. More specifically, in addition to the high heterogeneity of performances, in this context, we supposed that, if any, evidence of age-related deficits may in reality, be hidden.

2.3.2 Spatial delayed-alternation task in the radial arm maze

Given the ambiguity of our previous results, in a second experiment, we adapted the delayed-alternation protocol in the 8-arm radial arm maze. We expected an increase of task difficulty in this setting because of the spatial complexity of the to-be-remembered locations, which we assumed to require a higher cognitive effort to encode and maintain spatial locations with precision. Still, the protocol was adapted in such a way that, as in the Y-maze, only two spatial locations were to be encoded and updated within the scope of one session. Therefore, we assume this task to require WM in a context of a low-interferent encoding but high cognitive effort. Therefore, we assumed this protocol to increase cognitive load in the encoding of spatial locations within the 8-arm radial arm maze, in comparison to the Y-maze, but not on the number of items to be maintained in WM.

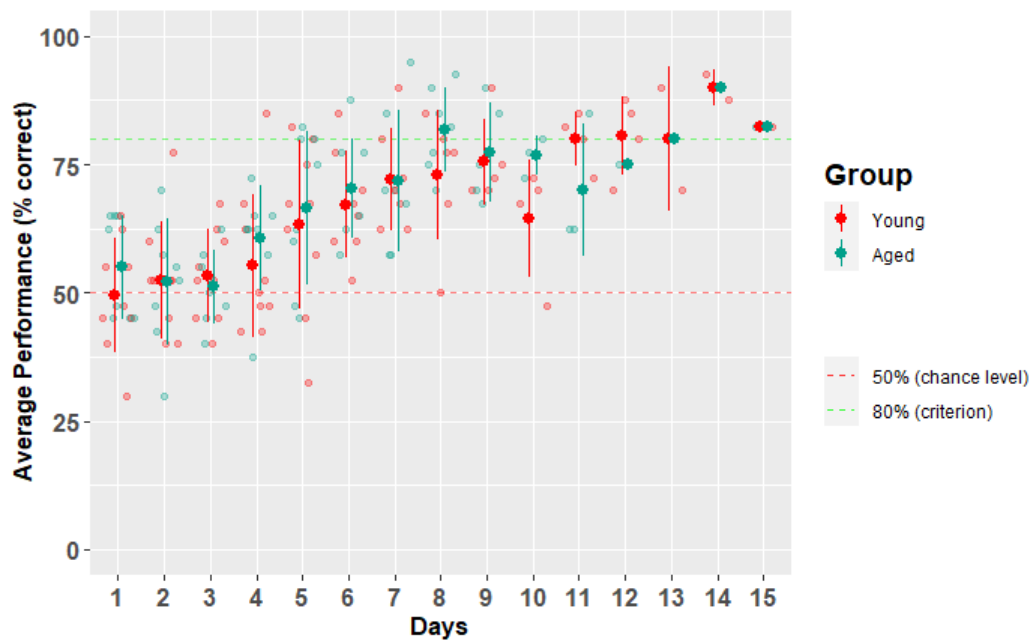


Figure 2.13: Learning in the delayed-alternation task set in the radial arm maze. Each point represent the average performance of a mouse over 4 sessions during learning with a 5s delay until criterion is reached.

Similar to our previous experiment, we compared the performance of young and healthy aged mice to test whether aging had an effect on the ability to maintain and update a single information within WM, in a context of minimum encoding

interference, but increased encoding effort. Based on our criterion, all mice completed training in 5 to 15 days (Fig. 2.13). We observed heterogeneity between mice in the number of days and sessions required to reach criterion, regardless of age. Overall, we did not find any evidence of an effect of age on learning performances and we observed that training until criterion in this task was critically longer than in the Y-maze setting. As initially predicted, we confirmed that the adaptation of the delayed-alternation protocol in the 8-arm radial arm maze increased task difficulty and we concluded it was adapted to the testing of spatial WM based on age and delay.

We tested mice in three delays (i.e. 5s, 30s, 60s) and we considered the 5s delay as the baseline performance. For all delay testing, mice were tested consecutively in a 5s delay then either a 30s or 60s delay, in their respective phase. We used such a sequence of testing as a control of the stability of mice baseline performance (Fig. 2.14). To analyze our data, we fitted a **Bayesian logistic mixed model**. Estimation were made using MCMC sampling with 4 chains of 5000 iterations and a warmup of 1000. Similar to the Y-maze, we fitted the performance of mice in the task and tested the effects of age, delay and interaction of age depending on delay. To

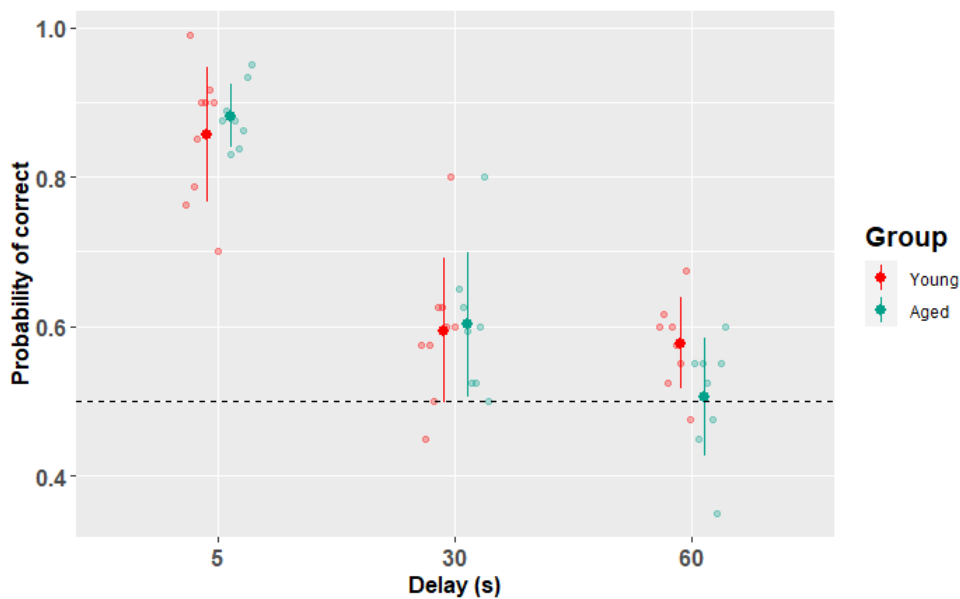


Figure 2.14: Delay performance in the radial arm maze delayed-alternation task. On the y-axis is represented the probability of each mouse to have a correct answer in a trial for each tested delay.

minimize bias, we included mice as a random effect (formula: Performance \sim Group x Delay + (1|Mouse)). An uninformative prior as student-t distributions (location = 0.00, scale = 2.50) was set over parameters, meaning that the generated probability distribution were modeled uniquely based on the data distribution. Our model's explanatory power is $R^2 = 0.13$ (95% CI [0.10, 0.15]) and the part related to the fixed effects alone (marginal R^2) is of 0.12 (95% CI [0.10, 0.15]). All our parameter estimations successfully converged and, using the ESS method (2.2.6), all the indices were considered as reliable. We set our threshold at 95% to consider the probability of an effect to be credible.

In our model, the sole effect of age (Median = 0.07, 95% CI [-0.29, 0.46]) had a 65.02% probability of being positive (> 0), 46.38% of being significant (> 0.09), and 1.00% of being large (> 0.54). This indicates that overall performance of mice in this task cannot be differentiated based on age (Fig. 2.15).

For the overall effect of delays on performance, regardless of age, our model reports the following: the effect of a 30s delay (Median = -1.60, 95% CI [-1.93, -1.28]) had a 100.00% probability of being negative (< 0), 100.00% of being significant (< -0.09), and 100.00% of being large (< -0.54); the effect of a 60s delay (Median = -1.62, 95% CI [-1.94, -1.30]) had a 100.00% probability of being negative (< 0), 100.00% of being significant (< -0.09), and 100.00% of being large (< -0.54). These indicate that longer delays (30s and 60s) impair all mice performances and that this effect is strong.

When testing an interaction effect between the performance in delays based on the age group, our model reports the following : the effect of the interaction between age and a 30s delay (Median = 0.03, 95% CI [-0.43, 0.50]) had a 55.35% probability of being positive (> 0), 40.41% of being significant (> 0.09), and 1.59% of being large (> 0.54); the effect of the interaction between age and a 60s delay (Median = -0.33, 95% CI [-0.78, 0.13]) had a 92.12% probability of being negative (< 0), 84.62% of being significant (< -0.09), and 17.81% of being large (< -0.54). These indicate that healthy aged mice do not perform differently from adult ones in any of the tested delays.

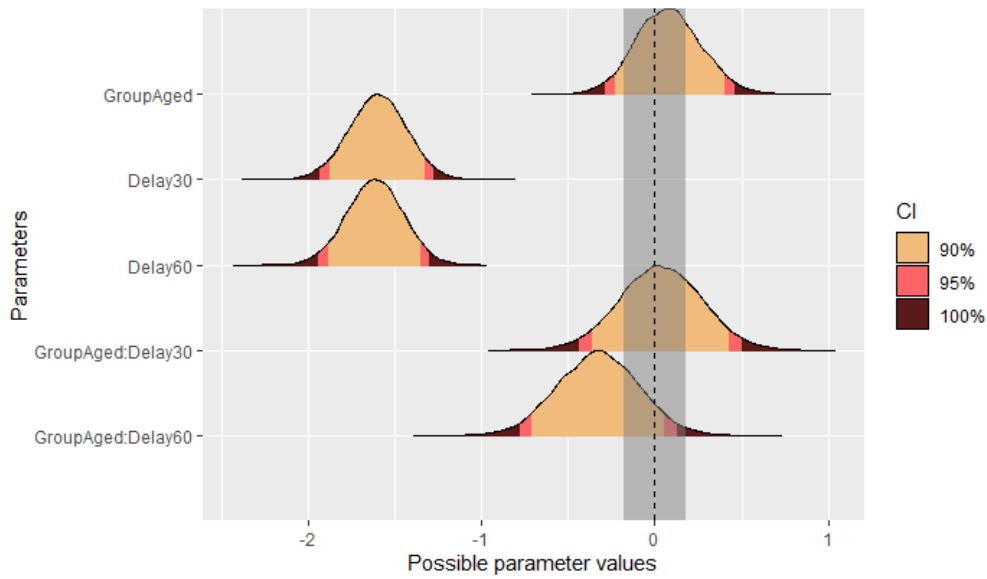


Figure 2.15: Bayesian estimation of parameters in the radial arm maze. Colors represent Credibility Interval. Dashed line represent 0 as a possible parameter value. The region shaded in grey represents the region of practical evidence (or ROPE) that represents the area around the null value enclosing values that are equivalent to 0. Therefore, when the posterior distribution encloses within a 95% CI these values, then the likelihood of the tested effect is marginal at most. If the posterior distribution do not encompass those values, then the variable has an effect on the data. Here, both longer delays had a significant impact on data, regardless of age.

Result summary

Overall, in the delayed-alternation set in the radial arm maze, our results put in evidence that age does not have an effect on the performance of mice.

As in both in the Y-maze and the radial arm maze version of the delayed-alternation task, age did not affect performance, in any of the tested delays, we concluded that in a low-interference encoding context (i.e. low cognitive load), the maintenance and stability of a unique information within WM is not affected by aging in the healthy aged mouse model, regardless of the required cognitive effort to encode the information to be stored in WM.

2.3.3 All-baited task in the radial arm maze

Next, we compared the performance of young and healthy aged mice in the all-baited task. We consider this paradigm to emphasize on the storage, maintenance, and manipulation of multiple information, here spatial locations, within WM. We assumed that this task creates a context of high encoding interferences and requires high encoding effort (2.1.3.2). We scored mice based on the number of correct entries in their first 8 runs within the maze (Fig. 2.16, Fig.2.21).

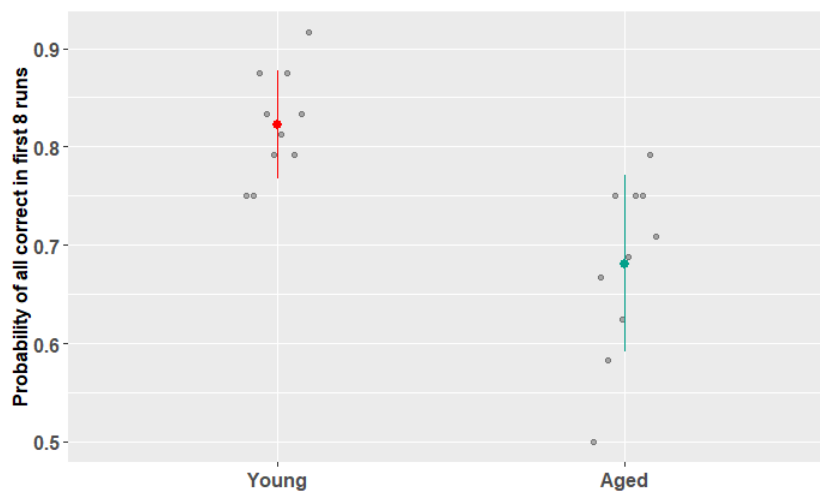


Figure 2.16: Performance in the All-baited task. On the y-axis is represented the probability for the mouse to correctly visit all arms of the radial-arm maze within the first 8 runs.

To analyze our data, we fitted a **Bayesian probit mixed model** (Fig. 2.17). Estimation were made using MCMC sampling with 4 chains of 5000 iterations and a warmup of 1000. We fitted the performance of mice in the task and tested the effect of age. To minimize bias, we included mice as a random effect (formula: Performance | Trials(8) \sim Group + (1|Mouse)). An uninformative prior as student-t distributions (location = 0.00, scale = 2.50) was set over parameters, meaning that the generated probability distribution were modeled uniquely based on the data distribution. The model's explanatory power is $R^2 = 0.45$ (95% CI [0.30, 0.58]) and the part related to the fixed effect alone (marginal R^2) is of 0.36 (95% CI [0.20, 0.49]). All our parameter estimations successfully converged and, using the ESS method (2.2.6), all the indices

were considered as reliable. We set our threshold at 95% to consider the probability of an effect to be credible.

Within this model, the effect of age (Median = -0.49, 95% CI [-0.66, -0.33]) had a 100.00% probability of being negative (< 0), 100.00% of being significant (< -0.09), and 26.01% of being large (< -0.54). This indicates that performance of mice in this task can be differentiated based on age. More specifically, aged mice show decreased performance in the all-baited task and this effect is considered as moderate.

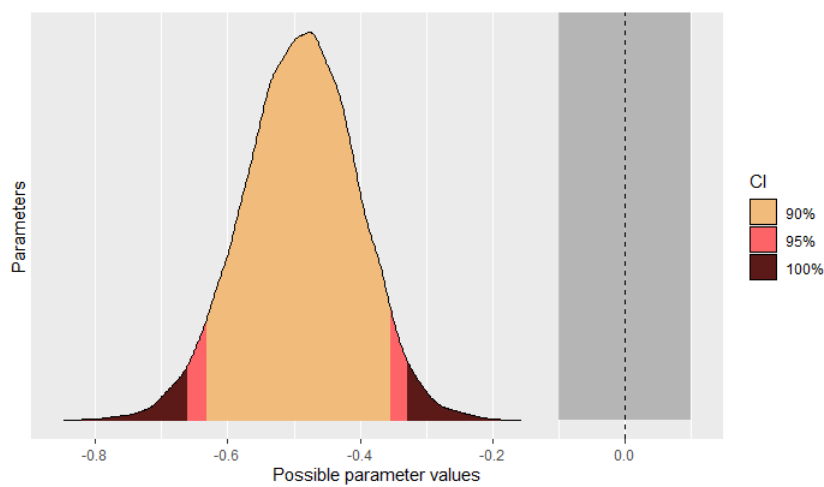


Figure 2.17: Bayesian parameter estimation in the all-baited task. Estimation of the effect of age on the data. Colors represent Credibility Interval. Dashed line represent 0 as a possible parameter value. The region shaded in grey represents the region of practical evidence (or ROPE) that represents the area around the null value enclosing values that are equivalent to 0. Therefore, when the posterior distribution encloses within a 95% CI these values, then the likelihood of the tested effect is marginal at most. If the posterior distribution do not encompass those values, then the variable has an effect on the data. Here, age has a significant effect on the data distribution.

Result summary

Overall, in the All-baited task set in the radial arm maze, our results put in evidence that age affects the performance of mice.

More specifically, we conclude that in a context of high encoding interference (i.e. high cognitive load), the maintenance and stability of stored information within WM is reduced during healthy aging in the mouse model.

2.3.4 Correlation between delayed-alternation and all-baited performances

We assume that optimal WM function relies on an equilibrium between stability and flexibility of mnemonic traces. Both of these aspects are tested and emphasized on, respectively, in delayed-alternation and all-baited tasks. As all mice tested in the 8-arm radial arm maze were subjected to both paradigms, we correlated individual performances to test the existence of a relation between the two performances in both age groups (Fig. 2.18).

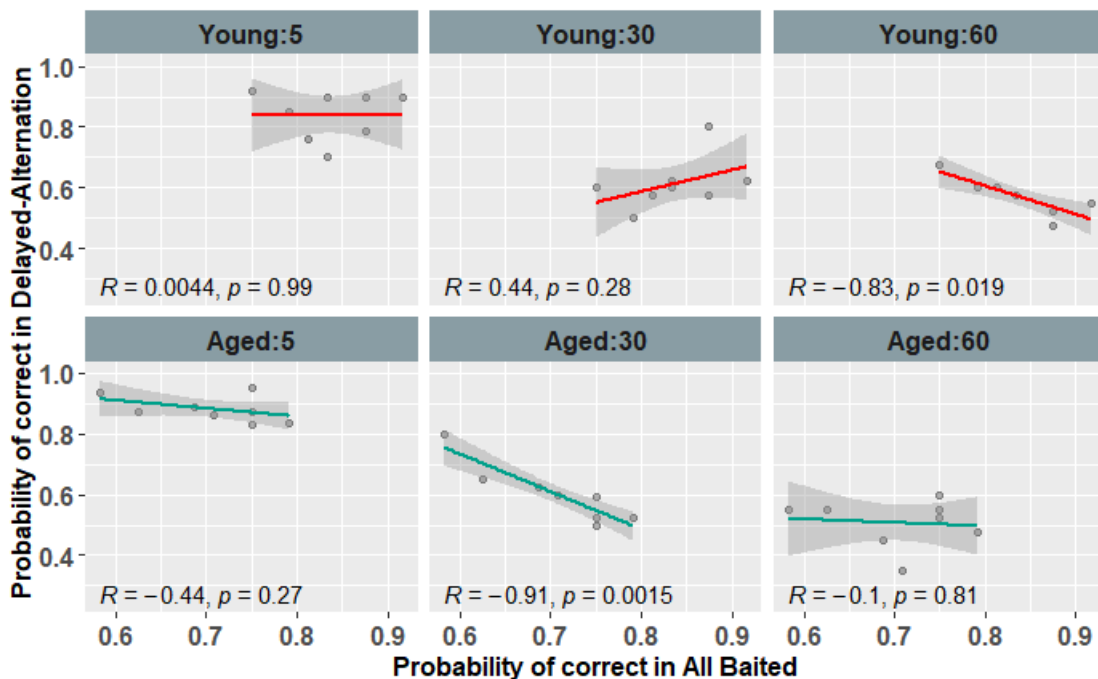


Figure 2.18: Pearson correlation of performance in the radial arm maze. Here, we show the correlation between performance in the all-baited task and delayed-alternation. For each, we indicate Pearson's correlation coefficient (R), and its associated p -value (p).

To do so, we used a **Bayesian correlation model** to test the existence of a relation between delay and all-baited performances. For both adult and aged groups, individual performances in each tested delay of the delayed-alternation task were unrelated to each other. However, our model indicated that there were evidence of correlations between specific delay performances in the delayed-alternation when compared to performance in the all-baited task. In adult mice (Fig. 2.19): correlation between

performance at the 5s delay and the all baited task had a probability of 50.42% of being negative ($\rho = -0.01$, 95% CI [-0.49, 0.54] and is considered as non-significant [27.52% in ROPE]); correlation between performance at the 30s delay and the all baited task had a probability of 79.95% of being positive ($\rho = 0.24$, 95% CI [-0.30, 0.68] and is considered as non-significant [19.85% in ROPE]); **correlation between performance at the 60s delay and the all baited task had a probability of 95.33% of being negative** ($\rho = -0.51$, 95% CI [-0.91, -0.05] and is considered as **significant** [4.30% in ROPE]).

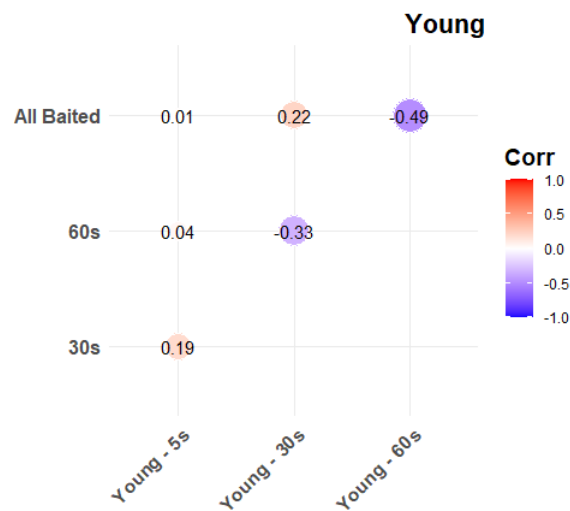


Figure 2.19: Bayesian correlation in the adult group. Estimation of the correlation between the performance in the all-baited task and each delay of the delayed-alternation task in adult mice. Here, there is a significant correlation of performance at a 60s delay.

In aged mice (Fig. 2.20): correlation between performance at the 5s delay and the all baited task had a probability of 79.97% of being negative ($\rho = -0.24$, 95% CI [-0.72, 0.28] and is considered as non-significant [18.12% in ROPE]); **correlation between performance at the 30s delay and the all baited task had a probability of 100% of being negative** ($\rho = -0.63$, 95% CI [-0.93, -0.28] and is considered as **significant** [0% in ROPE]); correlation between performance at the 60s delay and the all baited task had a probability of 57.25% of being negative ($\rho = -0.05$, 95% CI [-0.53, 0.47] and is considered as non-significant [29.78% in ROPE]).

These indicate that, collectively, performance in the all-baited task is correlated with performance in longer delays, respectively 30s in aged mice and 60s in adult mice,

when tested in the delayed-alternation task. However, based on the Bayes Factor (BF) (Fig. 1.12), only the evidence of correlation is moderate for the aged mice group ($BF=8.57>3$), whereas in adult mice, the correlation is considered as anecdotal ($BF=2.84<3$).

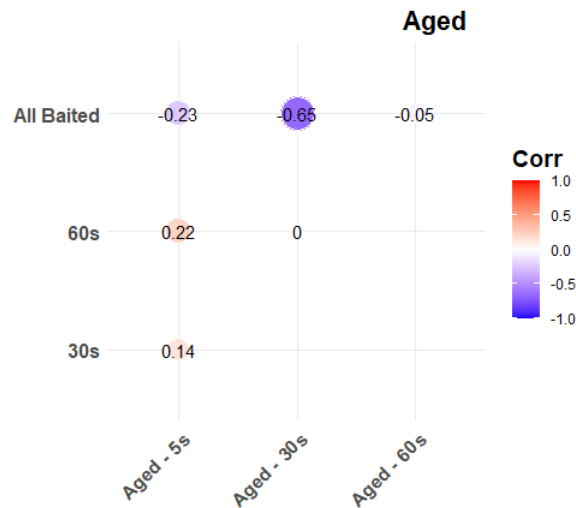


Figure 2.20: Bayesian correlation in the aged group. Estimation of the correlation between the performance in the all-baited task and each delay of the delayed-alternation task in aged mice. Here, there is a significant correlation of performance at a 30s delay.

Result summary

Overall, our results suggest that the more an individual performs well in a task requiring mnesic stability, the worse it is on a task requiring mnesic flexibility, and vice versa. This negative correlation is especially evident in aged individuals.

2.3.5 Decision-making related behavioral correlates

Finally, we tested whether mice behavior could explain performance in the all-baited task. More specifically, we were interested in exploratory and decision-making related behavior such as vicarious trial-and-error (VTE) and wall sniffing. We used DeepLabCut to track the position of mice bodyparts, including the nose (Fig. 2.21). We defined a region of interest within the maze that delimited the walls and entry of walls (Fig. 2.9) where we assumed these type of behaviors were most likely to occur. Number of entries of the bodypart 'nose' within this region were automatically counted, and considered as decision-making related behavioral correlates (Fig. 2.22).

We fitted a **Bayesian negative-binomial mixed model** (Fig. 2.23). Estimation were made using MCMC sampling with 4 chains of 5000 iterations and a warmup of 1000. We fitted the number of entries within the 'decision area' to test the effect of the age group, the performance and their interaction in the task. The model included each mouse as a random effect to control for bias (formula: $\text{entries} \sim \text{Group} \times \text{Performance} + (1|\text{Mouse})$). Priors over parameters were set as student-t distributions (location = 1.80, scale = 2.50). The model's explanatory power is $R^2 = 0.51$, 95% CI [0.25, 0.67] and the part related to the fixed effects alone (marginal R^2) is of 0.15 (95% CI [0.03, 0.30]). All estimations successfully converged and all the indices were considered as reliable.

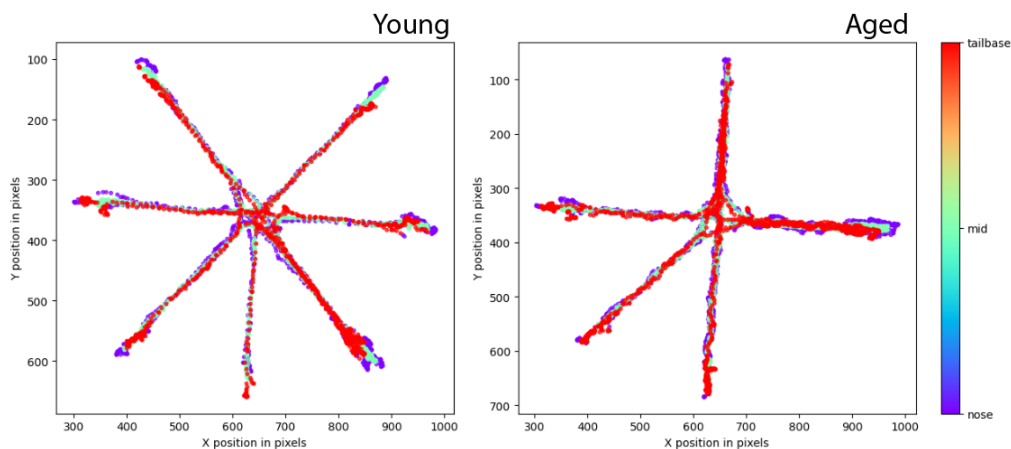


Figure 2.21: Example of trajectories in the All-baited task. Bodypart based trajectory extraction in the first 8 trials of the All-baited task from an adult (left) and aged (right) mice.

Within this model: The effect of age (Median = 3.05, 95% CI [0.25, 6.04]) had a 98.26% probability of being positive (> 0), 98.13% of being significant (> 0.05), and 97.31% of being large (> 0.30). This indicate that the number of entries can be differentiated based on age and that there is a strong age-related difference with an increase of entries for the aged group.

The effect of performance (Median = 0.41, 95% CI [0.05, 0.80]) had a 98.55% probability of being positive (> 0), 97.40% of being significant (> 0.05), and 72.97% of being large (> 0.30). This indicate that the performance is predictive of number of entries and this effect is moderate.

Finally, the interaction between group and performance (Median = -0.39, 95% CI [-0.86, 0.06]) has a 95.46% probability of being negative (< 0), 92.94% of being significant (< -0.05), and 64.72% of being large (< -0.30). This indicate that the respective performance of adult and aged mice is unlikely to influence on the number of entries in the decision area.

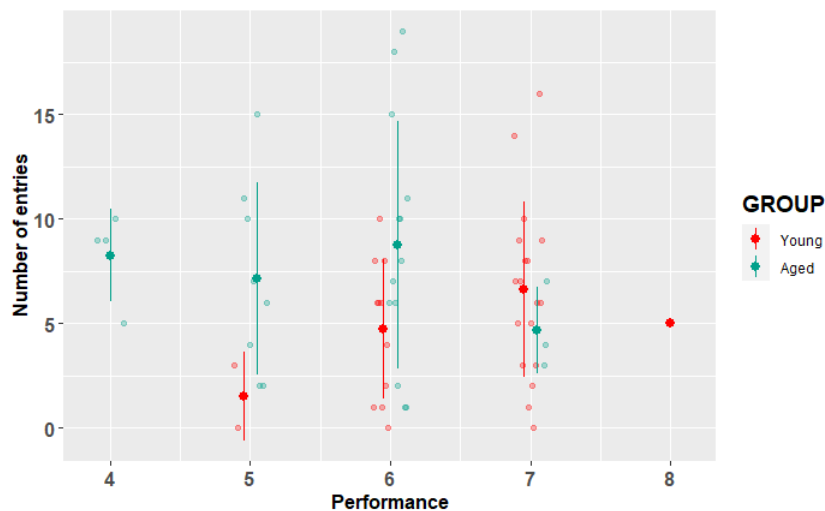


Figure 2.22: Number of entries in the 'decision area'. Number of entries within the labelled 'decision area' (see Fig. 2.9) extracted from the last three days of the all-baited task. We see here that overall aged mice tend to show higher numbers of entries compared to younger mice, regardless of performance.

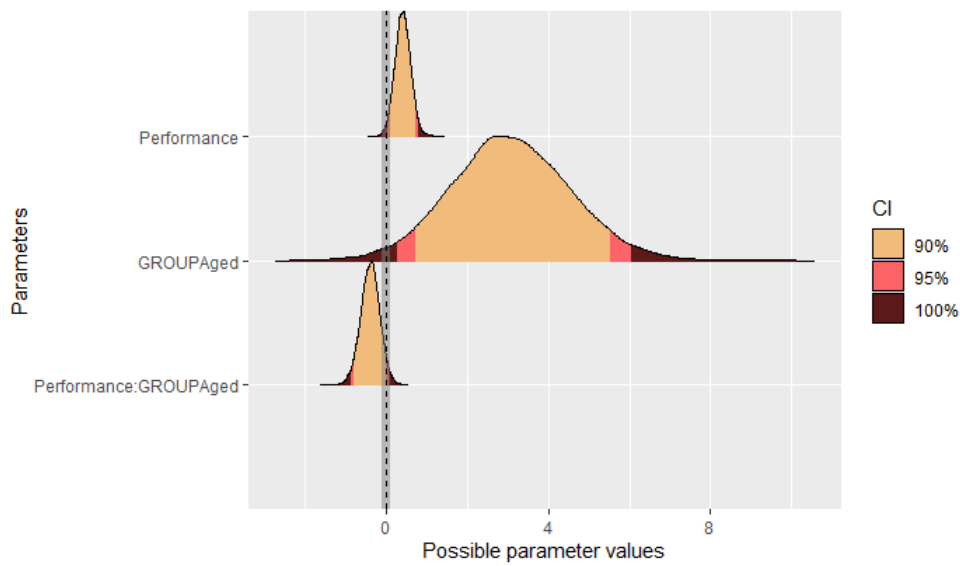


Figure 2.23: Bayesian parameter estimation of the number of entries in the 'decision area'. Estimation of the effect of age and performance on the data. Colors represent Credibility Interval. Dashed line represent 0 as a possible parameter value. The region shaded in grey represents the region of practical evidence (or ROPE) that represents the area around the null value enclosing values that are equivalent to 0. Therefore, when the posterior distribution encloses within a 95% CI these values, then the likelihood of the tested effect is marginal at most. If the posterior distribution does not encompass those values, then the variable has an effect on the data. Here, age and performance had a significant effect on the data distribution.

Result summary

Overall, analysis of mouse behavior in the all-baited task indicates that the better a mouse performs in the All-baited task, and the more they express decision-making related behaviors. We also show that these behavior correlates were overall more expressed in aged mice compared to adult ones but did not influence on their performance.

2.4 Discussion

Healthy aging is associated with a decline in WM in rodents. However, the extent to which the underlying subprocesses i.e. encoding, maintenance and manipulation are affected by healthy aging are not well characterized. Specifically, the extent to which WM maintenance was affected by healthy aging in the mouse model remained to be clarified. Here, we tested young (2-3months) and healthy aged (18-20months) mice in three behavioral paradigms that allowed to challenge the maintenance aspect of spatial WM in the following situations: (i) low encoding effort with minimum encoding interference, (ii) high encoding effort with minimum encoding interference and (iii) high encoding effort with high encoding interference.

We show that overall, in a context of low-encoding interference (low cognitive load), WM performance does not depend on age, regardless of the cognitive effort (precision) required to encode and maintain the relevant information. However, in a context requiring dynamic storage of multiple relevant information (high cognitive load), age affects WM performance and aged mice are more vulnerable to WM failure. Interestingly, we also observed that mice performance between a low cognitive load delay-dependent task and high-cognitive load task were negatively correlated, especially in older individuals. We found that the more an individual is susceptible to perform in a task requiring the temporal maintenance of a single information, the less it is likely to perform in a task requiring the flexible storage of multiple information within WM, especially in older mice. Finally, we show that aged mice are more likely to express VTE-like behavior, compared to younger mice.

Altogether, our results show that age-related WM deficits are evident in contexts requiring the maintenance of multiple information within WM, rather than the encoding effort required to maintain an information within WM. This suggests that the temporal maintenance of WM is affected by the number of items to be stored online (cognitive load) within WM rather than the sole ability to maintain the information within WM. More importantly, our findings suggest that inter-individual differences in the extent of age-related WM decline may be explained by the individual-dependent

tuning of the balance between neuronal states favoring the stability (*i.e.* less prone to interference) and those favoring the flexibility (*i.e.* more prone to interference)

2.4.1 The stability of mnesic traces encoded in working memory is not affected by healthy aging.

Delay-dependent paradigms are amongst the most widely used to test rodent WM. Their versatility allow either the emphasis on some sensory features (for ex: ODR task, Fig. 2.2) or not. Here, we chose a spatial approach in dry mazes which rely on high level integrations of multiple sensory features. This choice was justified because of (i) the explorative nature of mice, and (ii) we assumed that the integration of sensory information were not conflicting *per se*, but a precise encoding is required to register and differentiate spatial locations within an environment.

These paradigms emphasize on the temporal maintenance of an information within WM. We show that adult and aged mice share similar performances regardless of the cognitive effort required to encode spatial information. This suggests that the ability to hold and process a single information within WM is not affected by healthy aging. This implies that the mechanisms involved in the stability of an encoded mnesic trace within WM are preserved throughout healthy aging. Our result confirmed a previous study that tested mice in the delayed-alternation task in the T-maze (Bisaz et al., 2013). Still, the question remains on why do DNMTTP tasks lead to opposite conclusions on the effect of age during delay, when compared to the delayed-alternation task (Mizoguchi et al., 2009; Chisholm et al., 2013; Roy et al., 2019). Interestingly, in the DNMTTP, rodents are tested in pairs of trials consisting of a first information run where the animal is forced to enter in a rewarded arm of the maze, then a test run where the animal has both arm option available. In-between those runs, the animal is taken out of the maze for the delay of interest then put back in the maze to complete the trial. As in the delayed-alternation task, mice are rewarded when the alternation rule is applied.

Though both tasks are expected to challenge delay WM, as mentionned in Table 2.1, we suggest that the task contingencies differ and therefore affect the obtained results.

For example, forced encoding runs underlie a dynamic encoding of information. On the other hand, removing the animal of the maze can create a form of interference during maintenance, that would require a dynamic re-contextualization of the rule to be applied. This hypothesis is reinforced by our finding that VTE-like behavior are overall increased in aged mice, when compared to younger one. VTE is suggested be a behavioral output related to the recontextualization of spatial representations before decision-making. A previous study conducted in older rats show similar results as ours, with overall increased VTE behavior in a goal-directed task (Breton et al., 2015). Therefore, it is likely that the age-related WM deficits observed in older rodents during the DNMTTP may not be related to the delay itself, but to the age-related alterations in the maintenance of context representation.

2.4.2 The encoding of multiple mnesic traces in working memory leads to age-related cognitive decline.

Cognitive load is thought to have an influence on optimal WM function. Here, we show that the progressive increase in cognitive load of relevant information stored within WM is affected by healthy aging. Our study confirms results obtained in previous studies (Detoledo-Morrell et al., 1988; Ikegami, 1993) and suggests that increases cognitive load related to encoding interference increases the likelihood of WM failure in aged individuals.

One of the characteristics of WM is its limited storage capacity. Bond and colleagues tested the limits of WM capacity in young rats tested in the 8-arm radial arm maze (Bond et al. 1981). They found that up until the 7th choice, the probability of choosing a correct arm was above 80%. At the 8th choice it decreased to 70%. This suggests that the 8-arm radial arm maze covers a substantial amount of WM storage capacity in rodents. Thus, decreased performance of aged individuals in the all-baited task are suggestive of an age-related reduction in WM storage capacity.

While it is clear the aging affect the performance in the All-baited task, it is possible that the strategies used by mice may differ, specifically with the use of non-cognitive

strategies based on procedural or reference memory (for example, the mouse always chooses to go towards the arm on its right). We speculate that though possible, our data suggests otherwise. For instance, the VTE-like behavior observed during task are associated to the use of cognitive strategies to solve spatial task, and are not present when the task relies on non-cognitive procedures (Redish et al., 2016). Still, further work needs to be done using the extracted mice trajectories to differentiate patterned behavior and their VTE-like behavioral outputs.

2.4.3 There is an equilibrium between working memory sub-processes that is individual-specific and predictive of age-related deficits.

WM relies on a balance between subprocesses allowing the encoding, maintenance and processing of relevant information within their respective networks. Our result suggest a negative correlation between the ability to maintain an information within WM and the ability to flexibly store multiple information within WM. A study show a similar correlation in aged rats when correlating performance in an attentional set-shifting task, which requires a flexible shift of attention, and delay WM (Beas et al., 2013).

Maintenance of a mnesic trace within WM underlies a resistance to distractors and interferences whereas encoding and/or retrieval requires the availability of mnesic traces, and thus increase their vulnerability to interference. Specifically, our results suggest that the inner balance in the interplay between resistance and sensitivity is individual-dependent. Our data indicate that in younger individuals, these individual differences do not affect overall WM function, which can be explained by the fact that they are at their peak function. However, such is not the case in older individuals. We propose that during healthy aging, age-related alterations affect specifically the cellular mechanisms underlying cognitive flexibility. As those are thought to be for the most mediated through short-term synaptic plasticity, inter-individual differences in such inner balance may ultimately deepen gaps in between individuals in regards to their cognitive ability, especially in executive functions.

2.4.4 Healthy aging leads to age-related changes of neural networks during working memory: an increased involvement of long-term related neural processes?

Notably, the aforementioned correlation was evident at different delays in adult and aged mice. Indeed, the observed correlation is evident only at the 60s delay in adult mice and 30s delay in aged mice. Interestingly, performance of adult and aged mice did not differ in these longer delays. In WM models, the involvement of long-term memory (LTM) processes is thought to participate in the representation of context. Recently, the proposed activity-silent WM state suggests an active involvement of LTM processes in the maintenance of WM (Fig. 2.3). They are thought to act as a switch that participates in the selection for the 'online' availability of relevant mnemonic traces within WM, that are necessary for imminent processing (Beukers et al., 2021).

Based on this interpretation, it is currently unknown under which timescale 'online' signifies. We propose that the observed difference of delay correlation are reflective of the limits of 'online' WM maintenance. Specifically, we propose that in adult and aged mice, respectively at 60s and 30s, the observed correlations are reflective of the limits at which short-term WM involve the online maintenance of relevant information, without an involvement of LTM processes. Therefore, we suggest that during healthy aging, there is a shift towards an earlier involvement of LTM in WM.

This is further argued for based on the behavioral outputs of mice in the all-baited task. In our study, we found an overall increase of decision-making behavioral correlates in aged mice, regardless of performance. Those correlates can be assimilated as an approximation of vicarious-trial-and-error (VTE), a scanning behavior commonly attributed to animals prior to decision-making. It is considered as a sign of 'deliberation' or 'indecision' and associated with hippocampal activity, which is part of LTM networks. VTE is thought to reflect the representation of future outcomes prior to decision-making and action planning (Redish, 2016).

To reach optimal performance in the All-baited task, progression within this task is concomitant with an increase in cognitive load and a reduction in storage capacity.

Overall, mice with better performance in the all-baited task showed an increased expression of such behavioral patterns. One explanation could be due to the overcrowding of storage capacity leads to information loss. Such behavior may therefore be elicited to allow the recontextualisation of mnesic traces encoded in WM to compensate for this loss. We propose that the overall increase in expression of VTE-related behavioral correlates in aged mice further argues the age-related loss in WM storage capacity. Thus, the age-related increase of such behavior could be reflective of a mechanism elicited throughout healthy aging to compensate for storage loss and, overall, imply the increased involvement of LTM-related neural networks in WM function.

2.5 Conclusion

Executive functions and more specifically WM are vital processes that are found across species and are most likely to be evolutionary conserved. WM depends on the interplay between encoding, maintenance and processing of relevant information. Our study reveals that not all WM subprocesses are equally vulnerable to age-related alterations. Indeed, the ability to maintain a stable WM trace for goal-directed behavior is preserved during healthy aging, whereas the ability to store and maintain 'online' multiple relevant information within WM is impaired in the mouse model. Our study therefore further argues that age-related impairment in cognitive flexibility is one of the main predictor of age-related WM decline, and reflect common age-related decline of WM across species.

CHAPTER 3

Study 2: Age-related quantification of VTA DA subpopulations

Across species, healthy aging is associated with alterations in the DAergic system. In the PFC, reports indicate a decrease in prefrontal DA levels and reduced DA-related fibers. In the VTA, older individuals show reduced density in VTA DA neurons and reduced DA spontaneous activity. Still, VTA is a highly heterogeneous structure that is anatomico-functionally organized. VTA DA neurons are diverse in their molecular, electrophysiological and functional structures, which is reflected onto the topographic position of cell-bodies.

Interestingly, this diversity is thought to influence on the extent of vulnerability of DA neurons to neurodegeneration. In particular increased expression of Glutamate (Glu)-dependent markers is thought to emerge as a neuroprotective mechanism in DA neurons following neurotoxic lesions. Therefore, though reduced neuronal density of DA neurons in the VTA as a whole is reported in the literature, it still remains unclear whether this is due to neurodegeneration.

In this chapter, we compared in young (2-3 months) and aged (18-20months) mice, the density of VTA subpopulations based on their density of their cell-bodies according

to their topography, their type (DA, Glu, etc.) and their connectivity (mesocortical neurons). To do so, we used (i) all-brain immunostaining to reconstruct and quantify VTA DA cell bodies in the whole VTA based on their topography, and (ii) stereotaxic injections to label mesocortical neurons that were coupled with triple immunostaining in VTA slices to quantify the density of VTA mesocortical DA, Glu and DA/Glu co-expressing neurons.

3.1 Introduction

Executive functions, including working memory, depend on an intricate interplay between a diverse set of neuronal networks involving cortical and subcortical structures, one of which is the mesocortical pathway (Fig. 1.1). The mesocortical pathway characterizes the anatomo-functional connectivity between the PFC and VTA, and its components are known to be affected during healthy aging, which are thought to underlie age-related WM decline. Specifically, alterations in the levels of DA in PFC are thought to be hallmark of healthy aging.

3.1.1 The ventral tegmental area

3.1.1.1 Anatomo-functional proprieties

DA release in PFC is for the most originated from VTA DAergic mesocortical neurons. VTA is a small heterogeneous structure part of the midbrain composed of neurons expressing DA, glutamate (Glu), γ -Aminobutyric acid (GABA), and co-releasing neurons (Fig. 3.1) (Morales & Margolis, 2017; Poulin et al., 2020). VTA is a densely interconnected region that share most of its connectivity with basal ganglia, limbic areas and to a lesser extent with cortical areas such as PFC (Beier et al., 2015; Beier et al., 2019; Morales & Margolis, 2017).

In the last decades, animal studies have highlighted the high diversity within mid-brain VTA neurons, including DAergic ones (Poulin et al., 2020). It is suggested that based on their topography within VTA on the mediolateral (ML) (Beier et al., 2019; Floresco et al., 2014) or anteroposterior (AP) (Sanchez-Catalan et al., 2014; Petzel et al., 2017) axis, DA neurons express distinct molecular (Poulin, 2020), electrophysiological (Lammel et al., 2014) and functional (Engelhard et al., 2019; Cai et al., 2020) signatures. Interestingly, such conclusion is also reflected in recent mapping studies (Beier et al., 2015; Beier et al., 2019). Some DA neurons are also able to co-release Glu (Steinkelner et al., 2018; ; Buck et al., 2021; Kouwenhoven et al., 2020; Shen et al., 2021) or GABA (Tritsch et al., 2014; Bariselli et al., 2016; Morales & Margolis, 2017).

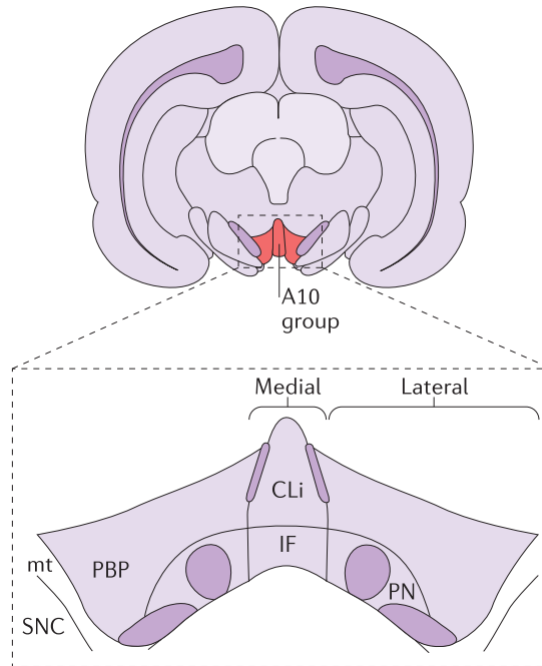


Figure 3.1: Schematic of VTA in the mouse brain. The A10 group, or VTA, (highlighted in red in the upper panel of the figure) is distributed across several subregions, including the parabrachial pigmented nucleus (PBP), paranigral nucleus (PN), caudal linear nucleus (CLi), interfascicular nucleus (IF) and rostral linear nucleus of the raphe (RLi; not shown); mt: medial terminal nucleus of the accessory optic tract; SNC: substantia nigra pars compacta.

From Morales & Margolis, 2017

Beier and colleagues reported that among all VTA neuronal subpopulation (*i.e.* DA, Glu, etc.), though segregated in their outputs, the localization of the soma is topographically clustered based on the receiving inputs (Beier et al., 2015; Beier et al., 2019). This suggests, for example, that neurons receiving inputs from PFC are most likely to be located close together. Based on this result, the authors highlighted four anatomo-functional clusters along VTA ML axis, regardless of the released neurotransmitter (Beier et al., 2019). Specifically, they report that PFC-receiving VTA DA neurons were located in medial VTA, whereas striatal-receiving tend to be found in lateral VTA (Fig. 3.2) (Beier et al., 2015).

3.1.1.2 Importance of the ventral tegmental area in working memory function

In goal-directed behavior, VTA DA activity is for the most attributed to reward processing. Electrophysiological recordings show that their activity encode unexpected,

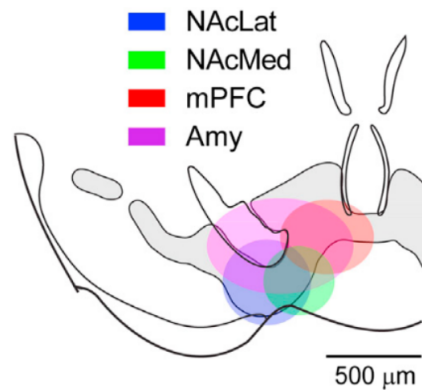


Figure 3.2: VTA input-based population clusters. NAcLat: lateral Nucleus Accumbens; NAcMed: medial Nucleus Accumbens; mPFC: medial Prefrontal Cortex; Amy: Amygdala
From Beier et al., 2015

anticipated and omitted rewards, all of which are referred to as the reward-prediction error (RPE) (Fig. 3.3) (Shultz et al., 1997; Morales & Margolis, 2017). VTA DA neurons are also thought to participate in the encoding of aversive stimuli (Lammel et al., 2014), novelty (Duszkiewicz et al., 2019), the processing of stress-related events (Matsumoto & Hikosaka, 2009; Chaudhury et al., 2013) and learning (McNamara et al., 2014).

Synchronization between VTA and PFC are essential for WM function (Fujisawa & Buzsáki, 2011) and require VTA-DA activity for its optimal use (Choi et al., 2020). Firing of VTA DA neurons follow along two types of firing dynamics: tonic and phasic (Grace, 1991; Goto & Grace, 2007). Phasic release characterises a large amplitude transient release (burst firing) whereas tonic release characterizes a spontaneous release of DA that participate in the maintenance of baseline levels of extracellular DA. In the PFC, phasic DA release is thought to participate in the recognition of relevant events for goal-directed behavior (Popescu et al., 2016; Ellwood et al., 2017) whereas tonic activity is thought to participate in the maintenance of behaviorally relevant associations (Ellwood et al., 2017).

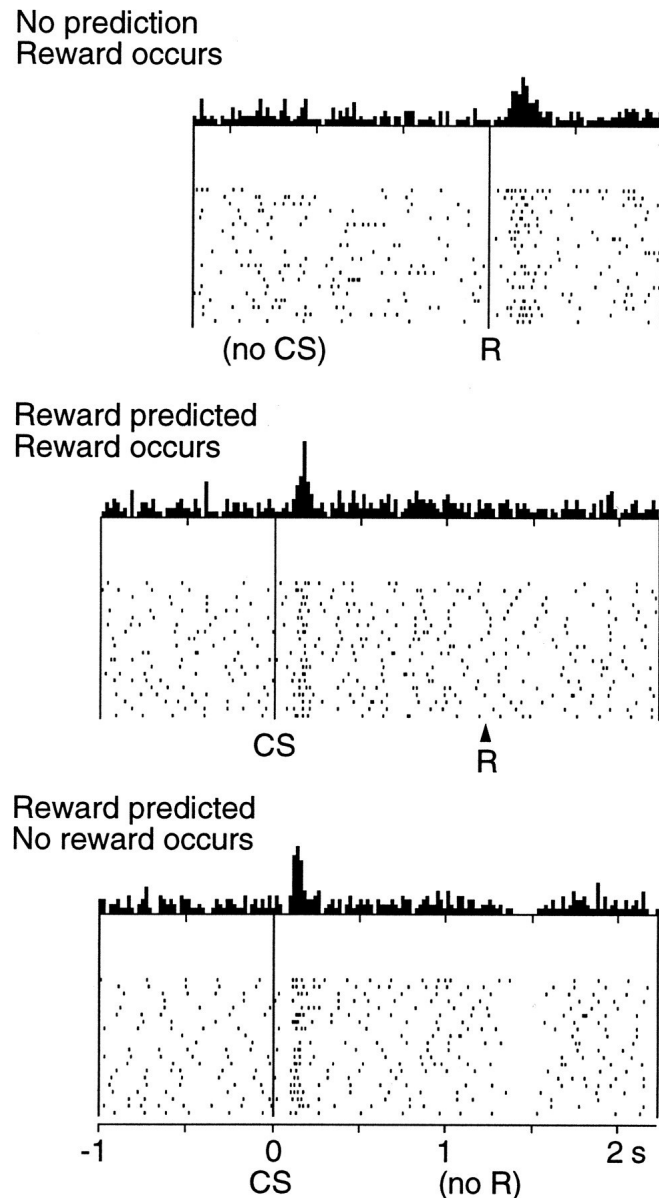


Figure 3.3: Reward-prediction error. Top: Unexpected rewarding stimuli. Occurrence of reward thus constitutes a positive error in the prediction of reward. DA neuron is activated by the unpredicted occurrence of the stimuli. Middle: Conditioned stimulus predicts a reward and the reward occurs according to the prediction. No error in the prediction of reward. DA neuron fails to be activated by the predicted reward (right). It also shows an activation after the reward-predicting stimulus, which occurs irrespective of an error in the prediction of the later reward (left). Bottom: Conditioned stimulus predicts a reward, but the reward fails to occur because of lack of reaction by the animal. Activity of the dopamine neuron is depressed exactly at the time when the reward would have occurred. CS, conditioned stimulus; R, primary reward.

From Schultz et al., 1997

3.1.2 How does healthy aging affect the mesocortical pathway?

As introduced in Chapter 2, healthy aging is characterized by the accumulation of numerous alterations, including in the mesocortical pathway. Age-related alterations of the DAergic system are thought to contribute in age-related loss of PFC integrity across species (Braver & Barch, 2002), which includes decreased tonic and phasic release of prefrontal DA (Mizoguchi et al., 2009; Ota et al., 2006; Segovia et al., 2007; Bäckman et al., 2006), decreased expression of D1R (Keyser et al., 1990), reduced DA-related fibers (Mizoguchi et al., 2009; Chisholm et al., 2013; Tomm et al., 2018; Brummelte & Teuchert-Noodt, 2007), losses of DA varicosities (Allard et al., 2011) and overall reduced VTA DA spontaneous activity (Shan et al., 2023).

As VTA DA modulation contributes to synaptic plasticity in PFC, its role is essential in executive functions, including WM (Goto et al., 2010; Bäckman et al., 2006; see 1.4.3). Therefore, it is hypothesized that during healthy aging, structural alterations in VTA DA neurons participate in age-related WM decline and prefrontal DA function is considered as a hallmark of healthy aging.

Midbrain DA neurons present diverse molecular profiles (Poulin et al., 2020), which are thought to differently influence their susceptibility to neurodegeneration, including in VTA (Di Salvio et al., 2010; Buck et al., 2021; Caudle et al., 2007). Interestingly, when neurotoxic lesions are made in the midbrain, though there is an overall loss of DA neurons, it is concomitant with an increase in expression of vesicular Glutamate Transporter 2 (vGluT2), found on Glu vesicles within Glu releasing cells (Steinkelman et al., 2018; Buck et al., 2021). vGluT2 expression in DA neurons is associated with axonal growth (Kouwenhoven et al., 2020) and its upregulation prior to neurotoxic lesions in DA cells increased survival (Buck et al., 2021). Thus, overall, the upregulation of vGluT2 is thought act as a neuroprotective effect.

3.1.3 Study 2: The effects of healthy aging on dopamine neurons in VTA

3.1.3.1 Rationale & Working hypothesis

DA release in the PFC is a critical factor underlying WM. Yet, during healthy aging, age-related WM decline is associated to alteration of the dopaminergic system, and DA is considered as a hallmark of healthy aging. However, the origin of the age-related decrease in prefrontal DA release remains unknown. A common hypothesis proposed to explain age-related decrease of prefrontal DA is that neurodegeneration, because DA neurons are more susceptible to oxidative stress and thus cellular degeneration. Yet, this hypothesis was never formerly tested in aged individuals. In Chapter 2, we confirmed that the effects of aging in WM function are similar in rodents and humans. Therefore, we assume that the underlying components participating in age-related cognitive decline may be similar between rodent and humans.

In previous studies, the quantification of age-related density of VTA DA neurons relied on the estimation of overall Tyrosine Hydroxylase (TH) expression, an enzyme participating in the synthesis of DA (Noda et al., 2020; Gür et al., 2020). However, this methodology relied on a biased quantification that could not give sufficient evidence to conclude on whether healthy aging contributed to age-related neurodegeneration of DA neurons. Specifically, these studies relied on the overall quantification of TH, which is known to be found in DA cell soma, fibers but also in noradrenergic neurons. Moreover, this quantification does not allow to distinguish between inputs and outputs DA fibers in VTA. Furthermore, given the heterogeneity of VTA DA neurons, it still remains unclear to what extent a loss of TH expression may be due to overall cellular degeneration across VTA or whether it is due to axonal degeneration, or even whether these changes in expression are a consequence of molecular shifts in neuronal identity, both at a somatic and fibre level. Therefore, we hypothesized that the observed decrease of TH expression in the mouse VTA is not due to DA neurodegeneration but due to a molecular shift towards the co-expression of Glu, as a neuroprotective effect.

3.1.3.2 Experimental approach

To test our hypothesis, we conducted two studies to quantify the age-related densities of VTA subpopulations in young (2-3 months) and aged (18-19 months) mice. We first aimed at characterizing the overall density of VTA DA soma. To do so, we took advantage of whole-brain immunostaining and clearing to preserve the integrity of the VTA structure. We developed a semi-automatic workflow to extract the number of DA soma in VTA, which allowed us to estimate the density of VTA DA neuron without the methodological bias described above. Furthermore, our quantification allowed to characterize the topography of DA neurons, and test whether there were age-related differences in their topological distribution.

Then, we aimed at comparing the density of DA and DA/Glu co-expressing neurons in VTA. To do so, we coupled stereotaxic injections with triple fluorescent immunostaining to quantify the density of DA and co-expressing neurons within VTA, but also their density within mesocortical neurons. As the tested antibodies were not compatible with clearing techniques, we used confocal microscopy on free-floating slices to identify and quantify the neurons of interest.

3.2 Material & Methods

3.2.1 Ethics

All experimental procedures are approved by French institutional and government animal care committees. Animals were all provided by Janvier Labs at either the age of 2 months or 18 months. Upon arrival and until sacrifice, animals were cared for by the staff of the animal facilities within the Vision Institute and the principles of the 3 R's, as stated in the European Directive 2010/63/EU were rigorously applied.

3.2.2 Animals

A total of n=25 male C57BL6J mice of either 2 (adult) or 18 (aged) months old were used. Upon arrival and prior to any manipulations, animals were left 5 days of acclimation. They were housed by groups of 1-3 per cage with enrichment (tunnel, cotton, wood stick) to minimize stress. However, when aggressive dominant behavior was observed, the dominant animal was isolated and extra enrichment was added to the cage.

3.2.3 Stereotaxic injections

Mice were anesthetized using isoflurane (5% to induce deep sleep, 1-2% for maintenance in anesthetized state). Mice were injected with 200nL of Cholera toxin-B (CTB), a retrograde tracer at a rate of 20nL/min. They were unilaterally injected in the right vmPFC, specifically targeting the prelimbic area adjusted from the mouse brain atlas. Mice were sacrificed 2-4 days following surgery.

3.2.4 Sample preparation

Mice were anesthetized with an intraperitoneal injection of Ketamine (0.2%) and Xylazine(0.1%) diluted in saline before sacrifice through intracardiac perfusion of paraformaldehyde (PFA) at 4%. Brains were extracted and post-fixed in PFA4% for 2 days before being stored in Phosphate Buffered Saline (PBS) until use. To prepare samples for staining, brains were first dehydrated through progressive increase of

methanol solution diluted in PBS (50%, 80%, 100%, 100%)? Each incubation lasted a minimum of 1h30 at room temperature. Samples were then bleached overnight using a solution of 10% H₂O₂ diluted in MeOH at 4°C and protected from light. The samples were then rehydrated through progressive decrease of methanol solution diluted in PBS (100%, 100%, 80%, 50%) then stored back in PBS at 4°C until use.

3.2.5 Whole-brain Immunostaining

For *in toto* immunostaining procedures, we used a solution of PBS with 0.2% gelatin and 0.5% or 5% Triton. For blocking and permeabilization, all samples were incubated for 4 days in PBSGT0.5% at room temperature. Primary antibodies (rabbit anti-TH 1/800, AB152, Millipore) were incubated for 2 weeks with PBSGT5% and 10X saponine at 37°C. Secondary antibodies were incubated (donkey anti-rabbit A594 1/500, Invitrogen) for 2 days at 37°C. Samples were rinsed in-between antibody incubations with PBSGT0.5% 6-7 times. Samples were then stored in PBSGT0.5% at 4°C until clearing procedure.

3.2.6 Triple-immunostaining on slice

Brains were sliced in a vibratome in coronal sections with a stepsize of 40µm. Free-floating slices were then permeabilized and blocked using PBSGT0.5% for 2h at RT. They were then rinsed 3 times with PBS, then incubated for 2 days in the primary antibody solution (Chicken anti-TH 1/500, rabbit anti-vGluT2 1/1000). Following incubation, slices were washed 3 times with PBS, then incubated for 2 hours in secondary antibody (Alexa-fluor 594 1/1000, 647 1/2000). In the last 10min, DAPI 1/1000 was added. The slices were rinsed 3 times in PBS, then mounted on slides.

3.2.7 Clearing

Samples were cleared using a methanol clearing protocol adapted from the iDISCO+ protocol (Renier et al., 2016). Samples were first dehydrated with progressive increase of MeOH dilutions (20%, 40%, 60%, 80%, 100%, 100%) for at least 1h30 at room temperature. Samples were delipidated using a solution of 2/3 dichloromethane (DCM) and 1/3 MeOH overnight at room temperature. Samples were then incubated

for a maximum of 30 minutes in pure DCM at room temperature, before transfer in dibenzylether (DBE). All samples were stored in DBE, protected from light at room temperature.

3.2.8 Imaging

Cleared samples were acquired using a light-sheet microscope (3.2.10) with a 4X objective at 1X zoom. All optical slices were acquired with a step of 1 μ m. Slices were acquired using a confocal microscope (Olympus) with a 40X oil objective with a step of 1 μ M. Images representative of all VTA were acquired.

3.2.9 Image analysis (whole brain)

Stacks obtained were stitched together using Imaris Stitcher. All imaged were then manually pre-segmented to isolate VTA in Imaris (v10.0). Using ImageJ, a top hat filter of a 10 pixel radius was applied on all images to enhance signal of interest. Images were then segmented in Imaris using a semi-automatic segmentation procedure for object detection. The images were then semi-automatically filtered upon visual inspection in order to eliminate non-specific signaling (background, lipofuscin, fibers). Only segmented soma were used for quantification of the density of neurons within VTA. Mediolateral, anteroposterior and dorsoventral were characterized based on half the distance from the origin (set on central VTA).

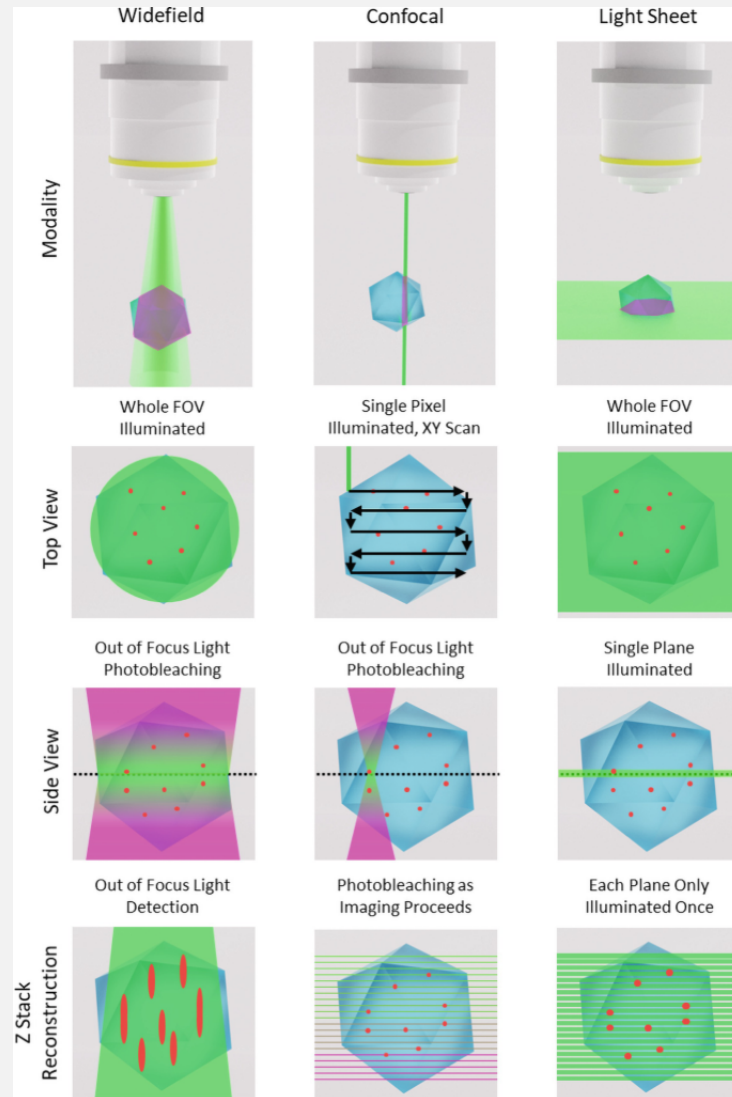
3.2.10 Statistical analysis

We used Bayesian inferences to analyze the data as it allowed to gather information about the hypothesis being tested based on the collected data. Our data structure consisted of independent count measures of the number of particles segmented in VTA of each individuals in each age group. We attributed a variable 'Topography' to characterize the localization of the number of segmented particles in either the mediolateral, dorsoventral or anteroposterior axis. For all mice, there was therefore 2 subgroups of total segmented particles based on the studied axis.

We used the variables Topography and Group as fixed effects. For all analysis, we used the R software. We used the STAN-based package brms for Bayesian inferences (Burkner, 2017). To test our data, we used uninformative priors.

Following the Sequential Effect eXistence and sIgnificance Testing (SEXIT) framework, we reported the median of the posterior distribution and its 95% Credibility Interval (Highest Density Interval), along the probability of direction (pd), the probability of significance and the probability of being large. The thresholds beyond which the effect is considered as significant (i.e., non-negligible) and large are $|0.09|$ and $|0.54|$. Convergence and stability of the Bayesian sampling has been assessed using R-hat, which should be below 1.01 (Vehtari et al., 2019), and Effective Sample Size (ESS), which should be greater than 1000 (Burkner, 2017).

Box 1: Types of microscopy acquisitions.



Widefield microscopy is characterized by an illumination of the whole field of view (FOV) which result in an out of focus light, and photobleaching of the FOV. **Confocal microscopy** has a single pixel point resolution. As imaging proceeds along the plane, photobleaching occurs throughout depth of sample. **Light-sheet microscopy** is based on a planar illumination over the whole sample. The plane is illuminated only once, which limits photobleaching and speeds up acquisition time, compared to confocal microscopy.

Adapted from Merces & Reynaud, 2022

3.3 Results

3.3.1 Whole-brain semi-automatic counting of DA soma

To quantify the age-related densities of VTA subpopulations in young (2-3 months) and aged (18-19 months) mice, in this first study, we used whole-brain immunostaining and clearing techniques to quantify the density of DA cell-bodies in VTA. To do so, it was first required to adapt the iDISCO+ protocol to immunostain and clear older brains. The quality of a whole-brain immunostaining relies on the following: the volume of the sample and its resistance to permeabilization as well as the ability of the primary and secondary antibodies to penetrate within the tissue and their compatibility with clearing solutions. Adult brains are highly myelinated which creates more resistance to the penetration of external molecules within neuronal cells. This required the adaptation of previous protocol in order to successfully use the TH antibody to visualize DA neurons within VTA, which was previously described as compatible with iDISCO+ in pups (Godefroy et al., 2017; Godefroy et al., 2019). To do so, we increased the concentration of permeabilization solutions to obtain iDISCO+ compatible brain (see 3.2.4).

The used TH antibody successfully labelled catecholaminergic neurons as previously described (Godefroy et al., 2017; Godefroy et al., 2019). Labelled regions included the locus coeruleus and the VTA, where whole cell-bodies and fibers were visible (Fig.3.4).

After imaging the whole VTA using a light-sheet microscope (see Box 1, 3.2.10), we obtained image stacks with a step size of 1 μ m for all animals in the form of a 2x2 mosaic that were manually stitched together using Imaris Stitcher, with a 10% overlap (Fig. 3.4).

Our semi-automatic methodology for the 3D quantification of VTA DA neuron soma relied first on the pre-processing of images using ImageJ with a top hat filter of a radius of 10 pixels to enhance the contrast between the soma and background (Fig.

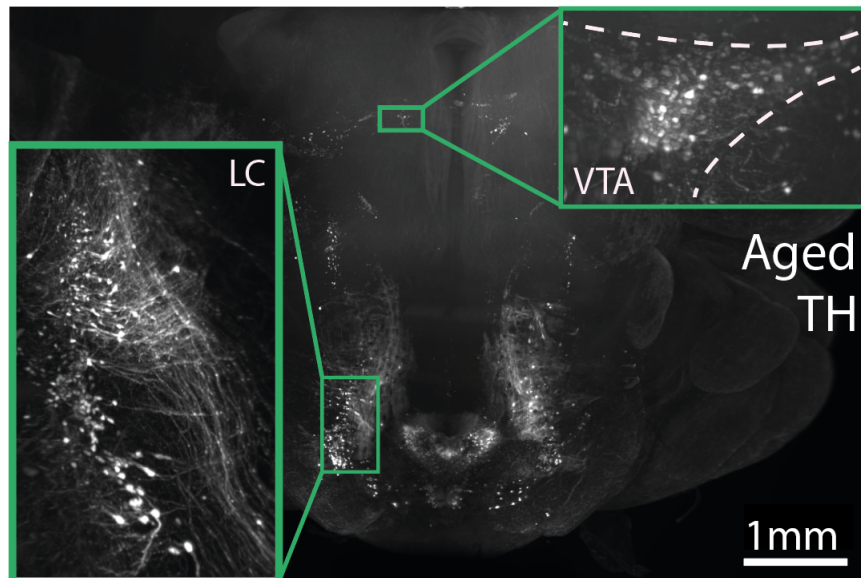


Figure 3.4: Whole-brain TH+ staining. Example of an aged mouse brain stained for Tyrosine Hydroxylase (TH). LC: Locus Coeruleus, VTA: Ventral Tegmental Area.

3.5). The filtered images were then segmented using Imaris (v.10.0). We identified based on the mouse brain atlas the location of VTA within each mouse brain, then applied local thresholding to segment the intensity of interest (here high intensity DA neurons within VTA). Each brain had to be manually thresholded based on visual inspections as the detected intensities and their contrast with background signal tend to differ between imaged brains. We observed a variety in expression of TH across neurons within VTA (Fig. 3.5). Here, only the high-intensity populations were taken into account for quantification.

Once particles were segmented, we needed to remove all segmented particles that were unrelated to neuronal cell bodies. Bright intensity particles could be associated with lipofuscin accumulation (especially in aged mouse brain), fibers and background vessels. To remove such noise, all segmented particles were manually filtered based on visual inspection in order to suppress those unrelated to cellular bodies. To do so, we used a combination of size, intensity and topographical filters. For each VTA, an 'origin' was manually set at the same position in order to determine the position of segmented particles based on either the mediolateral (ML), anteroposterior (AP) or dorsoventral (DV) axis (Fig. 3.6).

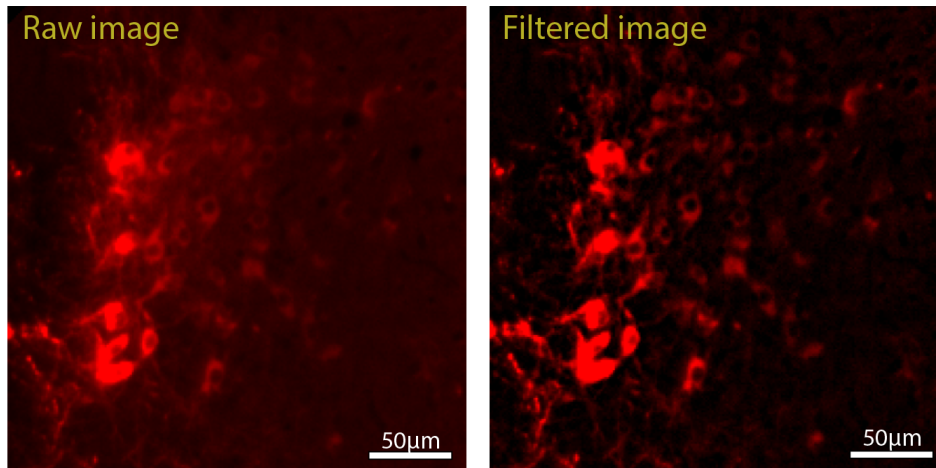


Figure 3.5: Pre-processing. On the left is the raw image, on the right the preprocessed image with a tophat filter. In each images, we can see a very bright neuronal population on the left, where the intensity of TH expression is at its peak, whereas on the right we see a less bright neuronal population, due to probably lower TH expression.

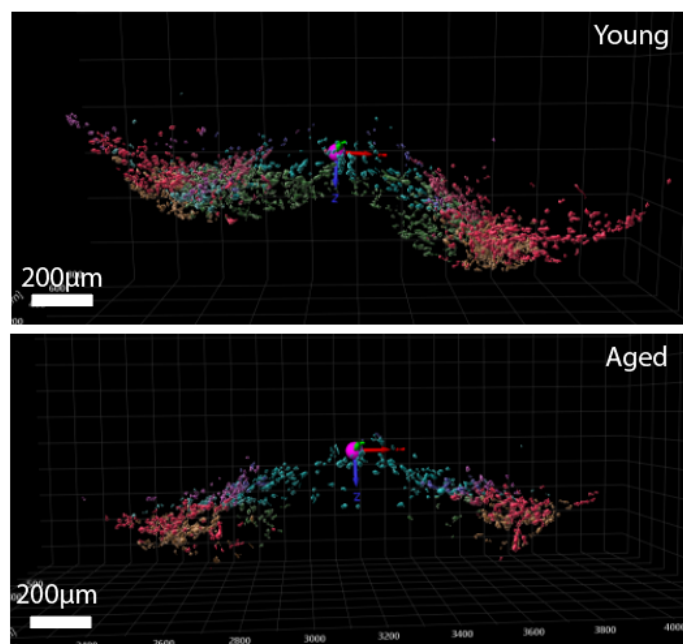


Figure 3.6: 3D visualization of TH+ cells in VTA. Colors represent distance-based clusters of segmented particles, determined based on their distance from the set origin (pink ball).

3.3.2 Age-related topography of TH+ neurons in VTA.

Based on the aforementioned methodology for the segmentation of DA soma-related particles in the VTA of adult and aged mice, we quantified the detected particles based on their topography on ML, DV and AP axis.

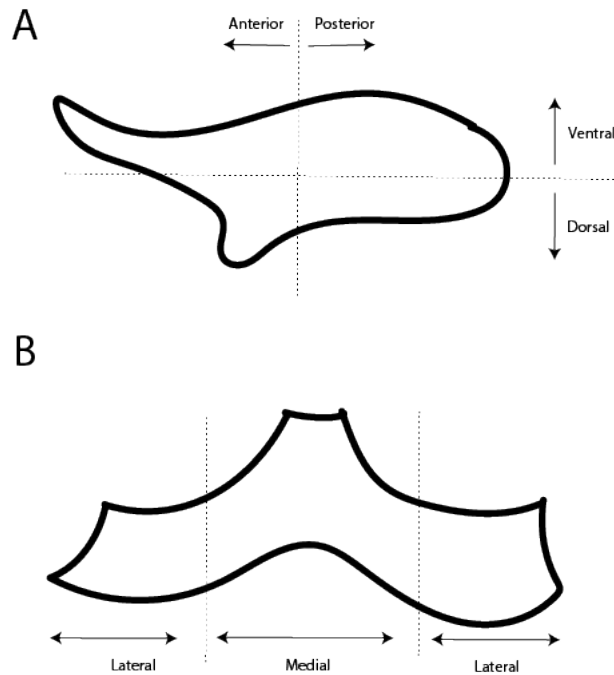


Figure 3.7: Schematic of the topography based segmentation of VTA. A. Sagittal view of VTA. B. Coronal view of VTA.

3.3.2.1 Overall

We fitted a **Bayesian negative-binomial model**, estimated using MCMC sampling with 4 chains of 5000 iterations and a warm-up of 1000 to predict the number of segmented particles with the topography on the mediolateral axis and the age group (formula: $\text{Count} \sim \text{Topography} \times \text{Group}$). Priors over parameters were set as student-t distribution (location = 6.30, scale = 2.50). The model's explanatory power is substantial ($R^2 = 0.40$, 95% CI[0.10, 0.58]). All the estimation successfully converged and all the indices were considered as reliable.

Within this model (Fig. 3.9), the overall effect of the age group (Median = 0.14, 95% CI [-0.59, 0.86]) had a 66.78% probability of being positive (> 0), 61.16% of being

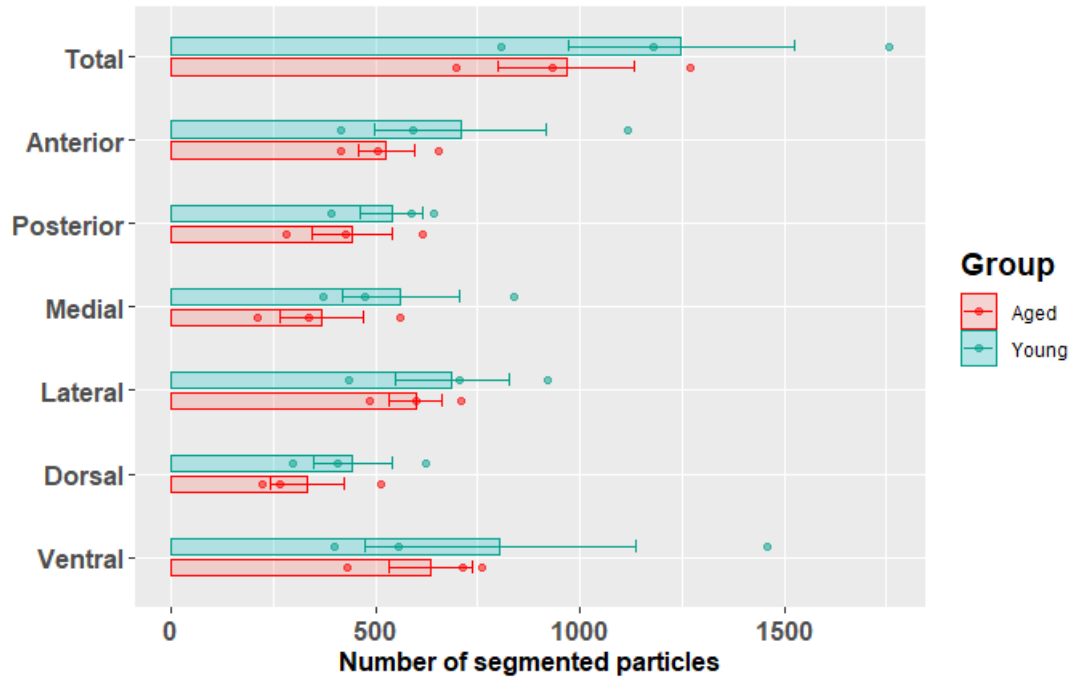


Figure 3.8: Topography of VTA TH+ segmented particles. We show here the estimated number VTA TH-expressing neurons in adult and aged mice based on the considered topography. The same total amount of segmented particles were attributed a label on either the AP, ML or DV axis.

significant (> 0.05), and 31.52% of being large (> 0.30). This suggests that age does not have an effect on the overall number of segmented neurons.

3.3.2.2 Medial versus Lateral

We fitted a **Bayesian negative-binomial model**, estimated using MCMC sampling with 4 chains of 5000 iterations and a warm-up of 1000 to predict the number of segmented particles with the topography on the mediolateral axis and the age group (formula: $\text{Count} \sim \text{Topography} \times \text{Group}$). Priors over parameters were set as student-t distributions (location = 6.30, scale = 2.50). The model's explanatory power is substantial ($R^2 = 0.40$, 95% CI [0.10, 0.58]). All the estimation successfully converged and all the indices were considered as reliable.

Within this model (Fig. 3.9), the effect of the overall topography on the mediolateral (ML) axis (Median = -0.48, 95% CI [-1.22, 0.22]) had a 92.22% probability of being negative (< 0), 89.98% of being significant (< -0.05), and 70.71% of being large ($<$

-0.30). This suggests that the number of segmented neurons were overall similar between medial and lateral VTA.

The effect of the topography on the ML axis per age group (Median = 0.28, 95% CI [-0.73, 1.29]) had a 72.74% probability of being positive (> 0), 69.09% of being significant (> 0.05), and 48.14% of being large (> 0.30). This indicates that there is no effect of age on the distribution of segmented neurons over the ML axis.

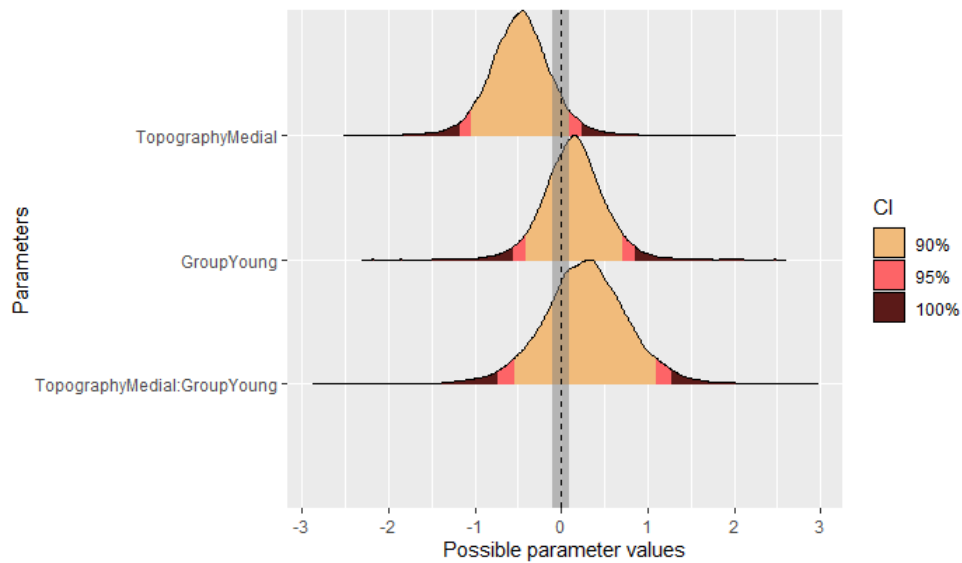


Figure 3.9: Bayesian parameter estimation on the ML axis.

3.3.2.3 Dorsal versus Ventral

We fitted a **Bayesian negative-binomial model**, estimated using MCMC sampling with 4 chains of 5000 iterations and a warmup of 1000 to predict the number of segmented particles with the topography on the DV axis and the age group (formula: $\text{Count} \sim \text{Topography} \times \text{Group}$). Priors over parameters were set as student-t distributions (location = 6.20, scale = 2.50). The model's explanatory power is substantial ($R^2 = 0.37$, 95% CI [0.10, 0.59]). All the estimation successfully converged and all the indices were considered as reliable.

Within this model (Fig. 3.10), the effect of topography on the DV axis (Median = 0.65, 95% CI [-0.21, 1.51]) had a 93.76% probability of being positive (> 0), 92.29% of

being significant (> 0.05), and 80.67% of being large (> 0.30). This suggests that the number of segmented neurons were overall similar between dorsal and ventral VTA.

The effect of the topography based on the age group (Median = -0.06 , 95% CI $[-1.25, 1.18]$) had a 54.24% probability of being negative (< 0), 50.68% of being significant (< -0.05), and 33.39% of being large (< -0.30). This indicates that there is no effect of age on the distribution of segmented neurons over the DV axis.

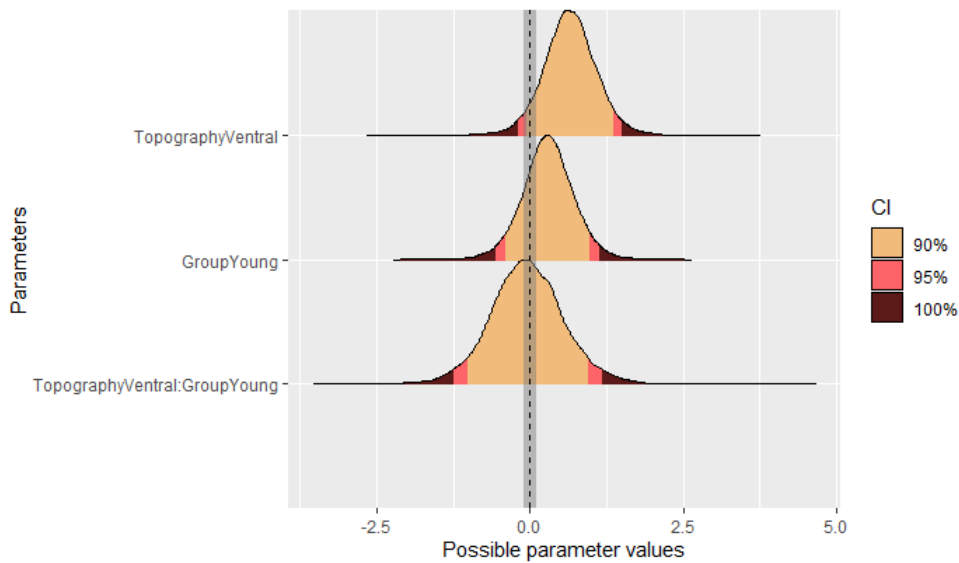


Figure 3.10: Bayesian parameter estimation on the DV axis.

3.3.2.4 Anterior versus Posterior

We fitted a **Bayesian negative-binomial model**, estimated using MCMC sampling with 4 chains of 5000 iterations and a warm-up of 1000 to predict the number of segmented particles with the topography on the AP axis and the age group (formula: $\text{Count} \sim \text{Topography} \times \text{Group}$). Priors over parameters were set as student-t distributions (location = 6.30, scale = 2.50). The model's explanatory power is substantial ($R^2 = 0.30$, 95% CI $[0.06, 0.56]$). All estimations successfully converged and the indices were all considered as reliable.

Within this model (Fig. 3.11), the effect of topography on the AP axis (Median = -0.18 , 95% CI $[-0.86, 0.51]$) had a 72.11% probability of being negative (< 0), 66.33%

of being significant (< -0.05), and 34.96% of being large (< -0.30). This suggests that there the number of segmented neurons were overall similar between anterior and posterior VTA.

The effect of the topography based on the age group (Median = -0.09 , 95% CI [-1.06 , 0.90]) had a 58.30% probability of being negative (< 0), 53.81% of being significant (< -0.05), and 31.68% of being large (< -0.30). This indicates that there is no effect of age on the distribution of segmented neurons over the AP axis.

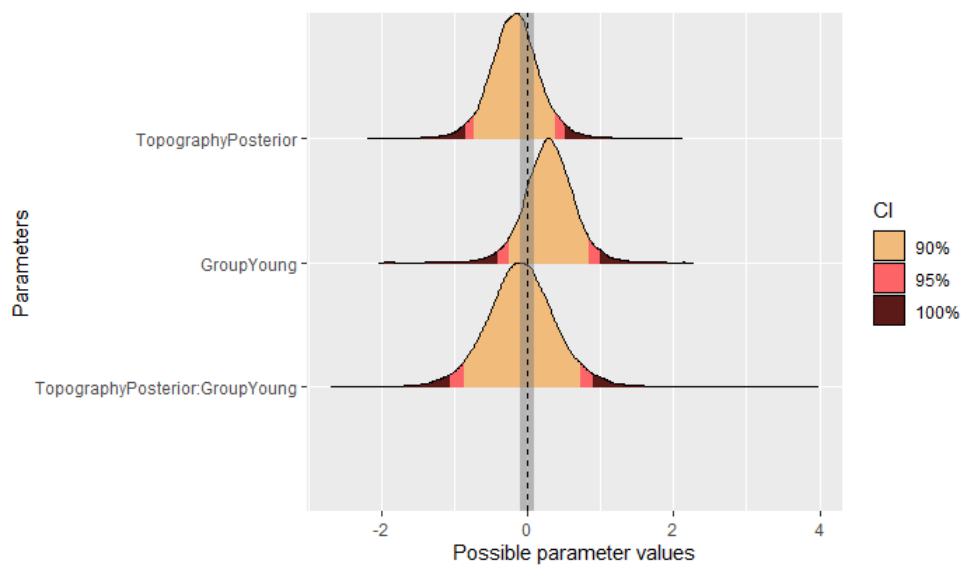


Figure 3.11: Bayesian parameter estimation on the AP axis.

Result summary

Altogether, we did not find an effect of age on the overall or on the topography-based distribution of TH+ neurons in VTA of mice.

3.3.3 Density of mesocortical subpopulations

In a second study, we attempted to quantify the age-related densities of VTA subpopulations in young (2-3 months) and aged (18-19 months) mice. To do so, we coupled stereotaxic injections of a retrograde tracer, CTB, within the vmPFC (Fig. 3.12). This tracer allowed the identification of soma in the VTA projecting to the injected region, *i.e.* mesocortical neurons. We initially were interested in using iDISCO+ to visualize VTA neuronal subpopulations in 3D, however, this procedure was not compatible with the antibody required to co-label DA and Glu subpopulations. Therefore, we used a triple-immunostaining approach on free-floating slices of VTA to quantify the density of DA, Glu and DA/Glu neurons in VTA (Fig. 3.13).

CTB injections successfully labeled mesocortical neurons in the VTA (Fig. 3.13). As related in the literature, mesocortical neurons are sparse in this region.

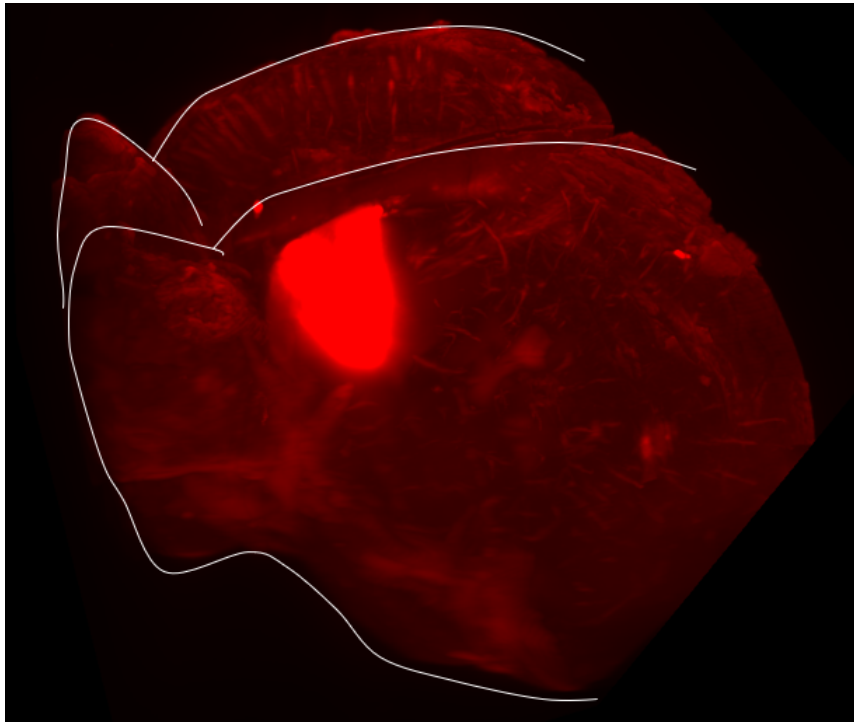


Figure 3.12: 3D visualization of a CTB injection in PFC.

We used a triple immunostaining approach to label DA, Glu and DA/Glu subpopulations within VTA. To do so, we labelled TH to identify DA neurons, vesicular Glutamate Transporter-2 (vGluT2) to identify Glu neurons, and DAPI to label nuclei

including neuronal ones (Fig. 3.13). All staining protocols were successful in the labelling of DA and Glu neurons within VTA. TH staining is diffused within whole neuronal cell bodies and fibers whereas vGluT2 staining is characterized by puncti that are found across cell bodies and fibers. CTB staining is also characterized by puncti, however those are for the most found in the neuronal cell bodies.

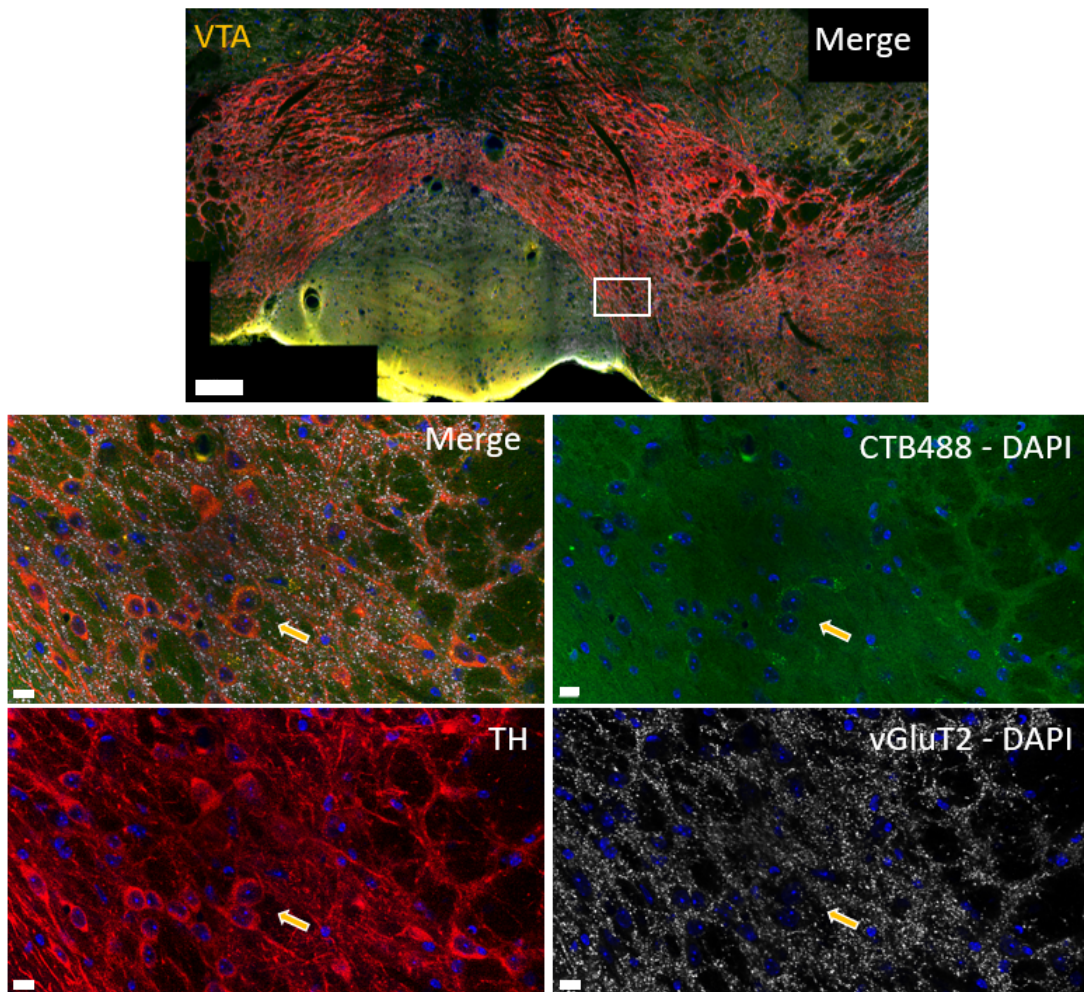


Figure 3.13: Triple staining of VTA neurons for DA, Glu, and DAPI. Confocal image where on top is the whole VTA, and below are the images for each immunostaining.

Due to the nature of the staining, though the identification of DA neuronal cell bodies from their fibers is evident, for Glu neurons it was more difficult to infer only based on visual inspections. Specifically, the segmentation of vGluT2 led to the extraction of clearly defined puncta, however since the labelling is not diffuse, the distinction between vGluT2 expression within a synaptic terminal targeting a cell body, and vGluT2 expression within a cell body was difficult to infer (Fig. 3.13; Fig. 3.14). To

circumvent this issue, it was therefore necessary to segment vGluT2 particles based on their density and proximity to nuclei. DAPI labels all nuclei, irrelevant of whether those are neuronal or not, therefore, to minimize false positives, the quantified vGluT2 particles had to be classified based on their proximity to DAPI-stained nuclei but also based on their clustering. The necessary steps required to develop the pipeline for this analysis could not be finalized within the scope of this thesis.

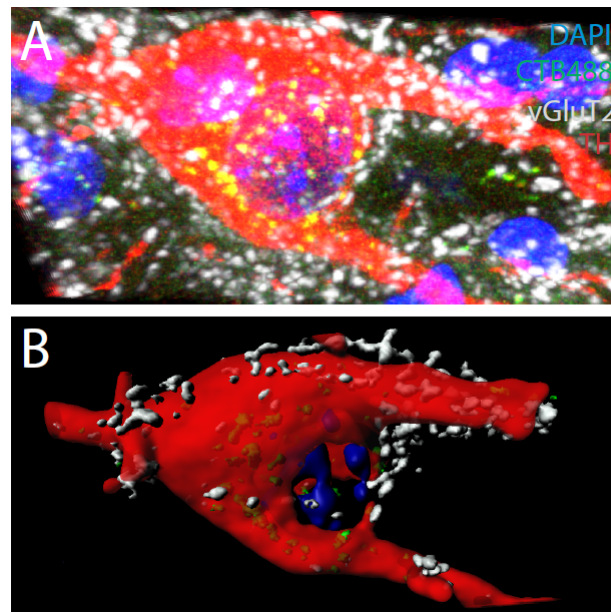


Figure 3.14: Example of 3D visualization of a TH+, CTB+, vGluT2+ cell in VTA.

Furthermore, the identification of VTA DA/Glu co-releasing subpopulations based on the identification of VTA soma may not be the optimal approach to quantify this neuronal population. Indeed, vGluT2 and TH were hypothesized to be localized in distinct compartments within VTA neurons, therefore not colocalized. We show here a mesocortical neuron labelled with CTB in its soma. If we based ourselves only onto the co-staining present within the cell body, and around the DAPI-stained nucleus, then we would characterize this cell neither as DA releasing nor Glu releasing as neither TH or vGluT2 staining is visible within the soma. Yet, we observe that the fiber emanating from this neuron, is associated with both TH labelling and vGluT2 labelling. Interestingly, we see this labelling to be characteristic of varicosities, from which either DA or Glu would be released. These varicosities appear compartmentalized from one another, yet located within the same fiber. Therefore, our result

indicate, for the first time, that mesocortical DA/Glu co-expressing neurons show a compartmentalization within their fiber. (Fig. 3.15).

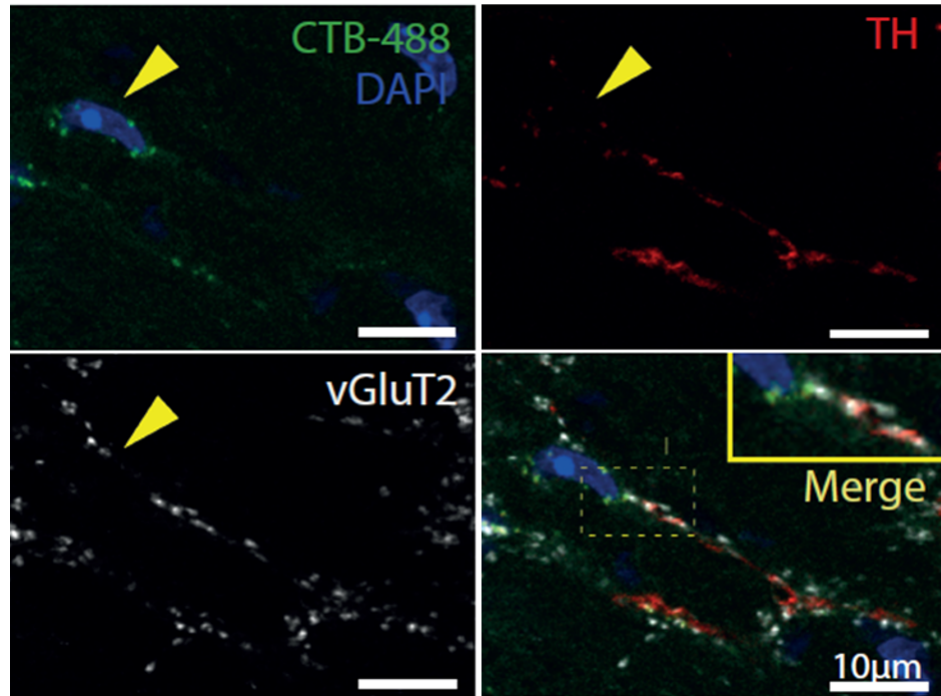


Figure 3.15: Example of a CTB+ cell expressing TH+ and vGluT2+ in its axon.

3.4 Discussion

Age-related alterations in the release of DA within the PFC is thought to participate in age-related WM decline. Yet, few studies investigated the origin of such alterations, and methodological limitations limited the conclusion on the origin of age-related DA decline. Specifically, the common assumption is that age-related neurodegeneration of VTA DA neurons underlie these alterations. However, given the heterogeneity of VTA and the recent findings concerning the molecular shift of DA neurons in response to lesions, previous views proposing the age-related neurodegeneration of DA neurons are challenged.

Here, we compared the density of DA soma within the VTA of adult and aged mice. Our study fail to put in evidence any age-related changes in the density of DA neurons within VTA, regardless of their topographical position, therefore suggesting that aging does not lead to age-related neurodegeneration of DA neurons, as previously hypothesized. In a second study, we aimed at quantifying the density of DA and DA/Glu subpopulations within VTA, including mesocortical neurons. Though we could not quantify the obtained data within the scope of this thesis, we identified, for the first time, the existence of mesocortical neurons that co-express DA and Glu in separate compartments within the same fibers, yet not in the soma.

Our result suggest that healthy aging does not affect the overall distribution of DA neurons in VTA, in opposition to what is proposed in the literature (Gür et al., 2020; Noda et al., 2020). Such opposition could be explained by the following reasons. Previous methodology failed to take into account that TH expression is diffuse within catecholaminergic neurons, therefore staining not only soma but also DA fibers and noradrenergic fibers within VTA. Therefore whether a decrease of TH expression is due to decrease of the whole cell, local axonal degeneration or reduced input from other regions, remain to clarified. Here, our approach allowed the extraction of data that are related only to the soma and not fibers. Still, we do observe a trend between groups, where older individuals seem to show overall reduction of VTA DA

neurons compared to younger individuals (Fig. 3.6). As the variance between individuals within our age groups is different (i.e. overdispersion) in addition to our small dataset, this could have prevented our statistical analysis from the detection of an effect. Finally, in our segmentation process, we observed a variability in expressions of TH within neurons in the VTA. Specifically, neurons were either very bright with clearly defined soma whereas others were low in intensity, smaller and shaped in a way that does not reflect the whole cellular body (Fig. 3.5). In our methodology, due to difficulties to delineate the low intensity neurons, only the brighter ones were taken into account. This bias may have influenced the presented results as not *all* TH-expressing neurons were taken into account. Still, we applied the same methodology for all samples which indicates that within this neuronal type (here bright TH+ neurons), age does not have an effect on the density of neurons.

The question remains as to why such diversity in intensities. This could underlie a methodological problem with the whole-brain staining, as the observed low-intensity population were located in deeper regions within VTA and could be the result of a lack of antibody penetration during whole-brain staining. However, we argue that such interpretation is unlikely, as the used TH antibody emitted fluorescent signal across all known TH related regions, regardless of their location within the brain (Fig. 3.4). Others have also validated the use of this antibody in adult and aged mice (Roostalu et al., 2019), and the TH antibody we used in our experiments is a marker well-known for its compatibility with whole-brain staining and clearing (Godefroy et al., 2017; Roostalu et al., 2019; Datta et al., 2023). This suggests that the differences in intensity may in fact be related to the level of TH expression within cells.

Therefore, another possible answer remains in our second hypothesis related to the diversity of VTA subpopulations. Indeed, as subpopulations of VTA DA neurons are able to co-release Glu or GABA (Morales & Margolis, 2017), it is possible that the reduced expression of TH could be due to a reduced expression of TH within co-releasing neurons. Though within classic slice staining, establishing a correlation between the level of intensity based on the object (here cellular bodies) can be difficult because of the difference in conditions and levels of saturation of between slices.,

in whole-brain immunostaining, the whole sample present similar and comparable conditions, therefore allowing to take into account these differences in intensities. Further work is required to characterize these different types of DA neurons, and relate to their molecular profile.

Interestingly, we show here for the first time the existence of compartmentalization within the fiber of a co-releasing mesocortical neuron. This was previously demonstrated in mesostriatal co-releasing neurons, where compartmentalization was observed in both the neuronal cell body and the projecting axon ((Fortin et al., 2019; Silm et al., 2019; Zell et al., 2020)). However, here the detected mesocortical cell did not present any staining within the soma but only in the fiber, suggesting the staining base identification of co-releasing neurons should take into account that the labelling may be present only within fibers and not visible in the cell overall. Additionally, lower TH intensities may be related to the co-expression of other co-releasing neurotransmitters within those cells. We observed that the morphology of the low-intensity neurons were also smaller compared to bright ones, which further argues that this difference may underlie a difference in neuronal type.

3.5 Conclusion

The DAergic system is affected by healthy aging and is thought to be involved in the in age-related loss of PFC integrity, that ultimately result in cognitive decline. One of the main release site of DA is the VTA, a highly heterogeneous structure characterized by the presence of a diverse set of neurons. The origin of decrease DA release is often thought to be due to age-related neurodegeneration, however sparse studies have attempted to test this hypothesis. Here we show that there is no evidence that age affects the density of DA neurons in VTA, which suggests that the origin of age-related DA decrease may not be related to neurodegeneration of DA neurons.

“We cannot direct the wind, but we can adjust the sails.”

Dolly Parton

CHAPTER 4

Discussion

Goal-directed behavior relies on the dynamic adjustment of oneself, based on internal needs and environmental contexts. Such type of behavior relies on executive functions, which are responsible for the planification and coordination of behavior and among the first cognitive processes to be declining during healthy aging. Within executive functions, WM and its underlying circuit are characterized as hallmarks of healthy aging.

WM is defined as the ability to store, maintain and manipulate relevant information for motivated actions. It is perceived as a function that orchestrates and maintains ‘online’ salient information to be used for current behavior thus contributing to the establishment and maintenance of associations for current and future behavior. WM requires dynamic coding of information that rely for the most in PFC activity, which is altered during healthy aging. Specifically, age-related WM decline is associated to a reduction of storage capacity driven by alterations in the ability to manipulate multiple information online within WM. We show here that such afflictions are also observed in older mice, where the maintenance of a single information within WM remained similar between younger and older mice, but the dynamic maintenance and

manipulation of multiple information resulted in a decrease of performance in older groups.

The similarities in the observed alterations of WM function in the mouse model, allowed us to assume that similar mechanisms could underlie age-related WM function across species. Specifically, the PFC networks underlying WM are especially dependent on the modulation by DA inputs from mesocortical neurons, issued from the VTA. VTA DA release contributes to the modulation of synaptic weights within prefrontal networks and is proposed to participate in both the selection and maintenance of ‘online’ information within WM. During healthy aging, there are multiple reports indicating a decrease of DA release in the PFC. Age-related WM impairment in the ability to maintain online multiple relevant information within WM may be in part due to alterations in the DAergic modulation of PFC networks for WM. Yet, the origin of such decrease remains unknown.

It is often hypothesized that, as DA neurons are vulnerable to oxidative stress, they are more likely to neurodegenerate and the latter was proposed to underlie decreased DA release. Here, our data suggest otherwise, where the density of DA neurons remained somewhat similar between young and aged mice. This suggest that other mechanisms rather than cellular death may be involved in alterations of DA release in older individuals. One hypothesis may be that healthy aging leads to a shift in the molecular profile of DA neurons, where as a neuroprotective mechanism, they may upregulate Glu release. Still, this hypothesis remains to be tested.

4.1 Age-related storage capacity and inter-individuality

Across species, healthy aging is associated in age-related WM decline. In humans, WM-related subprocesses e.g. flexibility, load and maintenance are usually distinguished, when in animal models, such distinction remain sparse. Most human studies indicate age-related WM failure in contexts that challenge cognitive flexibility and cognitive load but not temporal maintenance. However, in rodents, the circumstances in which age-related WM failure are more likely to rise remained unclear. Studies investigating delay-dependent WM failure, i.e. during temporal maintenance, result

in contradictory conclusions and the effects of healthy aging on WM subprocesses remained to be clarified. One of the objective of this thesis was therefore to uncover the extent to which the temporal maintenance of information in older individuals was dependent on the cognitive effort required during encoding and the cognitive load required to flexibly store information (Chapter 2).

We show that in the mouse model, age-related WM impairments are evident in context requiring the ‘online’ storage of multiple information within WM. We tested mice in three environments that tested temporal maintenance in a context of (i) low encoding effort and low encoding interference (Delayed-Alternation in the Y-maze), (ii) high encoding effort and low encoding interference (Delayed-Alternation in the 8RAM) and (iii) high encoding effort and high encoding interference (All-baited in the 8RAM). We show that in both low encoding interference tasks, the overall performance of mice was not affected by age, regardless of the cognitive effort required to encode information, here, spatial locations. Aged mice were impaired only in a high encoding interference context.

Interestingly, we observed that interindividual heterogeneity among low performing and high performing animals could be explained by the ability to perform in high or low encoding interference tasks (delayed-alternation versus all-baited). Indeed, in tasks requiring a high encoding effort, the WM subprocesses that were accentuated on differed between the low- and high-encoding interference paradigms. The prior emphasizes on the stability of a single information to be maintained ‘online’ whereas the second requires malleability of the encoded information for flexible ‘online’ storage and manipulation. Specifically, our results imply that overall WM function relies on a balance between resistance (stability) and yielding (flexibility) to noise, that are correlated with the extent of WM failure across individuals. Still, the strength of such correlation is stronger in older individuals, when compared to younger ones, but also shifted towards a shorter maintenance span (30s in aged versus 60s in young).

A study by Beas and colleagues report a similar correlation when comparing young and aged rats, in their performance in an attentional set-shifting task that relies on

a flexible shift of attention with Delayed-matching-to-place task set in an operant box (Beas et al., 2013). They also observe a strong negative correlation between performances, this time only in aged rats. As per their interpretation, they mention that the “opposing relationships [...] suggest that somewhat different neurocognitive mechanisms mediate performance at short and long delays in the delayed response task”. They propose also, as suggested here, the idea of a balance between stability and flexibility and suggest that, in some individuals, healthy aging could lead to “an impaired ability to hold information or representations stable across time [...] whereas others show an impaired ability to flexibly modify those representations as dictated by alterations in environmental contingencies”.

The longest delay in Beas’s study was 24s, while here, we tested animals at both a 30s and 60s delay. Our results therefore converge as we also observe a negative correlation in the older group at a 30s delay. This further argues for the above mentioned interpretations. Still, it remains unclear why such differences are observed within 30s in older individuals as opposed to a weaker one at 60s in younger adults. A possible explanation brings us back to the notion of ‘online’ WM. As discussed in the introduction of Chapter 2, though WM is defined as a short-term memory process, the timespan in which it operates is not well-established. To characterize WM, it was therefore proposed that it was effective based on the salience of the information to be maintained and processed for goal-directed behavior (Dudchenko, 2004).

Recently, the development of computational models for the understanding of WM imply the concomitant existence of “two types” of WM trace. Historically, the predominant persistent activity model, based on recordings in PFC, were thought to underlie the maintenance of information within WM (Goldman-Rakic, 1995). This suggests that the short-term memory of information were to always be maintained available for processing in neuronal populations within the PFC through a recurrent activation of their respective neuronal networks. More recently, such idea was challenged by the proposition of activity-silent models that suggest that prefrontal populations maintain the memory of salient information not through persistent firing, but through the phasic reactivation of neuronal networks encoding information

(Stokes, 2015). In this model, prefrontal neurons are thus not continuously activated, but ‘refreshed’, and underlie the maintenance of information.

Beukers and colleagues proposed that LTM (i.e. episodic memory) participate in such refreshing but more importantly, play an active role in the selection of the information to be available for processing (i.e. to be maintained ‘online’) while others salient information are access restricted until relevant for imminent behavior (Beukers et al., 2021). Based on this perspective, we propose that the tested delays in our tasks are representative of the timespan in which information are maintained ‘online’. Though young and aged mice had similar performances in all delays, (i.e. 5s, 30s and 60s), the delay-specific correlations with the all-baited task, suggest that the underlying cognitive networks involved in such performance may differ. Specifically, as no correlation were observed neither at 5s and 30s in younger mice, we suggest that these correlation are observable only when the “borders” of WM are reached, i.e. the limits of switching between ‘online’ WM and ‘silent’ WM. If such is the case, 60s would be the ‘normal’ temporal scale in-which information can be maintained ‘online’ within WM. As the strength of such correlation was considered weak in our analysis, we could further argue that this temporal scale could extend above 60s. On the other hand, in aged group, this timespan is shifted to the left towards an earlier timespan, namely 30s as accounted for in our and Baes’s studies, suggestive of an age-related impairment in the ability to maintain ‘online’ a given set of salient information.

Together with the possible shift towards shorter timespan in the availability of salient information, and alterations in the flexible use of WM, our study strongly suggests that healthy aging reduces the storage capacity within WM, which ultimately result in cognitive decline. The extent of such reduction may be individual-dependent, yet may be compensated for, to a certain extent, through behavioral adaptations (e.g. VTE; Redish, 2016). Still, it is possible that performance could be attributed to other non-WM dependent processes such as spatial memory or procedural strategies. For instance, difference in performance in the all-baited task could be in fact related to difference in strategies (cognitive versus non-cognitive) used. However, we observed the VTE-like behavior in both adult and aged mice, which are related to the use

of cognitive strategies in known spatial environments (Redish, 2016). Still, further work needs to be done in order to classify the strategies used by mice through the classification of trajectory patterns.

4.2 Anatomical insights from the dopaminergic system

As suggested above, most WM models rely on the decoding of activity within prefrontal networks. Among others, DA is a known key player in the modulation of PFC networks. Though most famous for its involvement in the coding of the “reward prediction error” (Shultz et al., 1997), DA plays also a key role in the regulation of WM subprocesses especially the selection and maintenance of information within WM. Prefrontal DA is proposed to participate in gating mechanism within the PFC, i.e. participating in the filtering of information to be processed by PFC, and the maintenance of the “salient” etiquette on a set of information to be maintained and therefore deemed as pertinent for behavior (Ott & Nieder, 2019). DA in PFC is suggested to act as a switch from activity-silent to persistent activity (see Chapter 2) (Froudust-Walsh et al., 2021). PFC D1R-activation is suggested to be involved in the selection of relevant information to be retained for current goal-directed behavior and participate in active forgetting of competing mnemonic traces (Gallo et al., 2022).

Such actions rely on the activity within mesocortical neurons in the VTA (Morales & Margolis, 2017). VTA DA release is thought to participate in both the short-term and long-term tuning of synaptic weights within the PFC through both phasic and tonic release of DA, that are thought to contribute differently to WM (Goto & Grace, 2007). Specifically phasic DA release is mediated through spiking of VTA neurons and involved in the filtering of salient stimuli whereas tonic release is thought to be mediated in axonal terminals, independently of firing and participate in the maintenance of associations between representations for WM.

During healthy aging, multiple reports indicate alterations in DAergic system, including age-related cellular degeneration. However, few have taken into account the diversity of VTA DA neurons, that overall is thought to influence the extent to which

they are resilient to degeneration. VTA is a highly organized structure characterized by topographical clusters of neurons based on their connectivity (that relate to their functional role) (Beier et al., 2019), their intrinsic properties (Lammet et al., 2014), their molecular (Poulin et al., 2020) and neurochemical profile (Morales & Margolis, 2017).

There is also an assumption of whole-cell degradation during healthy aging in VTA, however the extent of fiber degeneration compared to whole cell degeneration remains, along with a previously undetected shift in the neurochemical profile as a neuroprotective mechanism is currently unknown. Though our results indicate that healthy aging does not affect the density of VTA DA neurons, we do observe a trend that can be indicative of reduced DA cell bodies. Still, the differences between our observation and reports of decreased DA neurons in the literature could be reflective of a difference of methodological approach but also initial assumptions. As here we did distinguish cell-bodies from fibers, we propose that results found in previous literature were driven by a shift in the molecular profile of VTA DA neurons with an upregulation of vGlut2 and downregulation of TH as a compensation mechanism to minimize cellular death and therefore functional losses.

4.3 Limitations and future work

We used a behavioral and anatomical approach to infer how healthy aging may impact the WM function and its underlying dopaminergic network. Though we found evidence of a parallel between humans and the mouse model in age-related WM decline, our study is limited to the cognitive performance and behavioral outputs during our tasks. To optimally assess a parallel between the mouse model and human studies, future work needs to address this by developing shared paradigms that can be tested in both species in order to draw a proper conclusion. Furthermore, though we were interested in the relationship between DA age-related alterations and WM function, our study was limited in the establishment of a causal link between WM performance and DA alterations in PFC and VTA. Future work would involve, for example, the

monitoring DA release in PFC during the WM task, that can be coupled with a histology approach to assess and correlate the density of mesocortical DA neurons and the extent of DA release and the performance of mice. More importantly, the anatomical study of VTA subpopulations and the density of DA/Glu neurons be more easily investigated using other histology techniques such as RNAscope which would label only cell bodies. Still, our approach allowed the identification of co-releasing fibers and provided insight on the organization of such co-releasing mesocortical neurons. Overall this thesis project provided a basis for the exploration of age-related WM decline in the mouse model and provided key insights onto the underlying age-related alterations in VTA outputs to the PFC, that will be investigated in a recently funded project named DOPAGE.

4.4 Dopamine, Glutamate and Working memory: the DOPAGE project

Overall, the ability of DA neurons to co-release glutamate and their functional relevance remains poorly understood. Initially, the observation of DA release dynamics in the PFC were considered as too slow to support reward-related signaling by phasic DA activity (Seamans & Yang, 2004; Lavin et al., 2005). Therefore, it was suggested that co-released glutamate can signal precise-in-time goal-related information, while the role of DA was limited to signal a long-term state of the animal (motivation or stress) or to bias PFC network into a regime suitable to efficiently express persistent activity states allowing behaviorally important stimuli to be encoded into WM (Gorelova et al., 2012).

Still, the observation that the proportion of co-releasing midbrain DA neurons increased after neurotoxic lesions bring new information to take into consideration. Specifically, it is hypothesized that the homeostatic balance of VGluT2 expression in DA neurons plays a determinant role in cell survival (Steinkelman et al., 2018; Fortin et al., 2012; Buck et al., 2021), as vGluT2 promotes synthesis and release of growth factors in VTA, which can subsequently modulate DA neuronal growth and survival (Shen et al., 2021). Given that VgluT2-expressing glutamate neurons within the VTA

may form functional synapses with VTA DA neurons, this Glu-mediated release may also occur in the midbrain as a natural process during healthy aging.

Nonetheless, the relative functional significance of DA and glutamatergic projection to the PFC during behavior remains unclear. VTA DA is proposed to play a role as filter by switching prefrontal networks to a regime suitable for the encoding of behaviorally relevant information in WM (Seamans & Yang, 2004; Lavin et al., 2005). It is thought to do so through tonic activation of D1R and D2R, that play distinct roles in both the regulation of neuronal activity and synaptic plasticity (Sheynikhovich et al., 2011; Sheynikhovich et al., 2023; Durstewitz & Seamans, 2008). It has also been suggested that DA participates in communication-by-synchrony between spatial memory networks of the hippocampus and PFC during WM tasks (Fujisawa & Buzsáki, 2011; Benchenane et al., 2010).

Overall, it is suggested that DA neuromodulation is critically involved in the synchronization of oscillatory activity, which, in turn, is assumed to serve as a mechanism by which information are bound and held active in WM storage (Bäckman et al., 2006; Li et al., 2010; Seamans & Yang, 2004). However, the encoding and maintenance of goal-directed information necessitates precise temporal signals about the observed stimuli, for example during learning. In PFC, it was shown that during WM, prefrontal DA expresses slow ramping dynamics that are unlikely to encode events on a precise temporal scale (Durstewitz & Seamans, 2008; Garris & Wightman, 1994). In contrast, fast temporal dynamics of glutamate fit such a coding scheme and it was theoretically suggested that VTA Glu and DA activity may complement each other during behavior (Lavin et al., 2005). However, thus far, this has never been tested in a WM paradigm (Eskenazi et al., 2021).

These questions are addressed in the DOPAGE project, a project funded by the National French Agency (ANR) in 2022 (AAPG2022). This project was conceptualized and designed as a follow up of this thesis, to provide a better understanding of (i) the effects of healthy aging in VTA subpopulations, especially in mesocortical neurons and (ii) the functional role of mesocortical DA and Glu projections to the PFC in both young and aged mice.

To meet the presented objectives, the project was divided into three workpackages (WP). WP1 aimed to further investigate the behavioral correlates of decision-making among mice during spatial WM. To do so, mice are tested, as in this thesis, in WM and PFC-dependent spatial paradigms in which a combination of bodypart tracking (Fig. 2.8; Fig. 2.21) and behavioral clustering (see Appendix A) is proposed to entail an accurate depiction of how healthy aging modifies behavior during motivated actions. WP2 relies on anatomical approaches as used here (see Chapter 3), where stereotaxic injections are combined with immunostaining to label and quantify the density of VTA mesocortical subpopulations (DA, Glu, GABA, co-releasing) both at a somatic level (Fig. 3.13) and at a fiber level in PFC, in adult and aged mice. Finally, WP3 relied on an optogenetic approach to characterize functional connectivity and significance of VTA DA and Glu inputs to the PFC, where each projection type will be stimulated or inhibited to uncover their role in relation to WM subprocesses (i.e. encoding, maintenance, decision-making) (see Chapter 2).

4.5 Conclusion

In conclusion, this thesis has undertaken a comprehensive exploration into the dynamic interplay between healthy aging, working memory, and the dopaminergic system, with a particular focus on elucidating age-related cognitive decline in the mouse model. Our study revealed significant parallels in WM impairments across species, highlighting the importance of understanding WM subprocesses and their vulnerability to aging.

Our findings support the hypothesis that age-related alterations in WM subprocesses are indeed homologous between humans and mice, with flexibility-related subprocesses emerging as the primary contributors to age-related WM deficits. Specifically, we found that while the maintenance of information within WM remains relatively robust with age, the manipulation and dynamic adjustment of multiple information within WM are significantly compromised in older individuals. Interestingly, interindividual heterogeneity in WM performance was negatively correlated with performance in tasks challenging either maintenance or manipulation, especially in older

individuals, suggesting that there is fine individual-dependent balance in neuronal state that may underlie the extent of age-related WM deficit.

Moreover, our research delved into the underlying effects of healthy aging on the dopaminergic system, particularly focusing on the origins of the potential decrease in prefrontal dopamine levels. Through the quantification of the density of DA neurons in the ventral tegmental area of adult and aged mice, we aimed at testing the possible mechanisms driving decreased DA release in the PFC, specifically the hypothesis of DA neurodegeneration. We found no significant evidence of an age-related decrease of DA densities in older mice, suggesting that decreased DA release may not be related to neurodegeneration. Instead, we propose that DA neurons may shift their molecular profile towards an upregulation of Glu components as a neuroprotective compensation mechanism. More importantly, our research put in evidence for the first time the compartmentalization of mesocortical co-releasing fibers with distinct DA and Glu-related varicosities.

Overall, this thesis contributed to the characterization of age-related WM decline in the mouse model and further argue for the investigation of anatomical markers underlying WM function. Future works would need to now establish functional relationship between the anatomical results found here in relation to WM performance, that we propose, may participate in the unravelling of the complexities of age-related cognitive decline and identify novel avenues underlying the heterogeneity of the aging process.

Moving forward, the insights gleaned from this thesis lay the groundwork for future research endeavors, including the DOPAGE project, which aims to deepen our understanding of the functional significance of dopamine and glutamate projections to the prefrontal cortex in both young and aged mice. Through collaborative efforts and innovative methodologies, future work will allow to pave the way for targeted interventions aimed at preserving cognitive function and enhancing quality of life in aging populations.

APPENDIX A

Behavioral motif segmentation

One of the advantage of the tracking data extracted from our behavioral study (Chapter 2) was the ability to extract behavioral sequences (i.e. 'motifs') to test (i) the effects of aging on the overall behavior of mice (motivated exploration versus unmotivated exploration) (sniffing, VTE, rearing); (ii) the existence of behavioral predictors that reflect decision-making, (iii) the existence of behavioral sequences that reflect performance (i.e. delayed-alternation versus all-baited).

Though all data is available, the model used for the extraction behavioral features could not be validated within the scope of this thesis.

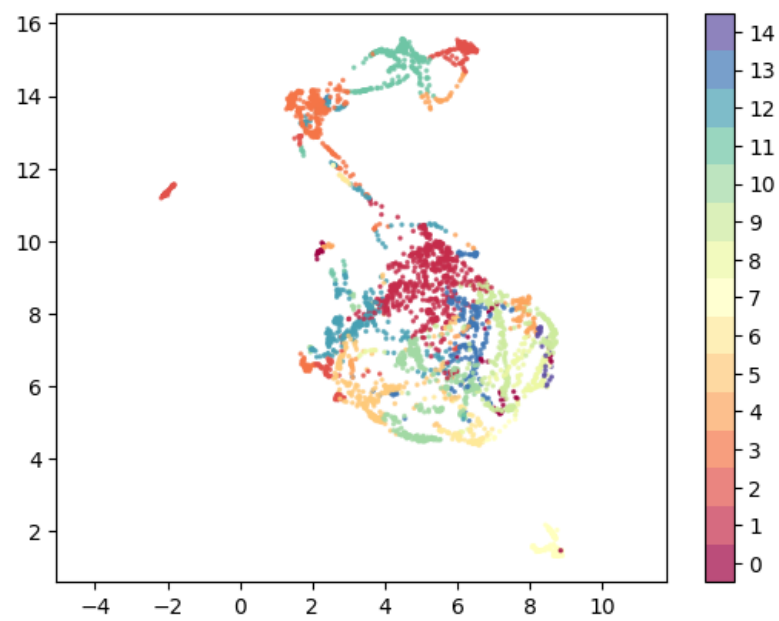


Figure A.1: Example of extracted behavioral clusters. Here is the visualization of 14 extracted behavioral clusters using Uniform Manifold Approximation and Projection for Dimension Reduction, or UMAP, based on DeepLabCut pose estimation of a mouse exploring the 8-arm radial arm maze.

APPENDIX B

Additional statistics for Chapter 2

Here, we report indicative statistics for the data obtained in Chapter 2. We report parameters from both mixed-model linear regression (*i.e.* ANOVA) and mixed-model logistic regressions.

ANOVA tables report the following:

- **Sum Sq:** Sum of squares, which is a measure of the total variability in the data.
- **Mean Sq:** Mean square, obtained by dividing the sum of squares by its degrees of freedom.
- **NumDF:** Numerator degrees of freedom, representing the degrees of freedom associated with the factor being tested.
- **DenDF:** Denominator degrees of freedom, representing the degrees of freedom associated with the error term.
- **F value:** The F-statistic, which is calculated as the ratio of the mean square for the factor to the mean square for the error.

- **Pr(>F):** The p-value associated with the F-statistic, indicating the probability of obtaining the observed results under the null hypothesis (i.e., the probability of observing these results if there is no true effect).

Logistic regression tables report the following:

- **Estimate:** These are the estimated coefficients for each predictor variable in the logistic regression model. They represent the change in the log odds of the outcome for a one-unit change in the predictor, holding all other predictors constant.
- **Std. Error:** Standard errors associated with the estimated coefficients. They measure the variability of the coefficient estimates.
- **z value:** The z-value is obtained by dividing the coefficient estimate by its standard error. It assesses the significance of the estimated coefficient.
- **Pr(>|z|):** The p-value associated with the z-value, indicating the probability of obtaining the observed results under the null hypothesis (i.e., the probability of observing these results if there is no true effect).

B.1 Delayed-alternation in the Y-maze

| | Sum Sq | Mean Sq | NumDF | DenDF | F value | Pr(>F) |
|-------------|--------|---------|-------|---------|---------|----------|
| Group | 0.08 | 0.08 | 1.00 | 7.46 | 0.40 | 0.5444 |
| Delay | 2.92 | 0.73 | 4.00 | 1247.71 | 3.68 | 0.0055** |
| Group:Delay | 0.28 | 0.07 | 4.00 | 1247.71 | 0.35 | 0.8432 |

Table B.1: ANOVA in the Delayed Alternation in the Y-maze. (.) $p < 0.1$, (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$

| | Estimate | Std. Error | z value | Pr(> z) |
|--------------------|----------|------------|---------|----------|
| (Intercept) | 0.68 | 0.18 | 3.78 | 0.00 *** |
| GroupAged | 0.30 | 0.26 | 1.16 | 0.25 |
| Delay20s | -0.11 | 0.19 | -0.54 | 0.59 |
| Delay30s | -0.23 | 0.18 | -1.28 | 0.20 |
| Delay45s | -0.32 | 0.18 | -1.78 | 0.07 . |
| Delay60s | -0.30 | 0.17 | -1.70 | 0.09 . |
| GroupAged:Delay20s | -0.13 | 0.28 | -0.48 | 0.63 |
| GroupAged:Delay30s | -0.31 | 0.25 | -1.24 | 0.21 |
| GroupAged:Delay45s | -0.16 | 0.25 | -0.63 | 0.53 |
| GroupAged:Delay60s | -0.11 | 0.24 | -0.45 | 0.65 |

Table B.2: Binomial logistic regression in the Delayed Alternation in the Y-maze. (.) $p < 0.1$, (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$

B.2 Delayed-alternation in the 8-arm radial maze

| | Sum Sq | Mean Sq | NumDF | DenDF | F value | Pr(>F) |
|-------------|--------|---------|-------|---------|---------|-----------|
| Group | 0.07 | 0.07 | 1.00 | 23.40 | 0.37 | 0.5477 |
| Delay | 61.07 | 30.53 | 2.00 | 2505.51 | 172.13 | 0.0000*** |
| Group:Delay | 0.80 | 0.40 | 2.00 | 2503.28 | 2.26 | 0.1050 |

Table B.3: ANOVA in the Delayed Alternation in the 8-arm radial maze. (.) $p < 0.1$, (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$

| | Estimate | Std. Error | z value | Pr(> z) |
|-------------------|----------|------------|---------|----------|
| (Intercept) | 1.13 | 0.07 | 16.56 | 0.00 *** |
| GroupAged | 0.03 | 0.10 | 0.35 | 0.73 |
| Delay30 | -0.93 | 0.10 | -9.67 | 0.00 *** |
| Delay60 | -0.94 | 0.09 | -10.07 | 0.00 *** |
| GroupAged:Delay30 | 0.02 | 0.14 | 0.16 | 0.87 |
| GroupAged:Delay60 | -0.20 | 0.13 | -1.50 | 0.13 |

Table B.4: Binomial logistic regression in the Delayed Alternation in the 8-arm radial maze. (.) $p < 0.1$, (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$

B.3 All-baited task

| | Sum Sq | Mean Sq | NumDF | DenDF | F value | Pr(>F) |
|-------|--------|---------|-------|-------|---------|----------|
| group | 5.92 | 5.92 | 1.00 | 28.32 | 10.10 | 0.0036** |

Table B.5: ANOVA in the All-baited task. (.) $p < 0.1$, (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$

| | Estimate | Std. Error | z value | Pr(> z) |
|-------------|----------|------------|---------|----------|
| (Intercept) | 1.57 | 0.17 | 9.03 | 0.00*** |
| groupAged | -0.52 | 0.24 | -2.19 | 0.03* |

Table B.6: Binomial logistic regression in the All-baited task. (.) $p < 0.1$, (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$

B.4 VTE-like behavior

| | Sum Sq | Mean Sq | NumDF | DenDF | F value | Pr(>F) |
|-------------------|--------|---------|-------|-------|---------|----------|
| Performance | 31.02 | 31.02 | 1.00 | 46.96 | 3.03 | 0.0881 . |
| GROUP | 29.01 | 29.01 | 1.00 | 45.24 | 2.84 | 0.0990 . |
| Performance:GROUP | 12.96 | 12.96 | 1.00 | 46.33 | 1.27 | 0.2661 |

Table B.7: ANOVA in the number of entries in the 'decision area' during the All-baited task. (.) $p < 0.1$, (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$

| | Estimate | Std. Error | z value | Pr(> z) |
|-----------------------|----------|------------|---------|----------|
| (Intercept) | -1.03 | 1.01 | -1.02 | 0.31 |
| Performance | 0.38 | 0.15 | 2.57 | 0.01* |
| GROUPAged | 2.71 | 1.11 | 2.45 | 0.01* |
| Performance:GROUPAged | -0.32 | 0.18 | -1.85 | 0.07 . |

Table B.8: Poisson logistic regression of the number of entries in the 'decision area' during the All-baited task. (.) $p < 0.1$, (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$

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In memory of Amala & Timothy