Exploring the role of Amyloid-β in the formation and maintenance of object recognition memory

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ABSTRACT

Background: Dysregulated accumulation of the amyloid-beta (Aβ) protein in the brain is one key event in the development of Alzheimer's disease (AD), characterized by severe synaptic dysfunction and memory impairments. Yet, Aβ is also constitutively present in the healthy brain: its secretion is related to synaptic activity, and it can affect memory processes and subsequent synaptic activity. While low concentrations of Aβ peptides promote long-term potentiation (LTP) and memory consolidation, high concentrations as well as complete or partial depletion of Aβ impair these processes and instead promote long-term depression (LTD) (Puzzo et al., 2013).

Objectives: The role of endogenous Aβ on hippocampus-dependent memory processes remains poorly understood and available findings are partly conflicting; additionally, previous research exclusively used male animals in predominantly aversive conditioning tasks. Therefore, we here aimed to explore the role of endogenous hippocampal Aβ in a rat model of incidental (i.e., non-reinforced) declarative memory (a one-trial novel object recognition task), using both male and female animals.

Methods: Male and female Long-Evans rats (3-5 months old) were exposed to two identical unfamiliar objects in an open field for 5 min, followed by a test for long-term memory for these objects 24 h, 48 h or 72 h later. We infused either the anti-Aβ monoclonal antibody 4G8, the BACE1 inhibitor IV, a control antibody (MG2a-53) or saline (PBS) into the dorsal hippocampus before (i.e., to affect encoding) or after learning (i.e., to affect memory consolidation), or during the memory retention interval (i.e., to affect memory maintenance/forgetting).

Results: Object memory retention was not impaired by blocking Aβ or the β-secretase BACE1 during memory acquisition and consolidation. However, blocking Aβ during a memory retention interval prevented the natural forgetting of long-term object memories. Furthermore, females displayed more persistent object memory at baseline compared to males.

Conclusion: Our results suggest that endogenous amyloid-beta in the hippocampus mediates the maintenance but not the formation of object recognition memory.

RÉSUMÉ

Justification : L'accumulation dérégulée de la protéine amyloïde-bêta (Aβ) dans le cerveau est un événement clé de la maladie d'Alzheimer (AD), caractérisée par un dysfonctionnement synaptique et des troubles sévères de la mémoire. Cependant, Aβ est également présent de manière constitutive dans le cerveau sain : sa sécrétion est liée à l'activité synaptique, et peut affecter la mémoire et l'activité synaptique subséquente. Alors que de faibles concentrations de peptides Aβ favorisent la potentialisation à long terme (LTP) et la consolidation de la mémoire, de fortes concentrations ainsi qu'une déplétion complète ou partielle altèrent ces processus et favorisent plutôt la dépression à long terme (LTD) (Puzzo et al., 2013).

Objectifs : Le rôle endogène de Aβ sur les processus de mémoire dépendant de l'hippocampe reste mal compris et les résultats disponibles sont en partie contradictoires ; de plus, les recherches précédentes ont exclusivement utilisé des animaux mâles, principalement dans des tâches de conditionnement aversifs. Par conséquent, nous avons cherché à explorer le rôle du peptide dans l'hippocampe dans un modèle de mémoire déclarative incidentelle chez le rat (tâche de reconnaissance d'objets), en utilisant des animaux mâles et femelles.

Méthodes : Des rats Long-Evans mâles et femelles (âgés de 3 à 5 mois) ont été exposés à deux objets identiques inconnus dans une arène à champ ouvert (open-field) pendant 5 minutes, suivi d'un test de mémoire à long terme pour ces objets 24 h, 48 h ou 72 h plus tard. Nous avons injecté l'anticorps anti-Aβ 4G8, l'inhibiteur BACE1 IV, un anticorps contrôle (MG2a-53) ou une solution saline (PBS) dans l'hippocampe dorsal des rats avant (pour affecter l'encodage) ou après l'apprentissage (pour affecter la consolidation de la mémoire), ou pendant l'intervalle de rétention de la mémoire (pour affecter le maintien/l'oubli de la mémoire).

Résultats : La rétention de la mémoire des objets n'a pas été altérée par le blocage de Aβ ou de la sécrétase BACE1 pendant les phases d'acquisition ou de consolidation de la mémoire. Cependant, le blocage de Aβ pendant un intervalle de rétention de la mémoire a empêché l'oubli naturel de la mémoire d'object à long-terme chez les femelles, mais pas les males. De plus, les femelles présentaient une mémoire des objets plus persistante que chez les mâles.

Conclusion : Nos résultats suggèrent que l'amyloïde-bêta endogène dans l'hippocampe intervient dans le maintien mais pas dans la formation de la mémoire de reconnaissance des objets.

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Contributions of Authors

The author of this thesis collected and analyzed all the work presented in this thesis, under the guidance of Dr. Oliver Hardt. Outside of Dr. Oliver Hardt, all other contributors to this work were recruited, trained and supervised by Célia Sciandra.

Contributions by the author include full management of the breeding and housing colony, all surgeries, euthanasias, preparation of drug solutions for infusions, infusions and experimental procedures, behavioural testing, statistical analyses and preparation of figures. Célia Sciandra as well as volunteer undergraduate students Iris Liu, Juliane Huynh and Xinran Gao sectioned brains for placement checks of all animals included in this work. Behavioural experiments were performed with the equal help of undergraduate research students in the lab, Xinran Gao, An Leng, Doobhashi Devi Dewan, Kaitlyn Capano, and Hannah Buchanan.

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Commonly Used Abbreviations

Aβ – Amyloid-beta

$A\beta_{42/40}$ – Amyloid-beta (epitope 1-42 or 1-40)

AD – Alzheimer's Disease

α7-nAChR – Alpha-7 nicotinic acetylcholine receptor

AMPAR –*α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate* receptor

dHC – Dorsal Hippocampus

E_2 - Estradiol

ICV – Intra-cerebroventricular

ISF – Interstitial fluid

LTP – Long-term potentiation

LTD – Long-term depression

NMDAR – *N-methyl-D-aspartate* receptor

NOR – Novel Object Recognition (task)

PKMζ - protein *kinase C isoform M-zeta*

INTRODUCTION

While Aβ is better known for its pathological role in Alzheimer's Disease (AD), where its aggregation is linked to severe cognitive impairments, recent research suggests that it may be involved in multiple physiological roles, most notably in normal memory processes. However, its precise contribution to memory formation or memory maintenance is still unclear.

An enduring debate surrounding the profound memory loss displayed by AD patients is whether it reflects in inability to acquire and form new memories or an inability to maintain them. Traditionally, memory impairments in AD have been linked to synaptic loss induced by Aβ accumulation in the hippocampus and medial temporal lobe, leading to deficits in the encoding and consolidation of new information and preventing the stable formation of long-term memory. However, recent research suggests that accelerated forgetting, where previously formed memories are lost at an abnormal rate, could also contribute to the mnemonic impairments observed in AD patients. Therefore, the memory deficits observed in AD could reflect both dysregulation in memory formation and memory maintenance mechanisms. Moreover, repeated failed therapeutic interventions targeting reductions of Aβ levels in the brains of AD patients along with significant negative cognitive side effects in both patients and healthy controls suggest that elucidating the role of Aβ in normal memory function might be fundamental to understanding how it could contribute to pathological memory loss seen in AD.

Therefore, we here explore in rats whether Aβ contributes to the formation and forgetting of long-term memory, using a task that models human declarative memory, which is severly imparied in AD. Considering well-documented sex differences in memory function, Aβ regulation and AD outcomes, we will also explore whether there are sex differences in the endogenous role of Aβ in memory in rats. First, we will review the role of the hippocampus in declarative memory, and the role of Aβ and in synaptic plasticity that underlies memory processing. Chapter 1 will present findings on how blocking endogenous Aβ in the hippocampus affects memory formation of male and female rats; Chapter 2 will present findings on how blocking endogenous Aβ in the hippocampus affects memory maintenance of male and female rats. In the Discussion, the broader implications of these findings for understanding the physiological role of Aβ in normal memory processes will be highlighted.

1. Memory

Memory is a fundamental aspect of human cognition, allowing humans to acquire and remember information that reflects their experiences of the world. In that sense, memory reflects a multidimensional and dynamic process deeply entwined with one's sense of self, that has fascinated people and the scientific community alike for centuries (Tulving, 1985; Zlotnik & Vansintjan, 2019). Memories are not static – they reflect the dynamic interplay between ongoing memory encoding, maintenance and retrieval processes that contribute to both their persistence and, often, their transience.

According a widely used taxonomy, human memory can be distinguished into declarative and non-declarative memory, based on the neural structures involved as well as the capacity for conscious recall (Squire & Zola, 1996). Non-declarative memory refers to memories that do not require explicit or conscious retrieval, such as procedural memory or types of associative learning (e.g., skills and habit). On the other hand, recall of declarative memory is usually explicit or intentional, accompanied by awareness of memory recall. There are two forms of declarative memory. Semantic memory reflects general knowledge, i.e., facts about the world, including facts about our own lives, stripped of the context in which this knowledge was acquired; episodic memory refers to specific events or episodes we experienced, including the spatial-temporal context in which they were encountered (Squire et al., 1993; Squire & Zola, 1996; Tulving, 1985).

1.1. The role of the hippocampus in declarative memory

The current understanding of memory reflected in this taxonomy began with the characterization of the peculiar memory deficit of Henry Molaison, more widely known as patient HM, who in the 1950s received bilateral hippocampectomy as a treatment for intractable epileptic seizures. The surgery removed most of his hippocampus and amygdala, as well as surrounding cortical areas, which resulted in severe anterograde amnesia – the inability to form new memories – as well as retrograde amnesia – the loss of recently acquired memories. Specifically, patient HM displayed an impaired ability to form and remember memories for specific events and experiences, but his motor learning and intellectual functions were unaffected, suggesting that memory is not a unified process (Scoville & Milner, 1957; Squire, 2009). This seminal work established that there are different types of memories, supported by different regions of the brain.

Evidence from patient HM, other amnesic patients and subsequent animal models have illustrated the importance of the structure of the medial temporal lobe, most specifically the hippocampus, which is structually highly conserved across species, in forming declarative memories (semantic and episodic), but not non-declarative memories, such as procedural memories (Bayley & Squire, 2003; Rudy, 2008). The hippocampus consists of three distinct areas: the dentate gyrus (DG), hippocampus proper, and subiculum. The hippocampus proper has further subdivisions, and is separated into the four fields CA1, CA2, CA3, CA4 (it should be noted that somtimes DG is considered part of the hippocampus proper). These regions are connected through the tri-synaptic processing loop in which excitatory inputs from the neighbouring entorhinal cortex first reach the DG via the perforant path. The granule cells in DG then project signals onto pyramidal neurons in CA3 via the mossy fibers. Neurons in CA3 then propagate the signal to principal neurons in CA1 via the Schaffer collateral. Lastly, CA1 neurons project to the subiculum, and both CA1 and subiculum project back to the entorhinal cortex, which then relays the signal back to the neocortex (David & Pierre, 2006; Rudy, 2008).

There is currently no consensus as to whether other animals possess declarative memory as humans, as it cannot be determined whether animals experience conscious recollection. There is, however, clear evidence that animals can form declarative-like memories, allowing them to remember *what* (fact) happened *where* (spatial context) and *when* (temporal context) (Lang et al., 2023). For example, the food-storing Western scrub jays can remember what, where and when they hid their favourite food. In one study, the birds were allowed to store either their preferred food (wax worms, which spoil fast) or peanuts, another well-liked food (which do not spoil quickly) at different locations. The birds chose to retrieve the wax worms from their locations after short, but not long delays, indicating the scrub jays remembered not only where they had stored each food, but also when they did that, as longer delays between cashing and retrieving implicated that the worms likely have spoiled (Clayton & Dickinson, 1998). Later research in another type of food-storing bird, the Passerine bird, indicated that this episodic-like memory for food locations was impaired by hippocampal lesions (Hampton & Shettleworth, 1996). Similarly, rats trained to associate the encounter with a preferred food (i.e., “what”, chocolate) to a specific time of the day (i.e., “when”) and a specific location of an 8-arm radial maze (i.e., “where”) were able to use these different types of information to obtain more chocolate in future trials, suggesting the presence of declarative-like memory (Zhou & Crystal, 2009). Rats were also shown to be able to form

memories for the specific locations (where) of odorous spices (what) presented in a specific sequence (when) and all three aspects of this memory were shown to be dependent on an intact hippocampus (Ergorul & Eichenbaum, 2004).

Furthermore, research in primates, whose brains are anatomically closest to humans, has also shown that they possess declarative-like memory. In his seminal work, Mishkin (1978) demonstrated this type of memory in Rhesus monkeys, using the delayed non-matching to sample (DNMS) task. In this task, animals are exposed to an object, followed by a short delay, and then exposure to both the familiar and a novel object. The animals are rewarded for selecting the novel object, implying that they have previous memory for the familiar object. Interestingly, howeveer, hippocampal lesions alone had very little effect on performance on this task (Mishkin, 1978; Rudy, 2008). In rodents, similar models of declarative memory that depend on object familiarity have revealed an even more complex involvement of the hippocampus in such tasks. One such task is the Novel Object Recognition (NOR) paradigm, which relies on the natural and tendency of rats to explore novelty in their environments. Just like the DNMS task, it assesses recognition memory, the ability to identify what has been encountered before (Winters et al., 2008). Hippocampal lesions have revealed that while lesioned rodents could perform basic object recognition – just like the primates in the DNMS task –, they presented with more pronounced memory deficits when the memory load was increased, and temporal delay or spatial features were added to the task, suggesting a hippocampal involvement in more complex aspects of declarative memory, when several types of memory, or modalities, need to be bound together (Eichenbaum et al., 2007; Rudy, 2008). One study further demonstrated that rats with large selective bilateral hippocampal lesions only showed impairments in a task that assessed associative memory of the specific location and context an object had been presented in, but not tasks that assessed novel object recognition, the association between an object and its location, or the association between an object and its context alone, suggesting that the hippocampus is necessary for some, but not all forms of associative context-dependent recognition memory (Langston & Wood, 2010).

Overall, findings from lesion studies support the idea that the hippocampus is necessary for declarative-like memory in both humans and animals, although its involvement may vary depending on species and task demands.

1.2. Synaptic plasticity in the hippocampus

In addition to findings from early amnesia and lesion studies, evidence of the involvement of the hippocampus in memory processes comes from electrophysiological recording (mostly in rodents) studying activity-dependent synaptic plasticity, a process believed to be the cellular substrate of memory. First formulated as a biologically plausible concept within Hebb's notion of cell assemblies (1949) is the idea that persistent changes in behaviour are mediated by persistent changes in synaptic connectivity. It is now widely accepted that learning and memory are depend on changes to synaptic morphology (Bliss & Collingridge, 1993; Nadel & Hardt, 2011; Rudy, 2008), such as the weakening and strengthening of synaptic connections. This has been extensively studied using long-term potentiation (LTP), usually induced with high-frequency stimulation of neurons in hippocampal slice preparations that leads to long-lasting increases of excitatory post-synaptic potentials (Bliss & Collingridge, 1993; Bliss & Lomo, 1973). The related phenomenon of long-term depression (LTD), induced with weaker, more prolonged or low-frequency activation, leads to a long-lasting decrease in synaptic strength (Lüscher & Malenka, 2012). LTP and LTD, two experimental models of the cellular correlates underpinning learning and memory, provide a cellular basis for how experiences may be encoded at the synaptic level. Specifically, at the molecular level the strengthening and weakening of synaptic connections involve the regulated activity of glutamatergic receptors in the hippocampus, specifically *N-methyl-D-aspartate* receptors (NMDARs) and *α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate* (AMPARs), which mediate synaptic changes underlying different phases of memory.

A critical event for the induction of LTP is calcium influx through NMDARs, ligand-gated ionotropic glutamate channels, which activates several molecular cascades involved in long-term synaptic plasticity (Babaei, 2021; Hardt et al., 2013). While strong activation or high-frequency activation of NMDARs causes a large influx of calcium and the induction of LTP, weaker, more prolonged or low-frequency activation of these receptors can initiate LTD. Importantly, experimental manipulations have shown that pharmacologically or genetically blocking NMDARs in the hippocampus prevents the induction but not expression of LTP and LTD, as well as the formation of long-term memory (Rudy, 2008). Furthermore, overexpression of hippocampal NMDARs enhances LTP and memory formation in multiple behavioural tasks, underscoring the importance of these receptors to the acquisition of memory (Babaei, 2021; Rudy, 2008).

The long-lasting strengthening of synaptic connections and expression in LTP depends on the regulated trafficking of AMPARs at the postsynaptic membrane. AMPARs are ionotropic

glutamate receptors that mediate most of the fast excitatory synaptic transmission in the mammalian brain (Babaei, 2021; Rudy, 2008). They are usually composed of GluA1-4 subunits, and their subunit composition affects their trafficking and contribution to synaptic plasticity (Shi et al., 2001). Specifically, the induction of LTP requires the rapid insertion of GluA1-containing-AMPARs into the post-synaptic membranes and the maintenance of LTP requires GluA2-containing-AMPARs to replace the GluA1-AMPARs (Rudy, 2008). Interestingly, studies show that the amount of post-synaptically expressed GluA2-AMPARs in the hippocampus correlates with long-term memory strength and memory persistence. More specifically, the persistence of LTP depends on maintaining the inserted GluA2-AMPARs at post synaptic membranes, which requires the continued activity of the atypical protein kinase C isoform M-zeta (PKMζ) (Babaei, 2021; Hardt et al., 2013). Indeed, inhibiting this protein leads to rapid erasure of established long-term memory but it does not impair learning. This loss of memory induced by inhibiting PKMζ results from the activity-dependent removal of GluA2-AMPARs, suggesting that PKMζ regulates memory persistence and LTP maintenance (Migues et al., 2010; Pastalkova et al., 2006; Schuette et al., 2016). Consequently, the natural forgetting of hippocapmus-dependent long-term memory can be prevented by blocking the removal of GluA2-AMPARs from post-synaptic sites during the memory retention interval (Migues et al., 2016).

In the dorsal hippocampus, most NMDARs are formed of either GluN2A or GluN2B subunits and are found at both synaptic (sNMDARs) and extrasynaptic (exNMDARs) sites. While debated, there is evidence suggesting that synaptic sites are enriched with GluN2A-NMDARs, supporting LTP mechanisms, while extrasynaptic sites are enriched with GluN2B-NMDARs, which instead promote LTD (Babaei, 2021; Dalton et al., 2012; Hardt et al., 2013; D. Liu et al., 2013; Ow et al., 2008). Consistent with this notion, blocking NMDAR activation pharmacologically with the GluN2B-NMDAR selective antagonist Ro25-6981 in the dorsal hippocampus of rats during a memory retention interval increased the persistence of long-term object location memories. In comparison, infusing a GluN2B-NMDAR agonist during the memory retention interval significantly accelerated forgetting (Migues et al., 2019).

Thus, the regulated activity of NMDARs as well as the proper modulation of AMPAR expression is essential for synaptic plasticity and memory (Huganir & Nicoll, 2013; Takeuchi et al., 2014). Taken together, these findings suggest that there is a fine balance between the persistence and the loss of established memories in the hippocampus. Mechanisms involving

AMPAR trafficking, PKMζ activity, or NMDAR activation could play an important role in maintaining this balance between the strengthening and weakening of synaptic connections that underlie memory formation and maintenance. Furthermore, it is reasonable to infer that if stable changes in synaptic connections promote memory persitance and LTP maintenance, forgetting most likely occurs when these changes are destabilized (Richards & Frankland, 2017), as a well-regulated, constitutive memory process (Davis & Zhong, 2017; Hardt et al., 2013).

1.3. Forgetting

While the psychological and neurobiological mechanisms underlying memory formation and learning have been the focus of research for the past decades, comparably less attention has been given to the topic of forgetting, which is defined as the inability to express a memory, whether through actual memory loss or retrieval failure (Benfenati, 2007; Hardt et al., 2013; Richards & Frankland, 2017). Emerging neurobiological research suggests that most of our natural forgetting may be an active process, driven by an endogenous and well-regulated form of synaptic plasticity (Davis & Zhong, 2017; Hardt et al., 2013). One constitutive forgetting process, *active decay*, transpires at the synaptic level and gradually reverses the synaptic modifications implemented by learning and long-term memory formation, much like the experimental phenomenon LTD (Hardt et al., 2013, 2014; Migues et al., 2016). To date, it has been established that this process requires the activation of GluN2b-NMDARs as well as the activity-dependent removal of GluA2-AMPARs from post-synaptic membranes in the rodent hippocampus (Migues et al., 2016, 2019).

Research on forgetting has mainly studied the loss of declarative memories, which initially consist of two parts -- the actual content of the memory, dependent on cortical areas, and a representation of the spatial context, which depends on the hippocampus (Hardt et al., 2013; Rudy, 2008). The hippocampus is though to indirectly link the neocortical representations contributing memory content, essentially providing an "index" to this knowledge. According to active decay theory (Hardt et al 2013), most of our long-term memories are eventually forgotten, due to a constitutive forgetting process that continuously removes long-term memory representations in the hippocampus, essentially erasing the "indices" binding the content representations of episodic or event-like memories. Over time, this process impairs the ability to reactivate the content that was part of an event, effectively leading to forgetting (Hardt et al., 2013). Active decay theory

assumes that this process elminates most of the long-term episodic memories encoded during a day, with only few surviving this form of endogenous memory loss. The theory proposes that this forgetting promotes adaptive behaviour, not only because it allows separating the mundane from the extraordinary, but also because it promotes cognitive processes underpinning behavioural flexibility, such as response generalization (Hardt et al., 2013; Richards & Frankland, 2017; Rudy, 2008).

Modern active forgetting theories therefore stress that constitutive forgetting is a well-regulated form of synaptic plasticity. Like LTP and LTD, active forgetting involves activation of NMDARs and trafficking of AMPARs. Not suprisingly, dysregulation of the same forms of plasticity underpinning active forgetting has been observed in neurodegenerative disorders that involve memory loss, such as Alzheimer's Disease (AD) (Cai et al., 2023; Lüscher & Malenka, 2012; Walsh et al., 2002). A leading theory on the etiopathogenesis of AD proposes that the extracellular accumulation of the peptide amyloid-β (Aβ) strongly disrupts synaptic plasticity mechanisms, leading to neuronal degeneration and the characteristic memory impairments of AD, that could reflect both impairments in mechanisms underlying memory formation, or the ones underlying memory active forgetting (Babaei, 2021).

2. The dual nature of Amyloid-beta

2.1. Alzheimer's Disease

Alzheimer's Disease was first described as a "peculiar severe disease process of the cerebral cortex" by Alois Alzheimer in the early 1900s, when he observed abnormal plaques in the brain of an aged patient with severe memory disturbance (Hippius & Neundörfer, 2003). Since then, AD has become the most common neurodegenerative disorder, affecting over 50 million people worldwide (Kumar et al., 2025; Sajadi et al., 2016; Tartaglia & Ingelsson, 2025; Zhang et al., 2024). It is a form of dementia characterized by severe, progressive cognitive impairments, such as altered sleep patterns, the loss of judgment, of orientation, of the ability to communicate and understand properly but more notably, profound declarative and non-declarative memory loss (Zhang et al., 2024). The loss of hippocampus-dependent declarative memory is often the first and most common clinical symptom of AD, although clinical symptoms often appear decades after the first pathological events in the brain (Sajadi et al., 2016). The cognitive deficits of AD are often

preceded by early synaptic dysfunction and dendritic loss first in the hippocampus, then all over the cerebral cortex, affecting excitatory transmission in the brain (Benarroch, 2018). Because of the pervasive nature of the disorder, it is likely that the observed memory loss in AD results from impaired memory formation as well as dysregulated active forgetting mechanisms.

While multiple hypotheses have been proposed to explain AD pathogenesis, there is currently no commonly accepted theory, most likely due to the complex and pervasive nature of the disorder, which manifests in different forms. Around 1-5% of AD cases are familial forms of AD (FAD), characterized by an early onset (as early as 35 years of age) and genetic mutations in genes that encode the *amyloid-β precursor protein* (APP) or presenilin 1 and 2 (PS1/2), mutations which typically accelerate and increase Aβ production (Kanekiyo et al., 2014). The rest of AD cases (~95%) cases are sporadic forms of AD (SAD), developing later in life (after 65 years old, also called late-onset AD) and influenced by genetic and environmental factors, as well as various comorbidities, making it difficult to establish a precise etiopathogenesis for the disorder (Zhang et al., 2024). Currently however, the most widely accepted pathophysiological mechanism driving cognitive and synaptic dysfunction in AD involves the dysregulated extracellular accumulation of the amyloid-beta (Aβ), which forms amyloid plaques in the brains of both sporadic and familial AD patients and significantly disrupts synaptic plasticity mechanisms, causing neuroinflammation and ultimately leading to the death of neurons (Zhang et al., 2024).

2.2. From the Amyloid Cascade Hypothesis to the Oligomer Hypothesis

Aβ is produced from the cleavage of the large transmembrane *amyloid-β precursor protein* (APP), ranging from 695 to 770 amino acids in length (Shi et al., 2001). APP is expressed ubiquitously across various tissues in the body but more highly so on neurons throughout the central nervous system (Chasseigneaux & Allinquant, 2012). APP is processed in two main pathways, and many of the resulting metabolites are thought to be biologically active (Fanutza et al., 2015; Gulisano et al., 2018; Kummer & Heneka, 2014). In the non-amyloidogenic pathway, APP is cleaved by an α-secretase, recently identified as ADAM10 and ADAM17, within the Aβ domain into a soluble fragment (sAPPα) and an 83 amino acid carboxyterminal fragment (α-CTF / CTF83). The α-CTF is further cleaved by γ-secretase into the short peptide p3 ($A\beta_{17-x}$), a small

non-amyloidogenic peptide. Overall, this cascade precludes the formation of Aβ peptides, is believed to be neuroprotective, and responsible for most of APP processing in the brain (Gulisano et al., 2018). The amyloidogenic pathway, however, is thought to be involved in the etiology of Alzheimer's disease. In this cascade, APP is first cleaved by a β-secretase, recently identified as the β-site APP cleaving enzyme one (BACE1), at one of two specific sites of the Aβ sequence, either at Asp_1 or Glu_{11} (Hampel et al., 2021; Siegel et al., 2017). This cleavage results in a soluble ectodomain sAPPβ as well as either a 99 amino acid C-terminal fragment (CTF99) or 89 amino acids (CTF89). The further cleavage of CTF99 by γ-secretase within the transmembrane domain results in the formation and secretion of full length Aβ peptides ($Aβ_{1-x}$, referred to as Aβ), while the γ-secretase cleavage of CTF89 will produce a N-terminally truncated form of the peptide ($Aβ_{11-x}$) (Siegel et al., 2017). Most sequential cleavage by α- and γ-secretase or β- and γ-secretase results in soluble Aβ species of up to 50 amino acids in length, most often between 36-43 amino acids, that are cleared at a rate of 8.3% per hour (Kanekiyo et al., 2014). The most common Aβ species secreted in the brain is Aβ(1-40) ($Aβ_{40}$), representing around 50% or more of total Aβ levels. The other two major isoforms of the peptide are $Aβ_{38}$, accounting for 15%, and Aβ(1-42) ($Aβ_{42}$), accounting for around 10%. The remaining 25% represent isoforms 15, 16, 17, 34, 37, and 39 amino acids in length, each in minor abundance (Kummer & Heneka, 2014; Mawuenyega et al., 2013). While it is not the most commonly produced, $Aβ_{42}$ has a larger tendency to aggregate in the extracellular space of the brain, especially in old age, therefore being implicated in enhanced neurotoxicity and the formation of neurofibrillary plaques observed in diseased states and memory disorders (Cleary et al., 2005; Das & Yan, 2019; Fagiani et al., 2021; Fanutza et al., 2015; Garcia-Osta & Alberini, 2009; Gulisano et al., 2018; Kummer & Heneka, 2014, 2014; Morley et al., 2010; Puzzo & Arancio, 2013; Tamayev et al., 2012).

Under the amyloid cascade hypothesis, this soluble form of the peptide has been considered an intermediate fragment that becomes neurotoxic only once it starts aggregating in the aging brain (Puzzo & Arancio, 2013). In contrast, the Amyloid-Beta Oligomer (AβO) hypothesis proposes that brain damage in AD neuropathology is first caused by the soluble oligomeric form of Aβ, contributing even more to neurotoxicity and the disruption of learning and memory processes in AD than amyloid plaques (Cline et al., 2018.; Garcia-Osta & Alberini, 2009). This hypothesis has

been the focus of intense research activity and is currently considered a better candidate to explain Aβ toxicity in AD.

2.3. The dynamic regulation of extracellular Aβ levels is activity dependent

The efforts to focus on the soluble form of the peptide have provided evidence that the peptide is endogenously produced and released in the brain, throughout life and under constitutive conditions, and that it is dynamically and directly influenced by synaptic plasticity (Brothers et al., 2018; Cirrito et al., 2005; Puzzo et al., 2008, 2011, 2012). In the healthy brain, APP is most strongly expressed in the cortex and in the hippocampus, suggesting that these are regions with enhanced Aβ levels. While APP is expressed by neurons, glial cells, endothelial cells, and the meninges, neurons are the main source of Aβ production. Specifically, glutamatergic neurons are believed to express more APP than GABAergic or cholinergic terminals, suggesting Aβ is mainly released from excitatory synapses, in relation to synaptic activity. In the rodent hippocampus, neurons in the second layer of the entorhinal cortex and in the border between CA1 and subiculum express significantly more of the $A\beta_{42}$ species than other brain regions, with evidence suggesting that APP is transported from the entorhinal cortex to the hippocampus via axons of the perforant pathway (Cirrito et al., 2005; Kobro-Flatmoen et al., 2023).

While the cellular processes underlying Aβ production and release from neurons are not fully understood, there is strong evidence that they are strongly influenced by synaptic activity. Live microdialysis of hippocampal interstitial fluid (ISF) of 3-5 months old Tg2576 mice that express human APP (and therefore higher levels of Aβ), at an age where they do not present any Aβ deposits, revealed that hippocampal levels of $A\beta_{(1-x)}$ significantly increased (130%) within the first hour following stimulation of the perforant pathway of the hippocampus. Reducing local synaptic activity through administration of the sodium channel blocker tetrodotoxin (TTX) lead to a gradual decrease (60%) in Aβ levels over 6-12 h, overall showing a direct causal relationship between neural activity and ISF Aβ levels. Interestingly, the rapid activity-driven changes in extracellular Aβ levels were found to be directly modulated by processes related to synaptic vesicle release in acute hippocampal brain slices of Tg2576 mice, independent of neural activity alone, suggesting potentially distinct pathways for generation of extracellular Aβ (Cirrito et al., 2005, 2008; Jahn,

2013). As Aβ is not located within synaptic vesicles, this suggests that an event closely related to vesicle exocytosis might underlie Aβ production and release. Cirrito and colleagues (2008) found evidence that activity-dependent increases in Aβ production and release require APP endocytosis for Aβ generation. Inhibiting inhibitory neurotransmission (picrotoxin, $GABA_A$ receptor antagonist) increased endogenous murine ISF $Aβ_{40}$ and $Aβ_{42}$ in young wild-type mice by 130% over baseline, without affecting Aβ clearance mechanisms from ISF Aβ. Moreover, inhibiting synaptic activity by preventing endocytosis of synaptic vesicles (with a dynamin dominant negative inhibitory peptide) significantly reduced ISF Aβ levels, $Aβ_{42}$ significantly more than $Aβ_{40}$ (75 % and 60% over 12 h) (Cirrito et al., 2008). Taken together, these findings suggest that synaptic activity, specifically pre-synaptic vesicle exocytosis, is linked to extracellular Aβ release, by increasing APP endocytosis and therefore Aβ generation, in transgenic and wild-type animals, and that this might be a normal pathway of Aβ production. Interestingly, in humans, brain regions showing the most default metabolic and neuronal activity throughout life are also the regions most vulnerable to Aβ aggregation in AD patients, further supporting a possible causal relationship between synaptic activity and circulating Aβ levels (Buckner et al., 2005; Cirrito et al., 2008).

2.4. Hormesis: Evidence of a physiological role for Aβ

The dynamic regulation of Aβ by synaptic activity suggests that, rather than being solely pathological, Aβ may have a role in normal brain function. Evidence for the peptide's potential effect on mechanisms that underlie learning and memory processes comes from studies showing the apparent hormetic effect of the peptide as a modulator of synaptic activity, where higher concentrations, which mimic those found in the brains of AD patients, have a detrimental impact on synaptic plasticity, but low concentrations, which represent physiological levels of the peptide in the healthy brain, have the opposite effect, promoting synaptic plasticity.

Many studies show that high concentrations of soluble AβOs and monomers in the nanomolar range (nM), mimicking levels found in AD brains, strongly disrupt synaptic long-term potentiation (LTP) and enhance long-term depression (LTD) in rodent hippocampal slices, by primarily disrupting AMPAR and NMDAR activity in glutamatergic synapses (Benarroch, 2018; Haass & Selkoe, 2007; Li et al., 2011; Puzzo et al., 2008; Sanderson et al., 2016). Lower picomolar

concentrations (pM) of the same AβOs, which is believed to better reflect the physiological levels of the peptide in the healthy brain, have opposite effects, enhancing LTP *in vitro*. (Puzzo et al., 2008, 2012). A dose-response curve titration of the effect of Aβ on LTP showed that incubating hippocampal slices with concentrations between 20 nM and 200 nM preparation of $A\beta_{42}$ before high-frequency stimulation impaired LTP at Schaeffer Collaterals synapsing with CA1 neurons, but a preparation of 200 pM enhanced it. Application of $A\beta_{42}$ alone or 20 min after stimulation did not affect basal transmission, suggesting an impact of the peptide specifically on the induction phase of LTP (Puzzo et al., 2008). It is still unclear if this enhancing effect is due to a specific effect on post-synaptic AMPAR or NMDAR activity, or on other aspects of synaptic plasticity, such as pre-synaptic mechanisms (Puzzo et al., 2008; Puzzo & Arancio, 2013).

Studies on partial or complete Aβ depletion have further supported a beneficial role of the peptide, particularly the $A\beta_{42}$ species. The application of an Aβ antibody before, but not after a tetanus shock in electrophysiological studies resulted in impaired LTP, again supporting a role during the induction phase of LTP (Puzzo et al., 2008, 2011). Furthermore, studies with APP-knockout animals revealed impaired LTP of hippocampal neurons in culture, but interpretations are limited due to the role of APP in synaptic neurotransmission, as well as other confounding behavioural deficits observed in these animals (Fanutza et al., 2015). Very few studies have replicated these findings in LTD, but findings from AD studies seem to suggest that high concentrations of $A\beta_{42}$ drive synaptic impairments by disturbing the LTP/LTD balance and through mechanisms that engage LTD signalling, such as an alteration of AMPARs trafficking (Sanderson et al., 2021), but there are no corresponding hypotheses for lower concentrations of the peptide on LTD.

This duality in the effects of the supposedly toxic protein on synaptic activity raises important questions about its physiological role in the brain, highlighting the possibility that the peptide might have an adaptive function related to the modulation of memory processes in the central nervous system (Frackowiak & Mazur-Kolecka, 2023; Puzzo et al., 2008, Puzzo & Arancio, 2013).

3. Considering sex differences

3.1. Gender and Sex differences in declarative memory

The presence, magnitude, and reliability of sex differences in memory has long been debated (Asperholm et al., 2020). Research in humans is ambiguous, as the concepts of gender and sex are often conflated and bias the interpretation of results. While *gender* is a social construct that depends on psychosocial concepts such as self-perception and identity, *sex* is determined by the sex chromosomes and refers to a wide range of biological differences between male and female organisms (Szadvári et al., 2023). Notably, females have been widely underrepresented in neuroscience research, often because of potential modulation of neurophysiological processes by phases of the reproductive cycle (Becegato & Silva, 2022). While the effects of circulating sex hormones cannot explain all sex differences observed in the brain, they can be observed in a wide range of behaviours, notably in learning and memory processes (Cahill, 2006).

For example, some sex and gender differences have been reported in human episodic memory, where women seemingly outperform men on most types of episodic memory tasks. Women perform better than men in verbal tasks or tasks that require recollection of faces or odours, particularly when circulating estrogen levels are high, but men outperform women when the task assesses visuo-spatial episodic memory (Asperholm et al., 2020; Gall et al., 2021). Furthermore, there is also evidence of better object recognition memory in women compared to men (Koss & Frick, 2017).

Research in non-human mammals revealed mixed results, reflecting that females are highly underrepresented. In the context of rodent object memory, for example, there is a female advantage, similar to humans, although not as pronounced (Koss & Frick, 2017). The few existing reports on sex differences in spatial task performance or memory retention in rodents tend to indicate that males outperform females, but this interpretation remains controversial as not all studies report sex differences and findings vary widely depending on the species, task, testing protocol, and variables measured, making results difficult to reproduce and to validate (Becegato & Silva, 2022; Safari et al., 2021). It is also unclear whether these sex differences emerge due to dimorphic neural mechanisms or if they are related to other factors that can affect the outcomes of these tasks, such as experiential variables or strategies employed (Gall et al., 2021).

3.2. Sexual dimorphism in neural mechanisms of memory

There are multiple known anatomical, functional and structural sex differences in the brain (Arenaza-Urquijo et al., 2024). Imaging studies have shown, for example, structural differences between the male and female rodent hippocampus, and electrophysiological studies suggest differences in synaptic plasticity mechanisms as well (Cahill, 2006; Safari et al., 2021). For example, some studies suggest that the CA1 region of the hippocampus in males is larger in volume and in number of pyramidal cells compared to females (Cahill, 2006). Other findings support sexual dimorphism in LTP induction but not expression in the dentate gyrus and CA1 of hippocampal slice preparations, but findings are inconsistent, and no studies have explored sex differences in LTD to date. Evidence suggests that in females, but not in males, the engagement of some plasticity mechanisms requires local estrogen production (Gall et al., 2021).

Lastly, sex differences were identified in the glutamatergic system, although corroborating findings are small in number (Giacometti & Barker, 2020). For example, glutamate levels and glutamate receptor expression vary by sex and in females across the phases of the estrous cycle. Estrus females (i.e., high estrogen levels) have lower AMPAR and NMDAR expression in the hippocampus compared to male rats and diestrus (i.e., low estrogen levels) females, but higher expression of the GluN2B-NMDAR subunit (Giacometti & Barker, 2020; Wang et al., 2015). It is yet unclear how these differences translate to differences in memory acquisition or retention.

To conclude, while sex influences synaptic plasticity and memory, clearly more research is needed to elucidate how these moderate potential sex differences in episodic memory. (Koss & Frick, 2017; Safari et al., 2021). Emerging evidence suggests that sexual dimorphism in neural mechanisms might occur even in the absence of behavioural differences (Cahill, 2006; Szadvári et al., 2023), further supporting the need to include sex as a variable, specifically in pharmacological/pharmaceutical research that attempts to understand neurobiological mechanisms responsible for different aspects of behaviour and disease.

3.3. Sex and Aβ-related research

Epidemiological evidence suggests that women, compared to men, show a higher prevalence (up to a 2-fold) and vulnerability to develop AD (Arenaza-Urquijo et al., 2024; Lindbergh et al., 2020; Carroll et al., 2010). However, there is disagreement whether this sex

difference simply reflects women's greater longevity (suggesting age as the main risk factor of AD) rather than an underlying greater risk compared to men of the same age (Beam et al., 2018; Ungar et al., 2014).

Genetic factors contribute significantly to both familial and sporadic forms of AD. Particularly, the ε4 allele of the apolipoprotein E gene variant (APOE4) is one of the strongest known genetic risk factors of the late onset form of the disease, found in around 50 % of AD patients, and 15 % of healthy older controls. APOE is a protein mainly generated by glial cells, expressed in three main isoforms in humans (APOE2, APOE3, APOE4), with the APOE4 variant being more common. APOE functions as a carrier protein in the brain and liver, involved in the redistribution of cholesterol and lipids among cells (Raber et al., 1998). Carrying one copy of APOE4 (heterozygous) was found to increase vulnerability to AD between 2-3-fold, while possessing two copies (homozygous) increased the risk tenfold, also lowering the age of onset. Notably, the relationship between the presence of the APOE4 gene and the subsequent development of AD has been suggested to be stronger in women compared to men, where women APOE4 heterozygotes had a 4-fold higher risk for AD, and 12-fold for women APOE4 homozygotes (Ungar et al., 2014).

Rodent studies using human APOE4 transgenic mice show that female mice showed greater impairments in spatial memory and object recognition memory tasks compared to male mice, and treatment with exogenous androgens (testosterone) reduced these behavioural deficits, suggesting an interaction between sex-hormones and APOE4-induced memory impairments (Raber et al., 2002). APOE4 is believed to contribute to AD pathology and associated cognitive impairments through multiple different pathways involved in Aβ production, aggregation and clearance as well as other Aβ-independent pathways, but the exact mechanisms are complex and not fully understood (Kanekiyo et al., 2014). Additionally, various studies using AD rodent models report that older females display higher Aβ burden and larger behavioural and memory deficits compared to male mice, further implicating gonadal hormones, especially in the context of aging, in AD vulnerability (Carroll et al., 2010).

Despite identifying differences in male and female susceptibility to AD, there are no clear corresponding hypotheses for Aβ-driven sex differences in healthy brain function, highlighting the importance of studying the role of endogenous Aβ in both males and females. This could reveal

insights into the peptide's potential differential impact on normal memory processing in males and females.

4. Aims & Objectives

The role of endogenous Aβ in constitutive declarative memory remains unclear, and potential sex differences in this area are understudied. Therefore, we here address the role of hippocampal Aβ in male and female rats in a rodent model of human declarative memory (novel object recognition), considering cardinal memory phases, such as memory encoding, stabilization, and maintenance.

METHODS

Subjects. We used 3-5 months old in-house bred male and female Long-Evans rats as well as purchased Long-Evans from Envigo/Inotiv (Livermore, California) with average weight of 300-350 g before undergoing surgery. The animals were housed in groups of four in two-level polyethylene cages. The cages were filled with sawdust bedding and environmental enrichment (PVC tube, wooden gnawing block, nesting material). Each cage was individually ventilated and contained 2 water bottles. Throughout the experiment the animals were kept on a strict feeding regimen where they received 65 g of food pellets per cage daily to remain at about 85% of their free-feeding adult body weight. Water was provided *ad libitum* throughout the experiment. The temperature of the colony room was maintained at 23 ºC and the humidity level remained at 40%. The lights in the colony room turned on at 7:00 A.M. and off at 7:00 P.M. All experiments were performed during the lights-on phase, between 8:00 A.M. and 4:00 P.M. All procedures were approved by the McGill University Animal Care Committee and conform to the standards set forth by the Canadian Council on Animal Care (CCAC).

Apparatus

The animals were tested in 2 separate rooms, each containing an open field arena. The first room (Context A) measured 3 m x 3 m, while the second one (Context B) measured 3 m x 2 m. Each room had no windows and one red door. The lighting in the room was set such that light intensity measured on the floor of the open fields was 15 +/- 1 Lux. Both open fields measured 60

cm x 60 cm x 60 cm. The open fields in contexts A and B consisted of aluminium frames with walls of black or white laminated MDF, with the arena floors 65 cm above the room floors.

The cameras recorded 135 cm above the field. The floors were covered with around 4 cm of sawdust bedding. The objects used for object memories were glued on the outside base of glass Mason jars. The objects were secured in place on the open-field floors with screws and wing nuts in the lids of the jars. Each copy of the object had a unique number invisible to the animals. The objects and their locations (NW-SE, NE-SW) remained constant for each rat but varied and were counterbalanced between them.

Procedures

Pre-experimental handling. Pre-experimental handling occurred over 3 days, in 3-hour sessions on each of these days, the week before the experiment began. We placed the rats in groups of 8 in a large plastic bin (H: 48.3 cm , W: 50.8 cm L: 1.14 m) to get them familiarized with the general handling that the experiments require. The bin contained a 3 cm base layer of sawdust bedding also used in their home cage, as well as several PVC tubes and paper chew sticks for environmental enrichment. During these sessions, the experimenters repeatedly picked up each rat and put the animals on their lap, as well as walked around with the animal in their hands, to simulate handling involved in the experimental task.

Novel Object Recognition Paradigm. Behavioural procedures comprised 4 days of habituation, 1 day of sampling, a memory retention interval of up to 2 days with daily drug injections, and a probe trial. An hour before each experimental session, we placed rats from the same home cage into transparent transport cages and brought from their colony room to the holding room adjacent to the experimental procedure room. The transport cages were made of a polyethylene base, had a wire lid, and measured 45 cm in length, 25 cm in width, and 20 cm in height. Each cage had the same sawdust bedding as used for the home cages, contained a PVC tube, and a water bottle, but no food.

(1) *Habituation.* Rats were placed individually in one of the open fields for 10 min, during which they explored the field with no objects present while the experimenter was outside the room and observed the animal behaviour on a computer monitor. The rats were lowered into the field with their snouts facing a different corner every day of habituation, and the bedding was cleaned and mixed up between rats to disperse any odour traces and remove any fecal deposits.

(2) *Sampling.* The sampling trial started 24 hours after the last habituation day. During sampling, two identical junk objects were placed at opposing locations in the open field arena (NW-SE, NE-SW). The rats were lowered into the arena facing a corner that did not contain an object and were left in the open field for 5 min, after which they were immediately returned to their individual holding cage. After each sampling trial, the objects were cleaned with 70% ethanol in sterile water, and as in habituation, the bedding was cleaned between each rat. At the end of the sampling trial, the animals were left in the holding room for an additional hour before they were returned to their colony.

(3) *Probe.* During the probe trial, one of the two objects presented during sampling was replaced by a novel one. We counterbalanced old/novel object across sex and treatement group. The rats were placed into the same corner that they had seen during sampling and remained in the open field for 3 min. Following the trial, the rats were returned to their holding cages. We pseudo-randomized the order in which we placed the rats into the open fields from day to day throughout the experiment to prevent possible training effects that might arise from a particular participation sequence.

Surgeries

The rats were acclimatized to their colony room for around 2 weeks prior to undergoing surgery. We deeply anaesthetized the animals with isoflurane (4-5% in O_2) before surgery, for up to 12 minutes in a transparent PVC chamber. During surgery, anaesthesia was maintained with 1-3% isoflurane in O_2. Three to four jeweler screws were implanted into the skull. Using a Kopf stereotactic frame, two 22-gauge stainless steel guide cannulas were then implanted bilaterally into the dorsal hippocampus (coordinates A/P +/- 3.60 mm, M/L +/- 3.1 mm, D/V +/-2.4 mm, at an angle 10º away from midline (Paxinos et al., 2004). Dental cement was used to stabilize the cannulas and obturators were inserted inside to prevent blockage. The 28-gauge microinjectors used for infusions protruded 0.5 mm from the guide cannulas. Surgeries took around an hour to complete. Rats were housed individually for 3 days post-surgery with *ad libitum* access to food and water before being rehoused with their original cage mates in the standard colony cages.

Drugs

Purified anti-Aβ antibody, 17-24 Antibody (4G8) was purchased from BioLegend (Cat # 80070) at a concentration of 1 mg/ml in PBS. The control antibody (Purified Mouse IgG2a, k isotype Ctrl Antibody MG2a-53) was also purchased from BioLegend (Cat #401508) and diluted in PBS to a concentration of 1mg/ml. PBS 1X was also used as an inactive control. The BACE1 inhibitor IV was purchased from Millipore Sigma (#565788) and diluted to 1mg/ml in 5% DMSO in PBS.

All drugs but the BACE1 inhibitor IV were infused at a volume of 1 µL into each hemisphere at a rate of 0.25 µL/min. The BACe1 inhibitor was infused at a volume of 2 µL into each hemisphere at a rate of 0.4 µL/min. Drugs were infused with a 28-gauge microinjector connected to a Hamilton® syringe (Model 1701 N SYR) with polyethylene tubing. The injectors remained connected for an additional 90 seconds after the infusion had stopped to allow drug diffusion away from the injector tip. Each injector was disinfected with 70% ethanol and dried thoroughly between every injection.

Histology

After the experiments, we first anesthetized rats through isoflurane inhalation, then euthanized the rats through CO_2 inhalation followed by decapitation. Brains were removed and fixed in a mixture of paraformaldehyde and sucrose. We used a cryostat to obtain sections of 50 µm thickness, at a temperature of -22 ºC. Cannula placement was checked under a light microscope. All rats included in statistical analyses had guide cannulas located bilaterally with injection needle tips terminating in the dHPC, at a distance between -2.64 and -3.96 from Bregma.

Sex-Based Analysis

As discussed in section 4 of the literature review above, it is unclear how sex will affect behaviour or underlying neural mechanisms in our experimental tasks. For this reason, we included equal numbers of male and female adult rats in all experiments and included sex as a factor in all our statistical analysis. We did not house or align experiments according to females' estrous cycles, as estrous cycle monitoring is a highly stressful procedure that could affect the behavioural results, and we would need a larger number of animals to control for the cycle as well. The number of

sacrificed animals would be unethical considering the lack of clear hypotheses on how sex will affect outcomes in our experimental preparations.

Statistical Analyses

The videos recorded in the open fields were coded for the exploratory behaviour of the rats. Behavior was only classified as exploratory when the rats sniffed the objects with their noses at a maximum distance of 2 cm, not when they climbed or rested on it (Ennaceur et al., 1988). We scored the videos using an in-house written software (zScore) and processed for statistical analysis with another in-house software (zChop). Rats are naturally attracted to novelty, and this bias can be used as a proxy for memory expression, as without memory novelty cannot be detected. We calculated novelty preference (*d*) from the total amount of time rats spent exploring the object at the novel location (t_{new}) and at the familiar one (t_{old}) as follows: $d = (t_{new} - t_{old}) / (t_{new} + t_{old})$. The novelty discrimination ratio *d* can take any value between -1.0 and 1.0. A value equal to 0 denotes equal exploration of both object locations, and no exploratory preference, suggesting no memory for the original object locations, while a value above 0 indicates that rats preferred exploring the novel location. Other outcome measures included total exploration time, in seconds, as well as the time (in seconds) it took to reach 30 s of cumulated object exploration, which is considered as providing sufficient sampling of exploratory behaviour to detect novelty expression in rats. Therefore, we excluded from analyses animals that accumulated less than 30 s of exploration during either sampling or probe, and recorded the time it took animals to reach this exploration threshold (Cohen & Stackman, 2015).

All statistical analyses were conducted with Jamovi (version 2.3, https://www.jamovi.org). Provided data were normally distributed, we used *t*-tests to determine group differences and one-sample *t*-tests comparing *d* against 0 to determine whether groups expressed memory. We used two-way ANOVAs to explore differences between sexes and groups on multiple exploratory measures of behaviour. Alternatively, we used non-parametric tests (Welch's or Mann-Whitney U test) for group comparisons and Wilcoxon rank test to compare *d* against 0. All data are represented at mean +/- 1 standard error of the mean. Significance levels were defined as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Chapter 1 – Blocking endogenous amyloid-β during the acquisition or consolidation phases does not impair object recognition memory in male or female rats

1.1. Background

Very few studies have specifically investigated the role of endogenous Aβ monomers or oligomers in memory processes *in vivo*, and even fewer the role of the peptide in "everyday" memories (i.e., memories that are not forms of conditioning, reward, and punishment schedules), such as unreinforced recognition memory or unreinforced event memories, which form the majority of long-term memories formed and forgotten in human, and probably non-human animal life. Previous studies have found that chronic or acute intracerebral injections of high concentrations of soluble AβOs and monomers disrupted the retention and consolidation of both spatial and contextual fear memory, as well as short-term and working memory in rodents, which mimics cognitive deficits found in AD patients (Balducci et al., 2010; Dash et al., 2005; Flood et al., 1991, 1994; Garcia-Osta & Alberini, 2009; Puzzo et al., 2008).

Intracerebroventricular (ICV) injections of picomolar levels of $A\beta_{42}$ in male mice immediately after training (i.e. during consolidation) can enhance memory retention in the aversive T-maze task and in object recognition memory tests compared to saline controls (Morley et al., 2010). Similar effect were observed in the Morris Water Maze. Male mice were injected with either 200 pM (low concentration) of $A\beta_{42}$, scramble $A\beta_{42}$, or vehicle in the dHPC 20 min before training sessions in this task. Mice injected with $A\beta_{42}$, but not scramble $A\beta_{42}$ or vehicle showed significantly shorter latency to reach the hidden platform at the 6^{th} training session and spent significantly more time in the target quadrant during the probe test, indicating enhanced memory acquisition (Puzzo et al., 2008). Furthermore, in contextual fear conditioning, male mice injected with 200 pM $A\beta_{42}$, but not scrambled $A\beta_{42}$ or vehicle 20 min before training showed increased fear expression (i.e., freezing) 24 h later when exposed to the training context, suggesting that low concentrations of Aβ could have enhanced memory retention during the acquisition or consolidation phases (Garcia-Osta & Alberini, 2009; Puzzo et al., 2008; Puzzo & Arancio, 2013). Taken together, these findings suggest that the addition of low picomolar doses of Aβ, specifically the 1-42 epitope, can enhance spatial, contextual and reference memory, an effect potentially mediated by Aβ signalling mechanisms in the hippocampus.

Alternatively, studies on partial or complete Aβ depletion have also yielded inconclusive results on the endogenous role of the peptide in memory processing. Studies of APP knockout (APP-KO) mice reveal that although the mice grew up with no discernable differences in phenotype, performance on spatial and aversive long-term memory tasks (avoidance learning) nevertheless was affected (Senechal et al., 2008). Further investigations of these animals have revealed more complex impairments such as reduction in weight, agenesis of the corpus callosum, hypersensitivity to seizures, and overall impairments in grip strength, locomotor and exploratory activity, and synaptic plasticity (Puzzo et al., 2011). These effects are not limited to mammals, as APP-knockout *Drosophila melanogaster* (*Appl*, the fly homolog for APP) similarly show impaired avoidance learning (Garcia-Osta & Alberini, 2009). However, these results might be due to the lack of APP and not specific to Aβ depletion, as APP is believed to be critical for hippocampal neurotransmission and neuroprotection. Besides, the various locomotor impairments of APP-KO rodent models are confounds for the interpretation of behavioural tasks that assess memory (Fanutza et al., 2015; Puzzo et al., 2011).

Male mice that received drugs into the ventricles to decrease levels of Aβ right before training showed impaired learning to avoid a foot shock in a T-Maze 72 h later (Morley et al., 2010). Male Long-Evans rats displayed similar outcomes in long-term inhibitory avoidance (IA) memory following bilateral intrahippocampal infusions of an Aβ antibody 15 min before, but not after a training session (Garcia-Osta & Alberini, 2009). Interestingly, this amnesic effect was rescued by infusions of exogenous $A\beta_{42}$ into the hippocampus. Furthermore, the intrahippocampal infusion of picomolar doses of $A\beta_{42}$ alone post IA training enhanced memory retention for the task 24 h later, overall suggesting an important role for the peptide in mediating memory formation and consolidation. Lastly, another study found impaired consolidation of auditory fear conditioning memories when supressing the secretases required for Aβ production in the amygdala (BLA) immediately after a training session, once again supporting an adaptive function for the peptide in memory stabilization (Finnie & Nader, 2020).

Overall, findings are not consistent on whether Aβ contributes to memory stabilization at specific timepoints, such as during memory acquisition or consolidation, or whether this finding holds true for other non-aversive or non-reinforced types of memories. While there is evidence that the addition of low levels of Aβ might enhance memory, not much research has been conducted to test whether the opposite also holds true, where blocking Aβ production or depleting

Aβ levels during specific memory phases produces consistent impairments on memory. Furthermore, it is unclear whether these effects can be localized to a specific brain region, such as the hippocampus, or if these findings are comparable in female rodents. Therefore, here we test whether endogenous Aβ in the hippocampus is involved in the encoding and consolidation phases of object recognition memory in both male and female rats.

1.2. Results

1.2.1. Blocking endogenous hippocampal Aβ in the dorsal hippocampus of rats does not impair the acquisition of long-term object memory

We first assessed the effect of blocking the endogenous activity of the Aβ peptide in the hippocampus of both male and female rats on the acquisition of object memory. Cannulated rats underwent our standard novel object recognition protocol, where they were first habituated to an empty open field for 4 days. Twenty-four hours later, we bilaterally infused either the monoclonal Aβ antibody 4G8 or the control isotype antibody MG2a-53 into the dHPC 15 minutes before the 5-minute sampling session, during which rats were exposed to two identical copies of an everyday object placed in opposing corners of the open field. Memory for these objects was tested 24 h later in the probe trial by exposing rats in the same open field arena to one copy of the familiar object and a novel one, placed at the same positions as during sampling (*Fig*. 1).

Male rats infused with 4G8 (n = 13) showed a robust preference for exploring the novel object during the probe trial, $t(12) = 3.175$, $p < .01$, Cohen's d = 0.881, as did rats that received the control antibody MG2a-53 (n = 13) , $t(12) = 5.334$, $p < .001$, Cohen's d = 1.479 (*Fig*. 1A). Similarly, female rats infused with 4G8 (n = 15) also preferred exploring the novel object during the probe trial, $t(14) = 5.25$, $p < .001$, Cohen's d = 1.35. Surprisingly, and contrary to the findings in males, female rats that received infusions of the control antibody MG2a-53 (n = 11) had no significant novelty preference, exploring the familiar and novel object the same, although there was a trend, $t(10) = 2.0$, $p = 0.074$, Cohen's d = 0.602 (*Fig*. 1A). We conducted a two-way ANOVA to explore the effects of sex (Male, Female) and group (4G8, MG2a-53) on novelty preference values. There was no significant main effect of sex, $F(1, 48) = 0.654$, $p = 0.423$, $\eta^2_p = 0.013$, and group, $F(1,48) = 1.349$, $p = 0.251$, $\eta^2_p = 0.027$, and the interaction between sex and group was also not significant, despite a strong trend, $F(1,48) = 3.205$, $p = 0.08$, $\eta^2_p = 0.063$, likely reflecting the

adverse effect of the control antibody on memory expression in female rats that was absent in males (see *Fig*. 1A). A two-way ANOVA on total time spent exploring objects during the probe trial did not reveal a significant effect of sex ($F(1, 48) = 0.781$, $p = 0.381$, $\eta^2_p = 0.016$) or group ($F(1,48) = 0.406$, $p = 0.527$, $\eta^2_p = 0.008$), and the interaction also was not significant ($F(1,48) = 0.405$, $p = 0.528$, $\eta^2_p = 0.008$) (see *Fig*. 1B). There was also no significant effect of sex ($F(1,48) = 0.397$, $p = 0.532$, $\eta^2_p = 0.008$) or group ($F(1,48) = 0.064$, $p = 0.801$, $\eta^2_p = 0.001$), nor was there a significant interaction ($F(1,48) = 0.032$, $p = 0.859$, $\eta^2_p = 0.001$) on the time it took animals to accumulate 30 s of exploratory activity in the probe trial (see *Fig*. 1C). Finally, the total time spent exploring obects during the sampling trial was also not affected by sex ($F(1,48) = 0.374$, $p = 0.544$, $\eta^2_p = 0.011$), group ($F(1,48) = 0.061$, $p = 0.806$, $\eta^2_p = 0.002$), or the interaction of these factors ($F(1, 48) = 0.567$, $p = 0.456$, $\eta^2_p = 0.016$) (see *Fig*. 1D). Overall, the lack of differences for exploratory behaviour suggests that performance in the probe trial was unlikely affected by potential differences in motility or motivation.

Data from our lab shows that our standard novel object recognition paradigm usually leads to robust memory for objects 24 h after sampling, but these data were exclusively collected with male rats. In light of the outcomes of the first experiment, we therefore conducted a control experiment exclusively with females to determine whether the impaired memory of female rats infused with the control solution MG2a-53 reflected worse baseline object memory retention compared to males. Cannulated female rats underwent the NOR task as described above and either received bilateral infusions of phosphate-buffered saline (PBS) in the dHPC or no infusions at all before sampling, and long-term memory for objects was assessed 24 h later, as before. Female rats infused with PBS ($n = 7$) showed strong novelty preference during probe, $t(6)=3.986$, $p = 0.007$, Cohen's d = 1.507, as did those that received no injections at all ($n = 7$), $t(6) = 3.394$, $p = 0.015$, Cohen's d = 1.283 (*Fig.* 2A). There also was no difference between the two groups in their novelty preference scores, $t(12) = 0.819$, $p = 0.428$, Cohen's d = 0.438 (*Fig*. 2A), in their total exploration time during the probe trial, $t(12) = 0.939$, $p = 0.366$, Cohen's d = 0.502 (*Fig.* 2B), in the time it took to reach 30 s of exploratory activity during the probe trial ($t(12) = -0.951$, $p = 0.361$, Cohen's d = -0.508) (*Fig.* 2C), and in the average time exploring objects during the sampling trial ($t(12) = 0.414$, $p = 0.686$, Cohen's d = 0.222) (*Fig*. 2D).

These results suggest that impaired object recognition memory in female rats receiving infusions of the control antibody in the previous experiment does not reflect potential differences

in baseline performance of female compared to male rats in this task, nor different reactions to infusions. To better understand whether performance of the females who received MG2a-53 indeed impaired the formation of object memory significantly compared to the performance of the female rats in the control experiment, we compared only the novelty preference of the female rats (data not shown). This revealed that the novelty preference of female rats infused with 4G8, MG2a-53, PBS, or no infusions at all did not significantly differ ($F(3,35) = 2.35$, $p = 0.09$, $\eta^2_p = 0.167$). There was also no significant group difference in total exploration time ($F(3,35) = 0.789$, $p = 0.508$, $\eta^2_p = 0.063$) or in total sampling exploration time ($F(3, 23) = 0.239$, $p = 0.868$, $\eta^2_p = 0.030$). This suggests that, while the control antibody might have had a disruptive effect on memory, it did not affect exploratory behaviour, and it was overall not strong enough to be detected statistically.

1.2.2. Blocking the activity of β-cleaving enzyme BACE1 does not impair the acquisition of long-term object memory

We aimed to confirm the previous findings by inhibiting the activity of the β-cleaving enzyme BACE1 in the dorsal hippocampus of male and female rats before sampling, to block the cleavage of Aβ peptides from the Aβ precursor protein (APP). Cannulated rats underwent the same behavioural protocol as described above and were infused with either 1 mg/ml of the BACE1 inhibitor IV or of a control solution (5% DMSO in PBS) in the dorsal hippocampus 15 minutes before sampling (see *Fig*. 3).

Male rats infused with BACE1 inhibitor IV ($n = 6$) showed strong novelty preference during the overall probe exploration period, $t(5) = 3.85$, $p = 0.012$, Cohen's d = 1.571. Novelty preference for rats that received the control solution ($n = 7$) almost reached significance, $t(6) = 2.301$, $p = 0.061$, Cohen's d = 0.870 (see *Fig*. 3A). Females that received infusions of BACE1 inhibitor IV ($n = 6$) showed significant novelty preference during probe, $t(5) = 3.224$, $p = 0.023$, Cohen's d = 1.316, as did females infused with the control solution ($n = 8$), $t(7) = 3.197$, $p = 0.015$, Cohen's d = 1.130 (see *Fig*. 3A).

We analyzed the effects of sex and infused drug on novel object recognition with a two-way ANOVA. There was no significant main effect of sex, $F(1,22) = 0.409$, $p = 0.529$, $\eta^2_p = 0.018$, or group, $F(1,22) = 0.202$, $p = 0.657$, $\eta^2_p = 0.009$, and the interaction between sex and group was

also not significant, $F(1,22) = 0.004$, $p = 0.948$, $\eta^2_p = 0.000$ (see *Fig*. 3A). There was a significant main effect of sex, $F(1,22) = 5.707$, $p = 0.026$, $\eta^2_p = 0.206$ on the time animals explored objects during the probe trial, but not of significant effect of group ($F(1,22) = 1.1098$, $p = 0.304$, $\eta^2_p = 0.048$) nor a significant interaction ($F(1,22) = 0.0413$, $p = 0.841$, $\eta^2_p = 0.002$), suggesting females explored more during probe regardless of the drug they received (see *Fig*. 3B). However, there was no significant effect of sex on the time it took animals to reach 30 s of exploratory behaviour during the probe trial ($F(1,22) = 1.009$, $p = 0.327$, $\eta^2_p = 0.046$), no effect of group ($F(1,22) = 0.056$, $p = 0.816$, $\eta^2_p = 0.003$), nor significant interaction ($F(1,22) = 0.031$, $p = 0.863$, $\eta^2_p = 0.001$) (see *Fig*. 3C). This suggests a possible different pattern of exploration between males and females when facing novelty, where females show a longer engagement with the task. It could be that the females explore in shorter bouts of exploration overall, or take slightly more time to approach the novel object, but explore it more once they do. Regarding exploratory behaviour during sampling, there were no significant effects of sex ($F(1,22) = 2.803$, $p = 0.108$, $\eta^2_p = 0.113$) and group ($F(1,22) = 0.111$, $p = 0.742$, $\eta^2_p = 0.005$), and the interaction was also not significant ($F(1, 22) = 2.599$, $p = 0.121$, $\eta^2_p = 0.106$) (see *Fig*. 3D), indicating similar exposure to the objects during the encoding phase.

1.2.3. Blocking endogenous hippocampal Aβ in the dorsal hippocampus of rats after sampling does not impair the consolidation of long-term object memory

We then tested whether Aβ in the dHPC was involved in the consolidation of object recognition memory. Cannulated rats underwent the same protocol as described above and were infused with either 1 mg/ml of anti-Aβ monoclonal antibody or a control antibody (MG2a-53) in the dHPC immediately after sampling (see *Fig*. 4).

Male rats infused with 4G8 ($n = 7$) showed strong novelty preference during the probe trial, $t(6) = 3.33$, $p = 0.016$, Cohen's d = 1.257, as did male rats that received the control solution ($n = 8$), $t(7) = 3.40$, $p = 0.011$, Cohen's d = 1.201 (see *Fig*. 4A). Females that received infusions of 4G8 ($n = 13$) showed significant novelty preference during the probe, $t(12) = 4.76$, $p = <.001$, Cohen's d = 1.319, as did females infused with the control solution ($n = 13$), $t(12) = 2.54$, $p = 0.026$, Cohen's d = 0.704 (see *Fig*. 4A).

We conducted two-way ANOVAs to explore the effects of sex (Male, Female) and group (4G8, MG2a-53) on novelty preference and the exploration times during probe and sampling. There was no effect of sex ($F(1,37) = 0.234$, $p = 0.631$, $\eta^2_p = 0.006$) or group ($F(1,37) = 1.030$, $p = 0.317$, $\eta^2_p = 0.027$) and there was no interaction effect between sex and group ($F(1,37) = 0.416$, $p = 0.523$, $\eta^2_p = 0.011$) on novelty preference (see *Fig*. 4A). Additionally, there was a significant effect of sex on total probe exploration times, $F(1,37) = 10.196$, $p = 0.003$, $\eta^2_p = 0.216$, where males explored objects significantly more than females. This discrepancy in exploratory behaviour across the previous sections is notable, and could reflect a variety of factors addressed more in detail in the discussion section of this thesis, such as variability in behaviour induced by the females' estrous cycle or non-specific cohort effects.

There was no interaction effect ($F(1,37) = 0.505$, $p = 0.482$, $\eta^2_p = 0.013$), and no significant effect of group alone ($F(1,37) = 1.429$, $p = 0.240$, $\eta^2_p = 0.037$) (see *Fig*. 4B). A two-way ANOVA on the time it took animals to accumulate 30 s of object exploration during the probe trial revealed no significant main effect of sex ($F(1,34) = 0.5203$, $p = 0.476$, $\eta^2_p = 0.015$), nor of group ($F(1,34) = 0.922$, $p = 0.344$, $\eta^2_p = 0.026$), nor was their interaction significant ($F(1,34) = 0.061$, $p = 0.807$, $\eta^2_p = 0.002$ (see *Fig*. 4C). Lastly, a two-way ANOVA was conducted to assess the effects of sex and group on overall time spent exploring objects during sampling. There were no effects of sex ($F(1,37) = 0.238$, $p = 0.624$, $\eta^2_p = 0.006$) or group ($F(1,37) = 1.289$, $p = 0.317$, $\eta^2_p = 0.027$) and there was no significant interaction ($F(1,37) = 2.589$, $p = 0.116$, $\eta^2_p = 0.064$) (see *Fig*. 4D).

1.3. Figures

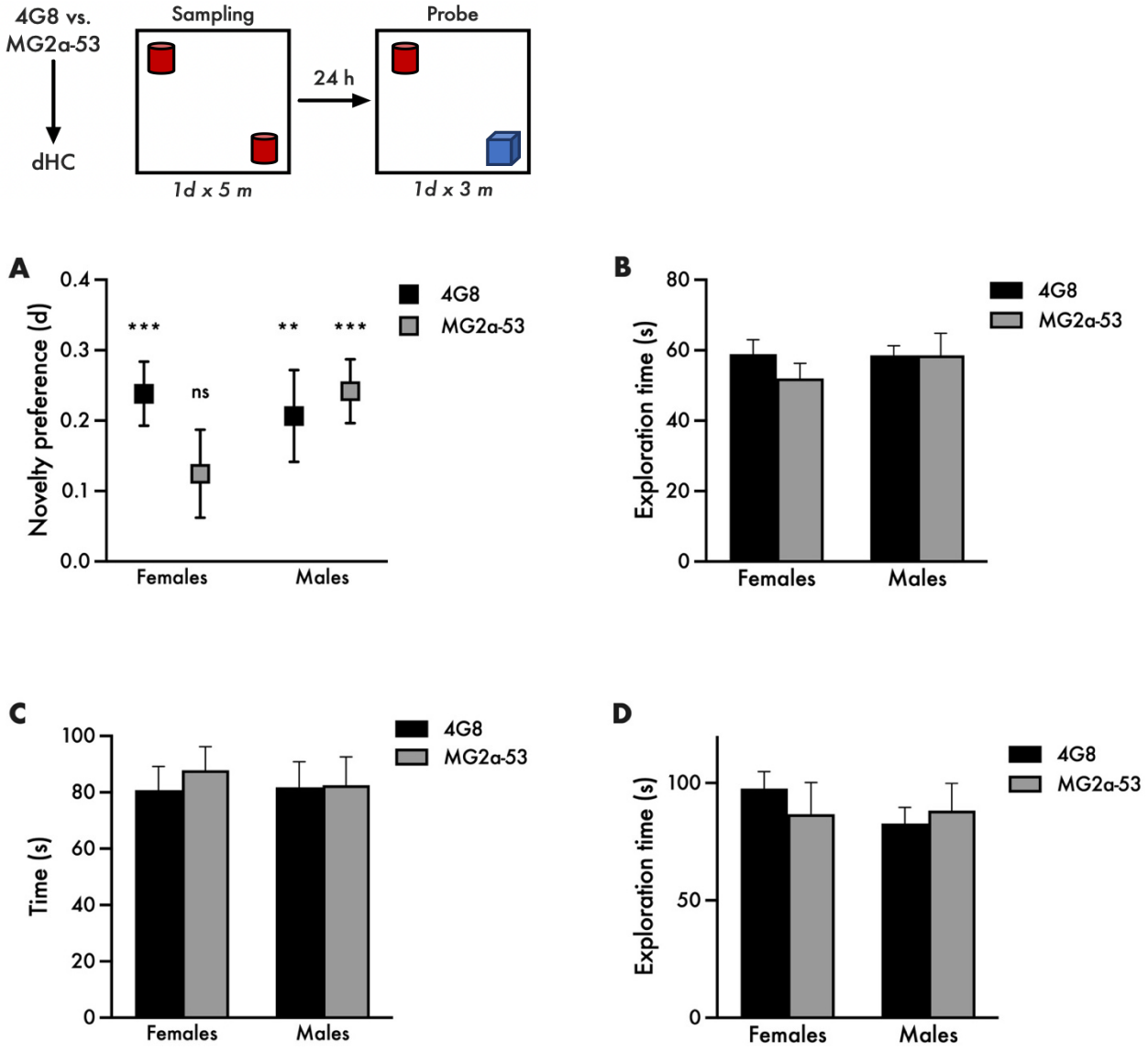

Figure 1. *Blocking endogenous hippocampal Aβ in the dorsal hippocampus of rats with 4G8 before sampling does not impair the acquisition of long-term object memory***.** Representation of experimental protocol. **A.** Novelty preference during probe for female rats infused with the anti-Aβ monoclonal antibody 4G8 (n = 15) or the control IGg2a isotype antibody MG2a-53 (n = 11), as well as male rats infused with 4G8 (n = 13) or MG2a-53 (n = 13). **B**. Total exploration time for each sex and group during probe, in seconds. **C.** Average total time it took for each sex and group to reach 30 s of accumulated exploration during the 3-minute probe, in seconds. **D.** Average total sampling exploration time per sex and group, in seconds. There were no significant differences in exploratory behaviour between the groups or sexes across sampling and probe. Error bars represent standard error of the mean (SE).

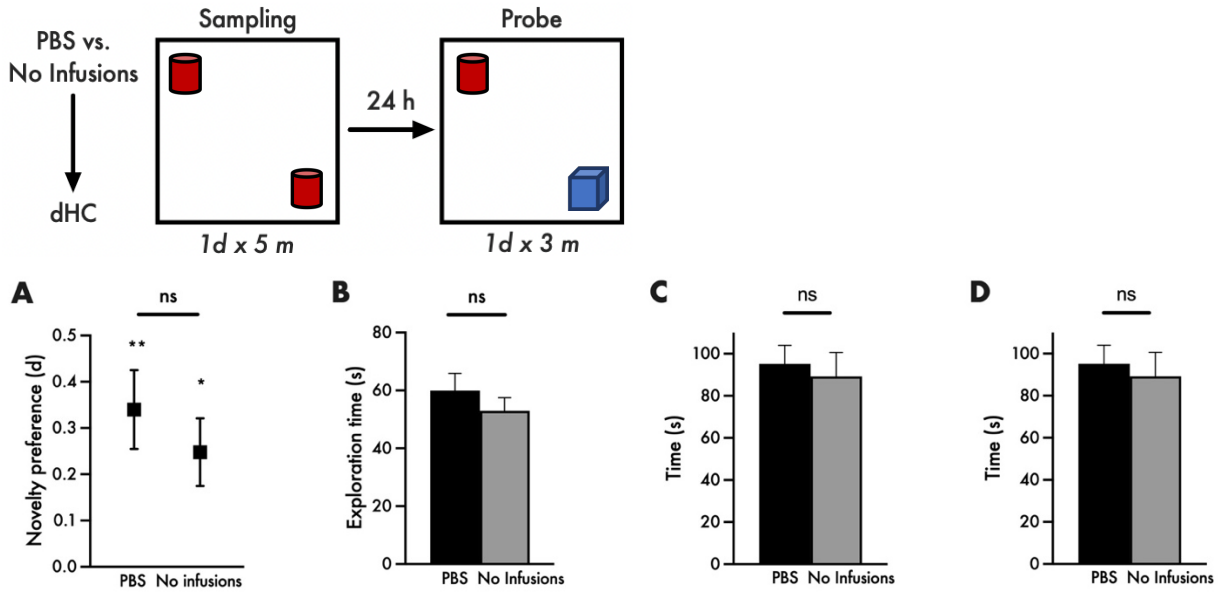

Figure 2. *Females show strong novelty preference at baseline***.** Representation of experimental protocol. **A.** Novelty preference during probe for female rats infused with the inactive solution Phosphate-Buffered Saline (PBS) (n = 7), as well as rats that received no infusions at all (n = 7). Both groups express significant novelty preference during probe. **B**. Total exploration time for each group during probe, in seconds. **C.** Average total time it took for each group to reach 30 s of accumulated exploration during the 3-minute probe, in seconds. **D.** Average total sampling exploration time per group, in seconds. There were no significant differences in exploratory behaviour between the groups or sexes across sampling and probe. Error bars represent standard error of the mean (SE).

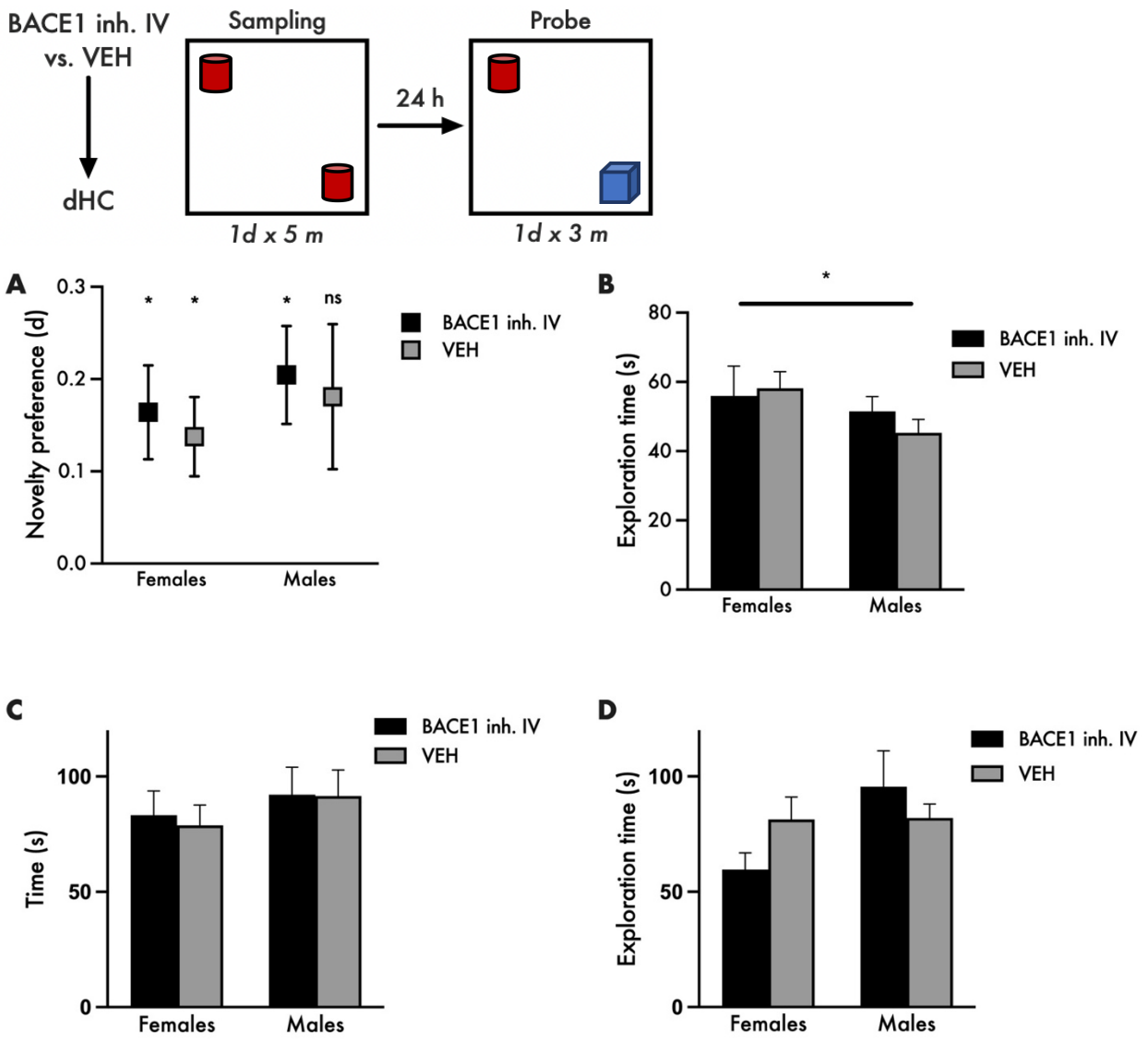

Figure 3. *Blocking the activity of the β-secretase BACE1 in the dorsal hippocampus of rats before sampling does not impair the acquisition of long-term object memory***.** Representation of experimental protocol. **A.** Novelty preference during probe for female rats infused with the BACE1 inhibitor IV ($n = 6$) and with the control solution 5% DMSO in PBS ($n = 8$), as well as male rats infused with BACE1 inhibitor IV ($n = 6$) and with 5% DMSO in PBS ($n = 7$). Both female groups expressed novelty preference during probe, as did male rats that received the BACE1 inhibitor. Surprisingly, male controls did not, although there was strong a trend towards significance. **B**. Total exploration time for each sex and group during probe, in seconds. **C.** Average total time it took for each sex and group to reach 30 s of accumulated exploration during the 3-minute probe, in seconds. **D.** Average total sampling exploration time per sex and group, in seconds. There were no significant differences in exploratory behaviour between the groups or sexes across sampling and probe. Error bars represent standard error of the mean (SE).

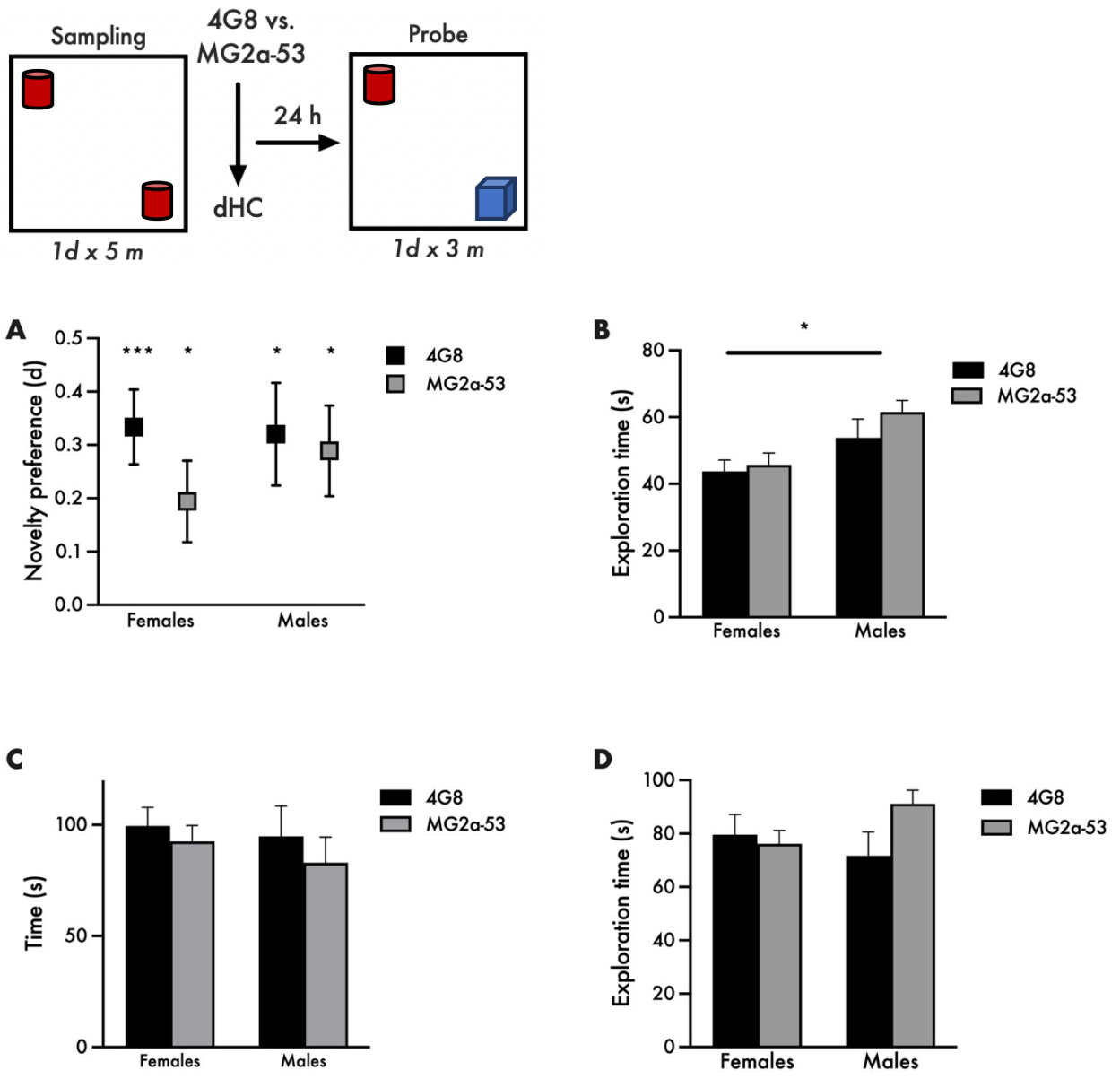

Figure 4. *Blocking endogenous hippocampal Aβ in the dorsal hippocampus of rats immediately after sampling does not impair the consolidation of long-term object memory***.** Representation of experimental protocol. **A.** Novelty preference during probe for female rats (4G8: n = 13, MG2a-53: n = 13) and male rats (4G8: n = 7, MG2a-53: n = 8). All groups showed significant novelty preference during probe. **B**. Total exploration time for each sex and group during probe, in seconds. **C.** Average total time it took for each sex and group to reach 30 s of accumulated exploration during the 3-minute probe, in seconds. **D.** Average total sampling exploration time per sex and group, in seconds. There were no significant differences in exploratory behaviour between the groups or sexes across sampling and probe. Error bars represent standard error of the mean (SE).

1.4. Conclusion

Our results suggest that blocking the endogenous activity of hippocampal Aβ does not impair the acquisition of long-term object memory, as neither an anti-Aβ monoclonal antibody or an inhibitor of the Aβ cleaving enzyme BACE1 affected long-term memory expression. While both males and females infused with 4G8 before sampling showed strong preference for exploring a novel object during the test 24 h later, the use of a purified isotype control antibody had an surprising adverse effect on memory in female but not male rats, possibly suggesting a sex-specific adverse neuroimmune reaction to the control antibody. Since the antibody did not seem to affect exploratory activity, its effect on long-term memory expression unlikely reflects differences on motility or motivation. Interestingly, the control antibody did not impair long-term memory in female rats when infused immediately after the sampling phase, suggesting that it imparied long-term memory because it affected encoding of object memory, but not its consolidation..

Furthermore, while both females infused with the BACE1 inhibitor and the control solution showed strong novelty preference, male control animals showed an unexpected slight memory impairment for objects 24 h later. Once again, this could suggest a sex-specific effect, albeit weaker, to the control solution.One possible explanation for this discrepancy might be the vehicle used to dissolve the drug. The BACE1 inhibitor and its vehicle control contained 5% Dimethyl Sulfoxide (DMSO)/PBS, a universal solvent that can exhibit toxic effects in high concentrations. In our experiments, the concentraiont of DMSO (5%) was higher than what is recommended as safe (1%) for *in vivo* experiments (Galvao et al., 2014). Although speculative, this higher concentration might have caused an unknown effect in combination with the inhibitor. Nevertheless, taken together these results suggest that the activity of the amyloid-β peptide immediately before or after memory encoding is not necessary for long-term novel object recognition memory.

Chapter 2. Blocking amyloid-beta in the dorsal hippocampus during a memory retention interval prevents the forgetting of object memories in female but not male rats

2.1. Background

Rrecent work in *Drosophila melanogaster* has shown that Aβ peptides may also contribute to constitutive forgetting. While pan neuronal expression of Aβ resulted in learning and memory impairments, the restricted expression of the peptides to the mushroom body of fruit flies did not produce memory formation impairments but instead significantly accelerated the forgetting of olfactory long-term memory (LTM) (Kaldun et al., 2021). The mushroom body (MB), like the mammalian hippocampus, is a multi-modal association hub with a prominent role in learning and memory, including active forgetting (Berry et al., 2012; Hourcade et al., 2010). In rodents, ICV injections of the monoclonal Aβ antibody 4G8 after memory acquisition and during a memory retention interval prolonged the duration of object location memories in male wild-type mice (Lee et al., 2018), suggesting that, at least in the context of declarative-like memories, Aβ might be involved in promoting the constitutive forgetting of long-term memory, and blocking its activity might prevent this process. In AD studies, the Aβ peptide, specifically the $A\beta_{42}$ species, are known to potently enhance hippocampal LTD in vitro, but it is unclear if depletion of the peptide also enhances mechanisms of synaptic weakening that might underpin constitutive forgetting, such as active decay (Frackowiak & Mazur-Kolecka, 2023). Interestingly, treatment with 4G8 in brain tissue samples of AD patients significantly ameliorated synaptic and dendritic loss, further supporting the hypothesis that Aβ peptides might act on mechenisms that promote synaptic weakening (Sandberg et al., 2022).

Taken together, these findings raise the intriguing possibility that under physiological conditions, endogenously released Aβ peptides, regulated by synaptic activity, might promote the natural forgetting of hippocampus-dependent memories without causing heightened neurotoxicity or cell death. Therefore, here we here aim to test part of this hypothesis by exploring the contribution of Aβ to memory retention in both male and female rats. We infused an Aβ antibody during the memory retention phase of an object recognition task, to validate and expand on the work by Lee and Choi (2018) and assess potential sex-differences in Aβ-mediated forgetting.

2.2. Results

2.2.1. Blocking endogenous hippocampal activity of Aβ during consolidation and a 48 h memory retention interval does not prevent the natural forgetting of long-term object memory in male rats.

We assessed whether blocking the endogenous activity of the Aβ peptide in the hippocampus affects the maintenance of object memory. We used our standard NOR protocol, and replicated the infusion protocol reported in a previous study (Lee et al., 2018). We bilaterally infused either the monoclonal antibody 4G8 or its vehicle PBS 1X as a control in the dHPC immediately after the 5-minute sampling session and then once more 24 h later. Memory was tested 24 h after the last infusion session (48 h after sampling), in a probe session as above (see *Fig*. 5). From previous studies in our lab, we know that this NOR protocol reliably results in strong memory for objects 24 h after sampling, but not 48 h after (Groves, I., 2023).

As expected, male rats that received infusions of the vehicle (n = 5) did not express novel object preference 48 h after sampling, $t(4) = 1.445$, $p = 0.222$, Cohen's d = 0.646. Interestingly, the rats that received 4G8 infusions (n = 6) expressed a stronger novelty preference, resulting in a strong trend, $t(5) = 2.21$, $p = 0.078$, Cohen's d = 0.901 (*Fig*.5A). Females that received injections of 4G8 (n = 12) showed strong novelty preference 48 h after sampling, $t(11) = 4.32$, $p = 0.001$, Cohen's d = 1.247, but, surprisingly, so did the females that received the inactive vehicle (n = 14), $t(13) = 3.78$, $p = 0.002$, Cohen's d = 1.011 (*Fig*. 5A).

To compare performance between the two sexes, we first conducted a two-way ANOVA to explore the effects and interaction of sex and group on novelty preference during the probe trial. There was no significant main effect of sex ($F(1, 33) = 1.273$, $p = 0.267$, $\eta^2_p = 0.037$) or group ($F(1, 33) = 0.157$, p = 0.694, $\eta^2_p = 0.005$) nor was the interaction significant ($F(1, 33) = 0.764$, $p = 0.388$, $\eta^2_p = 0.023$) (*Fig*. 5A). An ANOVA exploring the effects of sex and group on total time spent exploring objects during the probe trial revealed a significant effect of sex ($F(1, 33) = 18.762$, $p < .001$, $\eta^2_p = 0.362$), indicating that females showed more exploratory activity than males. There was no effect of group ($F(1,33) = 0.281$, $p = 0.599$, $\eta^2_p = 0.008$) or a significant interaction (F(1,33) = 1.582, p = 0.217, $\eta^2_p = 0.046$) (*Fig*. 5B). Females also accumulated 30 s of exploratory behaviour faster than males ($F(1,32) = 4.724$, $p = 0.037$, $\eta^2_p = 0.129$), regardless of drug received ($F(1,32) = 1.599$, $p = 0.215$, $\eta^2_p = 0.048$). There was no significant main effect of group on the time it took to

reach 30 s of exploration ($F(1,32) = 0.052$, $p = 0.821$, $\eta^2_p = 0.002$ (see *Fig*. 5C). Although there was a strong trend, the explained variance was low (<1%), suggesting a practically negligible effect of group on the outcome measure. Lastly, females also explored significantly more than males during sampling ($F(1,33) = 10.86$, $p = 0.002$, $\eta^2_p = 0.248$). There was no significant main effect of group ($F(1,33) = 2.84$, $p = 0.101$, $\eta^2_p = 0.079$) and no significant interaction ($F(1,33) = 2.01$, $p = 0.166$, $\eta^2_p = 0.057$) (see *Fig*. 5D).

This strong exploratory difference between females and males is unlikely to reveal a true sex difference however, as an additional comparison of exploratory behaviour across the different cohorts of animals included in this result revealed that a specific cohort of females included in this last analysis exhibited significantly higher probe and sampling exploration times compared to all other females (all p<.001), but not higher novelty preference scores ($F(2,34) = 1.10$, $p = 0.346$, $\eta^2_p = 0.061$), suggesting that there were cohort differences for the females driving the observed sex difference in exploratory behaviour, instead of systematic effects (data not shown).

2.2.2. Blocking the endogenous hippocampal activity of Aβ during consolidation and a 72 h memory retention interval prevents the natural forgetting of long-term object memory

In order to assess whether 4G8 might impact the forgetting of long-term object memories in females, who expressed memory independent of whether they received 4G8 or its vehicle, we repeated the previous experiment with females alone, but tested memory 72 h after sampling. As described above, animals received either 4G8 or PBS control after sampling and then then additional infusions, 24 h and and 48 h after the first one. Memory was tested 24 h after the last infusion (72 h after sampling) (*Fig*. 6). Females that received injections of the control solution ($n = 5$) did not show preference for the novel object ($t(4) = 1.054$, $p = 0.351$, Cohen's d = 0.472). However, females that received infusions of 4G8 ($n = 6$) strongly expressed novel object preference, $t(5) = 3.34$, $p = 0.021$, Cohen's d = 1.36. The group difference, however, was not significantly different, $t(9) = 0.601$, p = 0.562, Cohen's d = 0.364) (see *Fig*. 6A). The time spent exploring objects was the same during the probe trial for both groups ($t(9) = 0.003$, $p = 0.998$, Cohen's d = 0.001) (see *Fig*. 6B), as was the time required to accumulate 30 s of exploratory activity ($t(9) = 0.298$, $p = 0.772$, Cohen's d = 0.181) (see *Fig*. 6C). There were also no group differences for the time spent exploring objects during the sampling trial ($t(9) = 1.625$, $p = 0.139$,

Cohen's d = 0.984) (see *Fig*. 6D). Taken together, these rsults suggest that differences in motivation or exploratory ability do not account for the differences in novelty expression, indicating that the Aβ antibody attenuates natural forgetting that unfolds over time.

2.3. Figures

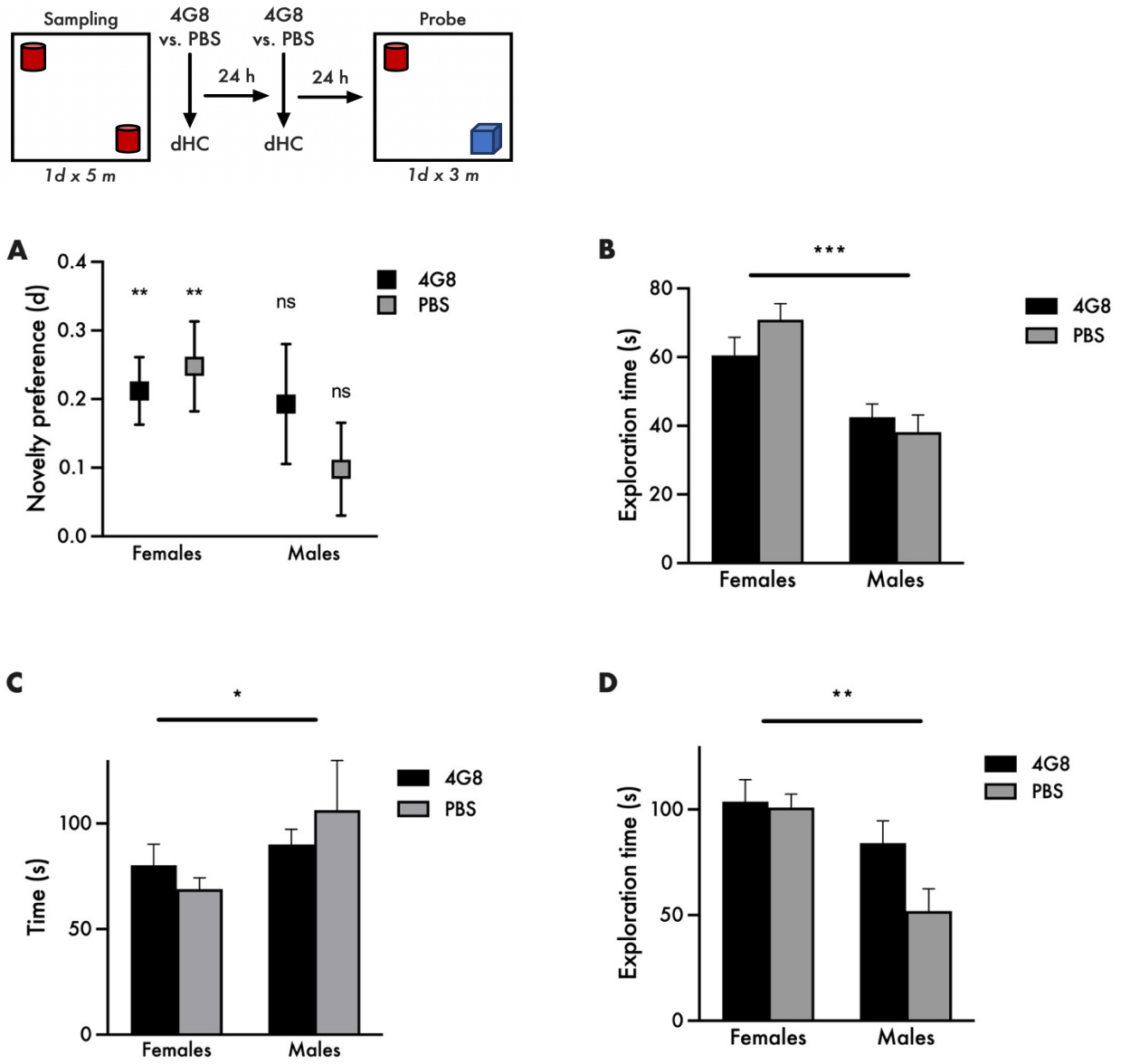

Figure 5. *Blocking endogenous hippocampal Aβ during consolidation and a subsequent 2 d retention interval does not prevent natural forgetting of long-term object memories in male rats.* Representation of experimental protocol. **A.** Novelty preference during probe for female rats (4G8: n = 12, PBS: n = 14) and male rats (4G8: n = 6, PBS: n = 5). Both female rats that received the 4G8 antibody and the PBS control expressed significant novelty preference 48 h after sampling. Both male rats that received 4G8 and PBS did not show significant novelty preference, although there was a trend towards significance for 4G8 male rats. **B**. Total exploration time for each sex and group during probe, in seconds. Female rats explored significantly more than male rats, but this effect was driven by one specific female cohort. **C.** Average total time it took each sex and group to reach 30 s of accumulated exploration during the 3-minute probe, in seconds. Females reached that exploratory threshold significantly faster than males. **D.** Average total sampling exploration time per sex and group, in seconds. Females explored significantly more during sampling compared to males. Error bars represent standard error of the mean (SE).

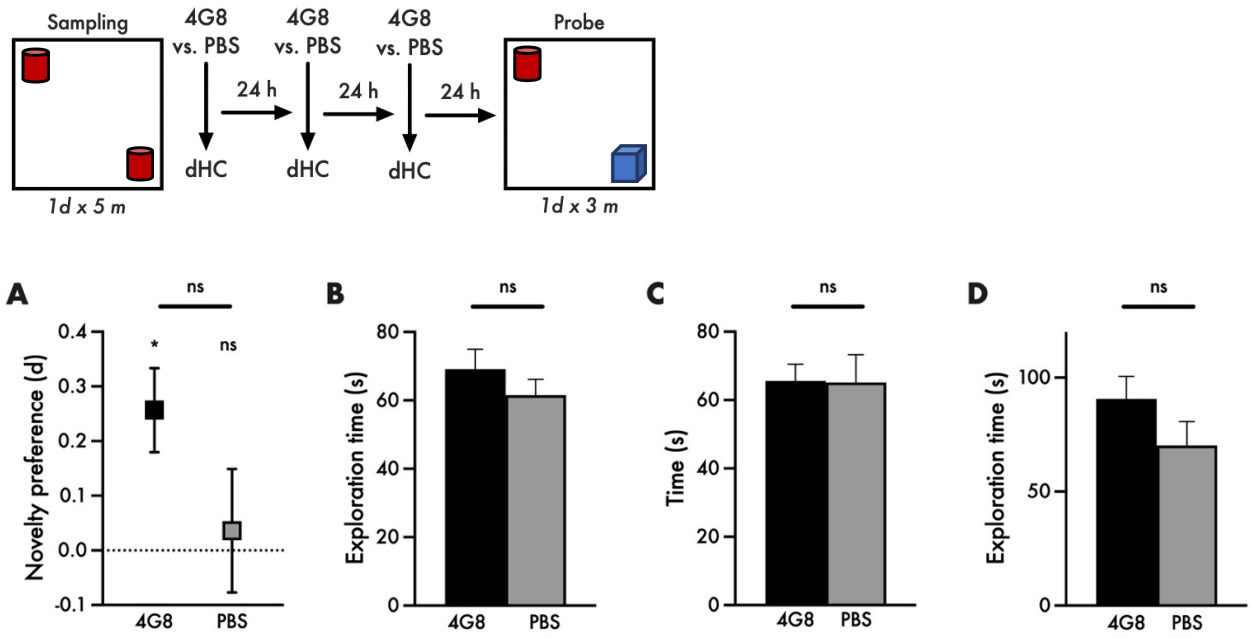

Figure 6. *Blocking endogenous hippocampal Aβ during consolidation and a subsequent 3 d retention interval prevents natural forgetting of long-term object memories***.** Representation of experimental protocol. **A.** Novelty preference during probe for female rats infused with 4G8 (n = 6) and rats that received inactive solution PBS (n = 5). Rats that received the 4G8 antibody, but not the inactive control, expressed significant novelty preference. **B**. Total exploration time for each group during probe, in seconds. **C.** Average total time it took each group to reach 30 s of accumulated exploration during the 3-minute probe, in seconds. **D.** Average total sampling exploration time per group, in seconds. There was no difference in overall exploratory behaviour between the two groups across probe or sampling. Error bars represent standard error of the mean (SE).

2.4. Conclusions

Our results suggest that female rats have more persistent long-term memory for objects than males given the same training experience, as the former expressed novel object preference 48 h after sampling, but the latter did not. This supports the view that female rats, compared to males, perform better at object recognition task (Cost et al., 2012; Sutcliffe et al., 2007). Additionally, and maybe more importantly, our results suggest that blocking the activity of the Aβ peptide in the hippocampus during the memory retention period can extend the duration of object memories beyond their natural lifespan, although the effect was only statistically significant in female animals.

Overall, our results seem to validate and expand on the effect found by Lee and colleagues (2018), where male mice that received intracerebroventricular 4G8 infusions immediately after exposure to objects in a NOR task, as well as 24 h and 48 h after expressed significant preference for a novel object when tested 3 d later. While we only find conclusive evidence for females, we provide further evidence that this effect on memory maintenance is specific to mechanisms underlying activity in the hippocampus. However, it remains unclear which mechanisms underpin the effect of Aβ on memory maintenance and whether this effect in our studies emerged because it affected memory consolidation, memory maintenance, or both.

DISCUSSION

We aimed to investigate the role of endogenous hippocampal Aβ in different phases of declarative memory in male and female rats. We found that infusing pharmacological agents that prevent the activity or release of the peptide before a period of sampling or immediately after did not impair the encoding or consolidation of object memory when tested 24 h later. However, we found that infusing an antibody during a memory retention interval prevented the natural forgetting of object memories in female, but not in male rats, suggesting a potential sex-specific mediating role of the peptide in long-term object memory maintenance or forgetting mechanisms. Lastly, female rats displayed longer lasting memory for objects compared to males rats given the same training protocol, suggesting potential sex differences in task-specific strategies or in the neural mechanisms underlying memory formation or maintenance.

1. Amyloid-β in memory formation

Contrary to findings showing a clear impairment of long-term memory formation when blocking endogenous hippocampal Aβ before training (Garcia-Osta & Alberini, 2009; Puzzo et al., 2011), we found that blocking Aβ in the hippocampus of male and female rats before a single training session in a novel object recognition paradigm had no effects on memory retention 24 h later. Furthermore, while some studies have shown that enhancing levels of hippocampal Aβ immediately after training augmented inhibitory avoidance memory, depleting Aβ levels at that same time point did not affect memory (Garcia-Osta & Alberini, 2009). In agreement with these findings, we found that blocking Aβ immediately after learning objects had no detectable effects on long-term object memory in male or female rats assessed in an object recognition task. Taken together, these findings suggest Aβ is not essential for the encoding or consolidation of object recognition memory in the hippocampus, suggesting the endogenous role of the peptide on memory could be task and manipulation dependent.

1.1. Does object recognition memory depend on the hippocampus?

Alberini and Garcia-Osta (2009) found a mediating role of Aβ on memory formation using an inhibitory avoidance task, a fear-conditioning-based paradigm in which rodents learns to

associate a specific context twith a foot shock, resulting in avoidant behaviour for that context. This specific task is known to require the hippocampus. Similarly, Puzzo and colleagues (2011) found a that Aβ is involved in the encoding (but not consolidation) of reference memory in the Morris Water Maze and in contextual fear conditioning, which are both hippocampus-dependent. On the other hand, it is to date unresolved in what form the hippocampus contributes to novel object recognition memory (Winters et al., 2008).

1.1.1. Recollection versus Familiarity: Distinct mechanisms for similar behaviour?

Human recognition memory is believed to be composed of two distinct processes that differ in subjective experience, functional characteristics and underlying supporting neural mechanisms. On the one hand there is *familiarity*, which reflects the experience of having encountered an object, independent of the context in which it was experienced, and on the other hand there is *recollection* – the explicit recall of the circumstances or spatio-temporal context in which something was experienced (Eichenbaum et al., 2007; Nadel & Hardt, 2011; Winters et al., 2008). Research suggests that the familiarity aspect of recognition memory relies principally on the perirhinal cortex, which encodes representations of distinct items, such as objects. The process of recollection on the other hand depends on the retrieval of both item representations from the perirhinal cortex, as well as associated context representations, an associative pattern encoded by the hippocampus (Brown & Aggleton, 2001; Eichenbaum et al., 2007). Hippocampal lesion studies in rodents have revealed variable deficits in object recognition tasks, with seemingly more pronounced impairments after extended memory retention delays. In contrast, lesion studies of the perirhinal cortex result in consistent and severe deficits in these tasks, suggesting that paradigms such as NOR require the use of a strategy that relies principally on familiarity judgments rather than recollection (Eichenbaum et al., 2007).

In that sense, it is likely that our paradigm includes only a minimal part of processes involved in this form of declarative memory. Standard NOR paradigms only assesses whether an object has been seen before, without other key aspects of episodic memory, such as time and context of where that object was previously encountered. While our paradigm assesses declarative-like memory, it might align more with semantic-like processing rather than episodic memory processing, reducing hippocampal involvement during memory formation. Considering we used the same animal strain, antibody, and concentration as in the study by Alberini and Garcia-Osta

(2009), it is therefore possible that we found no effects of blocking Aβ in the hippocampus during encoding because good performance on our task (i.e., obtaining a high novelty preference ratio) relies mainly on familiarity judgments, which would require little encoding of contextual cues and therefore little hippocampal involvement.

1.2. Dynamic fluctuation of Aβ levels in the brain: is it task dependent?

Evidence suggests that naturally secreted levels of Aβ fluctuate dynamically in many brain regions (Puzzo & Arancio, 2013). In the hippocampus, APP cleavage and Aβ release is highly activity-dependent, with studies showing that synaptic activity, such as it occurs during learning or memory formation, leads to transient rises in extracellular Aβ levels, and that reduced neuronal activity can also decrease Aβ levels in the brain (Cirrito et al., 2005). Since different behavioural tasks engage distinct synaptic activity patterns and neural circuits, we may not have found effects of Aβ depletion due to task-dependent variations in Aβ production.

Puzzo and collegues (2011) estimated that depleting murine hippocampal levels of endogenous APP via a small interfering RNA (siRNA) significantly impaired LTP both *in vitro* and *in vivo*, impairing performance on the Morris Water Maze task. Treatment with 200 pM of $A\beta_{42,}$ but not 100 pM was sufficient to rescue the observed LTP and memory impairments (Puzzo et al., 2011). Furthermore, they found a significant increase in $A\beta_{42}$ in the hippocampus but not cerebellum of mice sacrificed 1 min after contextual fear learning, but not after 5 or 30 min, and not in any other experimental conditions (groups that received no tone and no shock, tone alone, foot shock alone with no contextual association) (Puzzo et al., 2011). $A\beta_{42}$ levels were also found to be more elevated in the basolateral amygdala of rats after strong compared to weak auditory fear conditioning, overall suggesting very specific conditions for increases in Aβ production in various brain regions involved in the tasks (Finnie & Nader, 2020). These findings support the notion that Aβ production is strongly tied to synaptic activity. Specifically, Aβ levels increase in a transient, tightly regulated and highly localized way, shortly following a strong memory encoding event.

Interestingly, data from our lab show that activity in the hippocampus is necessary to consolidate, but not acquire or express long-term object recognition memory, as inhibiting hippocampal synaptic activity with $GABA_A$ and $GABA_B$ agonists during exposure to objects

significantly impaired their recognition 24 h later (Groves, 2023). While this suggests that the hippocampus critically contributes to forming object memories, it is possible that overall the engagement of the hippocampus was too low to produce a significant and behaviour-relevant change in extracellular Aβ – in that underlying hippocampal mechanisms during object memory formation do not rely on task-evoked Aβ release. Tasks that require a stronger involvement of the hippocampus such as spatial navigation, or contextual fear conditioning may invoke stronger synaptic activity in the hippocampus than a non-reinforced object recognition paradigm, most likely leading to increased production of extracellular Aβ that might be more relevant to the observed behaviour. Furthermore, the emotional salience of information during fear-based learning is well known to engage memory-modulating mechanisms which influence the strength of recently acquired memories, potentially further explaining the discrepancies between our results and these findings (Puzzo et al., 2011; Rudy, 2008). Considering the likely involvement of the perirhinal cortex in novel object recognition, it remains possible that memory-relevant changes in Aβ levels occur in that region during object memory acquisition, rather than the hippocampus.

1.3. Mechanism of Aβ-mediated effects on memory formation

While our findings do not support a role of Aβ in memory formation, previous studies have suggested that the peptide does have a mediating role during that specific phase of memory, and that it might be dependent on the presence and activity of *α7-nicotinic acetylcholine* receptors (α7-nAChRs). Aβ binds with picomolar affinity to these receptors, a subtype of acetylcholine receptors expressed widely in the hippocampus, modulating presynaptic neurotransmitter release and synaptic plasticity (Letsinger et al., 2022; Puzzo & Arancio, 2013). Interestingly, low concentrations of Aβ can act as an agonist to α7-nAChRs, allowing increases in calcium influx that facilitate neurotranmistter release. Furthermore, since α7-nAChRs is expressed in both excitatory and inhibitory (GABAergic interneurons) neurons, activation of α7-nAChRs increase presynaptic release of both glutamate and GABA, depending on the timing of activation (Letsinger et al., 2022). In contrast, high concentrations of the peptide inhibited the activity of the receptors, especially in the presence of the strong genetic predictor of AD apolipoprotein E4 (APOE4), which can increase the binding of Aβ and α7-nAChRs (Letsinger et al., 2022).

The role of these receptors in mechanisms underlying memory formation is further supported by findings showing that the constitutive effects of Aβ on memory were mediated by α7-nAChRs (Puzzo et al. 2011). Hippocampal slices from α7-nAChRs-KO mice perfused with an Aβ antibody did not show impaired LTP or post-tetanic potentiation (PTP), a type of short-term synaptic activity that reflects enhancement of neurotransmitter release following high-frequency stimulation; however, in slices from wild-type controls LTP was impaired, suggesting α7-nAChRs are involved in the impairment of synaptic plasticity induced by depletion of Aβ levels (Puzzo et al., 2011). Furthermore, while the α7-nAChRs-KO transgenic mice in these studies did not show impaired reference and contextual fear memory, they also did not show an enhancement in memory after infusions of picomolar levels of Aβ compared to controls, suggesting that the effect of Aβ on synaptic activity is partially mediated by the activity of α7-nAChRs (Puzzo et al., 2008, 2011). It is possible that the memory enhancement following exogenous infusions of Aβ depends on α7-nAChRs activity, but the interaction between Aβ and α7-nAChRs is not necessary for normal memory formation. Taken together, these findings suggest that Aβ acts as a memory modulator instead of a memory-permitting molecule, and that the interaction between α7-nAChRs and the Aβ peptides plays an important neuromodulatory role in synaptic dynamics (Puzzo et al., 2011).

Our lack of behavioural effects following Aβ depletion in the object recognition task does not contradict these findings. In fact, while our outcomes most likely reflect the low task demand on the hippocampus and a stronger involvement of other cortical regions in the acquisition of object recognition memory, they do support the view that Aβ may not be an essential molecule for memory formation in the hippocampus, even if its production is tightly connected to ongoing synaptic activity and plasticity mechanisms. Our previous findings show that hippocampal plasticity is required in our NOR paradigm, as infusing the selective NMDAR antagonist APV intrahippocampally before exposure to objects significantly impaired memory for familiar objects in male rats (Groves, I., 2023). Considering that synaptic plasticity is linked to Aβ production, it suggests that during memory acquisition of object recognition memory, Aβ might not critically interact with pre or post-synaptic mechanisms of long-term plasticity in the hippocampus, and that its impact on memory formation might be more critical in tasks that rely on α7-nAChRs modulation of synaptic activity. In that sense, it might be interesting to explore whether slightly increasing levels of the peptide in the hippocampus of rats during NOR would enhance memory

for objects, and whether this effect would also depend on α7-nAChRs for object recognition. Furthermore, it would be interesting to explore whether adding an emotional signalling to the NOR task, such as a stressor (e.g. very bright lights) or infusions of norepinephrine into hippocampus or amygdala, might promote involvement of hippocampal Aβ in spontaneous object recognition memory.

2. Amyloid-β in memory destabilization

Both in accordance and contrary to the results found by Lee and Choi (2018), we found that infusing an Aβ antibody during the consolidation period and every 24 h (for 72 h) during thereafter during the memory retention interval prevented the natural forgetting of object memories in female, but not male animals. Our sex difference should be interpreted with caution, however, as the absence of memory in the male rats is very likely due to a small sample size. Yet, the effect size was large (Cohen's d = 0.901), suggesting a potential effect of our pharmacological intervention on our outcome measure. Moreover, Lee and Choi (2018) did not include female rodents in their analyses, and opted for ICV infusions of the Aβ antibody instead of infusions in a partciular brain region.

Blocking the activity of Ab once a day during the memory retention inteval was sufficient to allow long-term memory to persist, suggesting that the peptide might be constitutively involved in a mechanism that promotes active forgetting, such as synaptic weakening or pruning. While our results are preliminary as they do not offer any insight into potential mechanisms, they suggest that this form of forgetting might involve Aβ to regulate synaptic homeostatic processes. Notably, we depleted levels of Aβ during the sleep phase of the rats when these homeostatic processes are assumed to occur. Thus, endogenous hippocampal Aβ might contribute to natural forgetting by promoting mechanisms that weaken synaptic strength during sleep.

2.1. Aβ as a neuromodulator of synaptic homeostatis

Briefly, learning and memory involve long-lasting modifications of synaptic connections. However, mechanisms like LTP or LTD that lead to changes in synaptic connectivity, if left unchecked, can destabilize neural networks over time by biasing neurons towards excessive or

minimal firing rates. Homeostatic plasticity is considered to be therefore a fundamental form of plasticity, which operates at slower rates than LTP and LTD, and which could provide the negative feedback mechanisms necessary to prevent synaptic strength and plasticity from exceeding a functional dynamic range, which preserves long-term network functionality. One form of homeostatic synaptic plasticity is synaptic scaling, which involve changing the strength of all synaptic connections of a neuron up or down proportionally, to preserve their relative weights and information coding from previous Hebbian learning events (Diering, 2022; Galanis et al., 2021; Pérez-Otaño & Ehlers, 2005; Turrigiano, 2012). According to some theories, synaptic downscaling is believed to occur mainly during sleep, a time when changes in synaptic activity is not driven by experiences with the environment (Tononi & Cirelli, 2006). Therefore, according to the synaptic homeostasis hypothesis of sleep (Tononi and Cirelli, 2003), behavioural experiences are firstly encoded in the brain through forms of LTP, leading to specific synaptic strengthening and an overall increase in network excitation. To prevent "run-away potentiation", synapse strength is brought back to baseline by homeostatic synaptic downscaling, specifically during periods of sleep and rest (Diering, 2022; Tononi & Cirelli, 2006).

In regards to the role of Aβ, synapse loss is the strongest structural correlate of cognitive decline in patients with AD and rodent models of the disease. Studies in mice models of AD report that soluble forms of Aβ contribute to the downregulation of synapse density and depression of glutamatergic transmission, and the effects of the peptide on synapses can be directly linked to apparent cognitive and memory deficits. These effects are observed especially in the early phases of the disease, when there is not yet global neurodegeneration, suggesting that the cognitive and memory impairments of AD may develop from a physiological impairment rather than the overall loss of neurons (Abramov et al., 2009; Hsieh et al., 2006; Kamenetz et al., 2003; Wei et al., 2010).

In the context of our findings, we therefore hypothesize that one of the constitutive functions of Aβ might be to regulate synaptic homeostasis, specifically by affecting downscaling of synaptic connections during sleep or resting periods (Galanis et al., 2021). Supporting this, Galanis and colleagues (2021) recently found *in vitro* evidence that Aβ is involved in homeostatic processes. Hippocampal cell preparations of APP knockout (KO) mice treated with tetrodotoxin (TTX) to block action potentials (i.e., suppress neural activity) did not express any compensatory AMPAR-mediated miniature excitatory postsynaptic currents (mEPSCs), a measure of spontaneous excitatory neurotransmission, taken to reflect functional adjustments (scaling) in

homeostatic plasticity mechanisms. However, treatment with both $A\beta_{1-40}$ and $A\beta_{1-42}$, but not the non-amyloidogenic APP fragment APPsα rescued homeostatic synaptic plasticity in APP-KO cells (Galanis et al., 2021). Pharmacological inhibition of both β- and γ-secretase in hippocampal tissue cultures of wild-type mice, which can express TTX-induced synaptic scaling (increases in mEPSCs), prevented homeostatic synaptic plasticty and could be rescued with $A\beta_{1-42}$ treatment, further implicating a role for APP and Aβ in homeostatic synaptic scaling mechanisms (Galanis et al., 2021).

There is evidence of a substantial overlap in the molecular mechansims employed by homeostatic synaptic processes and Hebbian learning. Both hippocamapal synaptic downscaling and forms of LTD have been reported to depend on the trafficking of postsynaptic NMDARs and the endocytosis of AMPARs to weaken synapses, suggesting that LTD and downscaling might overlap mechanistically. As structural synaptic plasticity are central to both, and given the strong tie between these forms of plasticity and memory processes, it is likely that homeostatic plasticity also contributes to memory processing (Mendez et al., 2018). Given this convergence, it is possible that during sleep, endogenous Aβ may constitutively serve as a modulator of both forms of plasticity in the hippocampus, and that both contribute to the natural forgetting of memories in the hippocampus, such as active decay, which progressively reverses the synaptic changes induced by learning and memory formation. Similar to LTD, it requires NMDAR activity and the postsynaptic removal of GluA2-AMPARs from hippocampal neurons, and it is also hypothesized to occur mainly during sleep (Hardt et al., 2013; Migues et al., 2016, 2019). It remains to be seen whether Aβ afffects neural network stability through adaptive synaptic weakening mechanisms and how in AD, the dysregulation of these processes could contribute to network hyperexcitability and general cognitive deficits, as well as increased rates of forgetting typical for this condition (Galanis et al., 2021; Styr & Slutsky, 2018).

3. Sex specific implications

A key, clear finding of this study was that female rats displayed longer-lasting memory for objects compared to males, despite being exposed to the objects for the same amount of time during sampling (5 min). This finding aligns with previous findings suggesting female superiority particularly in tasks with delayed recall, regardless of estrous cycle phase. It is unclear, however,

if this difference is the result of males and females using different encoding strategies that result in stronger memory for objects for females but not males, or if it reflects differences in neural bases underlying memory formation and maintenance.

3.1. Behavioural strategies

Male and female rodents express different behavioural strategies in the same tasks. Male rodents are more likely to navigate through their environment using allocentric spatial strategies, using the spatial relationship between distal cues to encode the location of proximal objects , while females are more likely to use egocentric strategies that rely on the spatial relationship between their current location and the objects, without considering unchanging spatial arrangements of distal cues. Additionally, males tend to engage in more risk-taking and impulsive behaviours, while females are more "cautious" and engage in more risk-assessment in the face of novel environments (Cavigelli et al., 2011). In that sense, females might pay more attention to the physical appearance of the objects or landmarks, and tasks that require memory for changes in landmarks or objects might be more behaviourally relevant, potentially leading to stronger or longer lasting memory for females compared to males (Zorzo et al., 2024).

We noticed that some females, but almost no male rats, tended to engage in a more pronounced avoidant behaviour towards the unexpected novel object during the probe trial, resembling novophobia commonly found in non-domesticated rats, but only in the first few seconds of exploration. Female rats that displayed this behaviour were consistent across phases of testing, as they also showed avoidance behaviours when first introduced to the two objects during sampling. This somewhat anecdotal observation suggest that under certain conditions, males and females may engage differently with objects and novelty specifically, which could result in different expression of behaviours taken as diagnostic for memory. However, while we generally found no consistent sex differences in exploratory behaviour across our experiments, the few results that did present differences seem to point towards greater female exploration, consistent with previous findings in the literature.

In general, female rats have been reported to display more exploratory activity in open field assessements and spontaneous recognition paradigms, including more ambulation, darting and rearing compared to male rats (Lovick & Zangrossi, 2021). Furthermore, some evidence suggests females move faster and travel farther distances than males, and that their behaviour is more

consistent across different phases of testing compared to males, suggesting the estrous cycle itself might not lead to a lot of variation in exploratory behaviour (McElroy & Howland, 2025). Overall, this seems to support the idea that males and females might accumulate information about their environment in different ways, suggesting different engagement and motivation in spontaneous recognition tasks. In the broader context of our study however, it is impossible to extract more conclusions about the contribution of differential behavioural strategies to the observed sex difference in memory retention duration, due to the limited scope of our behaviour and locomotor metrics, total and accumulated exploration time. Measuring only total exploration time may miss other aspects of exploratory behaviours that could reveal the use of different exploratory strategies during different phases of memory processing (McElroy & Howland, 2025).

Furthermore, the lack of consistent sex differences in exploratory behaviour in rodents could indicate that exploration or motivation to explore might reflect dimorphic sensitivity to multiple different factors, such as circulating sex hormone levels, task or rearing related factors (such as level of socialization of the rats, non-systematic cohort effects, possible drug interaction effects, handling of the experimenter), or statistical factors, such as unequal sample sizes with more females being tested in our study compared to males.

3.2. The role of gonadal hormones

Outside of behavioural strategies, longer-lasting object memory in females compared to males could reflect modulatory effects of gonadal hormones on hippocampus-dependent memory processes. Testosterone, estrogen and progesterone are the three major gonadal steroid hormones. Female gonads mainly produce estradiol and progesterone, while male gonads produce higher levels of testosterone. It is generally accepted that gonadal hormones, particularly estradiol (E_2), a potent type of estrogen sex steroid hormone that principally regulates female reproduction, can act as a strong neuromodulator of synaptic activity and long-term memory processes, especially in the hippocampus (Bimonte-Nelson et al., 2010; Taxier et al., 2020).

E_2 is synthesized both locally in the hippocampus and systematically by the ovaries, which both contribute to circulating levels of the gonadal hormone. Evidence shows that performance during novelty preference tasks is impaired by ovariectomy (ablation of the ovaries, which eliminates systemic E_2 production), and significantly improved by exogenous acute subcutaneous

admisitration of E_2 (in both males and females) (Becegato & Silva, 2022; Cost et al., 2012). Both systemic infusions of E_2 and local infusions into the dorsal hippocampus or perirhinal cortex of ovariectomized female rats immediately before or after training on the NOR task can enhance novelty preference, but not when administered 2 hours after, suggesting a time-specific effect of E_2 on object recognition memory that might be limited to memory consolidation and might exclude the later memory retention interval (Finney et al., 2020; Taxier et al., 2020).

Estradiol was shown to exert this modulatory effect on long-term memory through its effects on functional changes in excitatory activity, signaling changes in calcium dynamics, and synaptic remodelling (Finney et al., 2020). In female rats, the density of dendritic spines and synapses at the CA1 region of the hippocampus fluctuates naturally with the ovarian steroid levels, particularly E_2, during the estrous cycle, being highest during proestrus where ovarian steroid hormones are at their highest concentrations, and at their lowest 24 h later, during estrus. Ovarectomy impairs the modulatory effect of E_2, reducing CA1 spine density to similar levels as during estrus, and this effect can be rescued by exogenous treatment with the hormone (Woolley & McEwen, 1994). Overall, a large body of evidence supports the finding that estradiol increases total spine density and dendritic branching, and that it requires the co-recruitment of NMDARs and AMPARs to allow increased calcium influx (see Finney et al., 2020 for a full review).

Considering the natural fluctuation in estradiol levels in the female rat across the extrous cycle, and the differing levels and production patern of estradiol in males and females (Finney et al., 2020), estradiol might play a neuromodulating role that could explain potential differences in encoding or consolidation mechansisms, resulting in longer lasting memory in females, maybe independent of behavioural strategies.

To date, it remains unkown if memory maintenance mechanisms (such as the continuous activity of PKMζ) or active forgetting mechanisms differ between males and females. Considering the evidence presented in the previous section on the interplay between different forms of synaptic plasticity in the hippocampus and their shared reliance on NMDARs and AMPARs, it's possible that estradiol-mediated structural synaptic changes could influence the balance of processes involved in memory maintenance and forgetting. One study has explored the role of PKMζ specifically in the maintenance of remote long-term reference memory in both male and female animals. Using an 8 arm radial maze male and female rats were trained to locate food reward (Sebastian et al., 2013). The rats underwent either 30 or 60 trials over 3 and 6 days respectively,

and memory for the rewarded arms was tested either 1 day or 1 month after the last training day. While male rats in either group remembered the rewarded arms 1 d or 30 d later, females did not have significant memory at the later test point. Furthermore, while memory retention was highly correlated with the presence of post-synaptic AMPARs (specifically the GluA2 subunit), males showed significantly higher levels of synaptic PKMζ across all conditions compared to females, suggesting that females may be using different mechanisms to upregulate GluA2-AMPARs. This finding highlights a potential sex-dependent memory maintenance mechanism, one that could contribute to the observed difference in memory retention between the sexes.

Further explorations into the different molecular mechanisms that regulate memory maintenance or forgetting in females could reveal different molecular pathways by which Aβ interacts with neural processes, and how they could become dysregulated in disease.

4. Implications for AD

Our findings that endogenous hippocampal Aβ might promote natural forgetting align with recent evidence that the accelerated forgetting of long-term declarative memory might be an early marker of cognitive decline in preclinical AD patients, with impairments emerging up to 7 years before symptomatic onset of the disease, even earlier than other previously known cognitive changes (Weston et al., 2018). Interestingly, this suggests endogenous Aβ could serve as a biomarker for early detection and diagnosis of AD, and could maybe reveal a timepoint where preventive interventions could be more effective. Moreover, considering the sex differences in memory retention found through this work, it would be interesting to explore whether changes in natural forgetting constitute a better or more sensitive diagnostic marker for females compared to males, and whether changes in endogenous Aβ levels vary between the sexes in the context of early, preclinical cognitive decline.

Combined with evidence from the literature, our findings suggest that the timing of Aβ manipulation might be important, as "task-evoked" Aβ dynamics seemingly primarily regulate synaptic strengthening and memory encoding (Garcia-Osta & Alberini, 2009; Puzzo et al., 2011), but baseline levels of Aβ might conversely regulate mechanisms involved in synaptic weakening and memory forgetting (Kaldun et al., 2021; Lee et al., 2018). In the context of AD, impairments in cognitive flexibility are among the earliest observed cognitive impairments (Albert, 1996).

Considering the suppression of irrelevant old information in favour of new information is critical to cognitive flexibility, it would be very interesting to explore whether blocking the peptide post-encoding impairs performance on tasks such as spatial or contextual reversal learning, which rely on flexible behaviour and the ability to switch between old and new information, and whether this is mainly driven by the inability to forget old information or failure to acquire new one (or both).

Altogether, our findings highlight the need to further explore and understand the complex role of the Aβ peptide in normal memory processes, particularly in the context of natural forgetting and considering both sexes, to design targeted therapeutic strategies that can minimize maladaptive memory loss without disrupting the adaptive, physiological functions of Aβ.

5. Methodological considerations

There are a few considerations that limit the generalization of our results. First, the unequal sample sizes of male and female animals make comparing between sex and the detection of consistent sex differences more difficult. We tested fewer male animals compared to females, which could have reduced our statistical power for detecting sex differences, especially when trying to detect consistent differences in exploratory behaviour. Furthermore, females were used with no manipulations or interventions aimed at controlling or verifying their estrous cycle or circulating hormone levels. We cannot therefore exclude a role of gonadal hormones in the Aβ-mediated effect we observed in females, expecially since we did not obtain conclusive results with the males. It would be interesting to repeat our experiment and add extra infusions of a local E_2 inhibitor, to explore whether gonadal hormones and Aβ interact to produce the observed effect on forgetting, and to investigate further whether the longer memory rentention we observed in females at baseline is due to E_2 related mechanisms during the memory retention interval specifically, different behavioural strategies, or a combination of both.

Second, our study did not include a control to verify specifity of the 4G8 antibody's binding or a verification of target depletion. Therefore, we cannot fully rule out that there were potential changes at the cellular or molecular levels, even in the absence of explicit behaviour effects for the encoding and consolidation experiments (or that there were no changes at all). Furthermore, for experiments reported in Chapter 2, we used PBS as our control instead of the control antibody used in studies described in Chapter 1. Due to the unexpected impairment of memory it induced

in female rats, we decided to not use this drug for the repeated infusions when exploring forgetting, to avoid further adverse effects and confusion around the interpretation of our results. Despite this, 4G8 is a widely used antibody in AD research, and its binding specificity and target depletion effects are well established. 4G8 targets a specific epitope of Aβ within amino acids residue 17-24, a sequence that is identical between humans and rodents and that includes multiple species of interest, such as $A\beta_{42}$ and $A\beta_{40}$. Furthermore, 4G8 is not conformation specific and binds to monomeric, oligomeric, and fibrillar forms of Aβ, including full-length $A\beta_{(1\text{-}x),}$ N-termininally truncated $A\beta_{(11\text{-}x)}$, the small peptide p3, and to full-length APP (Siegel et al., 2017), potentially limiting the interpretation of our results to a specific form of the peptide. Replicating our effect on the forgetting of object memory with a β-secretase or γ-secretase inhibitor might confirm the Aβ specific role in that effect.

Lastly, for our experiments involving infusions of the BACE1 inhibitor IV, it is currently a candidate for clinical trials in humans as the first non-peptidic BACE1 inhibitor that robustly decreases total extracellular Aβ levels (L. Liu et al., 2019). We based our choice of the drug and its preparation on previous work where the drug was used both *in vivo* (bilateral amygdala infusions in rats) and *in vitro* (cultured neurons) (Finnie & Nader, 2020). However, the inhibitor was dissolved in 40% DMSO in PBS, which is relatively high for *in vivo* infusions and even considered toxic at that concentration. We chose to use a lower concentration to avoid negative side effects of high levels of DMSO (from 5 μg/μL to 1 μg/μL), and one that would still be above the reported half maximal inhibitory concentration (IC50) of the drug (10-15 nM *in vitro*, Millipore Sigma). Therefore, although we have no binding assays to support this, it is highly likely that our lack of effects with the BACE1 inhibitor was not due to insufficient concentration to inhibit the enzyme, but rather due to hippocampal Aβ mechanisms not being critical during the encoding period of object recognition memory. Since hippocampal Aβ mechanisms may be involved in the forgetting of this type of memory, it would be important to replicate and confirm our findings with the same BACE1 inhibitor.

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