THE EFFECTS OF AGE AND SEX ON PATTERN SEPARATION ABILITIES IN SPRAGUE-DAWLEY RATS

By

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Abstract

Pattern separation, which is important for episodic memory, minimizes the overlap between neural representations of similar experiences. This study explored age and sex differences in pattern separation using male and female rats at two life stages: young (4-5 months) and old (14-15 months). We employed a two-trial spatial Y-maze to assess spatial memory, where rats first explored two open arms (acquisition phase) and then were tested for their preference for a previously unexplored arm (24 h later). At this test, female rats regardless of age traveled greater distances and spent more time in the novel arm location, than male rats. In the spontaneous location recognition (SLR) task, rats were presented with objects at known locations during the sample phase and tested for object location memory after 24 h. Two versions of this test were performed: a Dissimilar SLR (DSLR) test where the location of a familiar object in the test trial varied greatly from the previous location of the object, and a Similar SLR (SSLR) task where the new location of a familiar object differed to a lesser degree from the original location. The DSLR and SSLR tasks were performed first in a circular arena, and again in a square arena. In the DSLR task in the square arena young rats spent a greater percentage of time exploring the familiar object in a new location than their older counterparts, also females showed higher discrimination ratios than males. However, results from the SSLR in the square arena did not reveal significant differences across age or sex in memory retention. Contrasts between circular and square arena performance suggest that task environment may influence memory retention and discrimination. Overall, this study highlights potential age and sex influences in tasks of pattern separation and suggests these influences may vary dependent on task.

General Summary

This study examined age and sex influences on pattern separation which is essential for reducing neural overlap in episodic memory, in adult and older adult rats. In the two-trial spatial Y-maze, female rats consistently demonstrated enhanced activity and memory retention at 24 h, showing a preference for novel arms regardless of age. The spontaneous location recognition (SLR) tasks in a circular arena revealed no significant discrimination ratio differences, indicating no preference for familiar objects in novel locations. However, in the square arena, females and younger animals showed superior spatial discrimination in the Dissimilar (DSLR) task, compared to males and older groups respectively. The Similar (SSLR) task was inconclusive, suggesting task or environmental complexities may affect outcomes. While the findings highlight memory retention in female rats, they do not conclusively establish consistent age and sex differences across tasks, pointing to the need for further investigation.

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Age and Sex Differences in Pattern Separation Abilities in Sprague-Dawley Rats

There is a longstanding global consensus that the hippocampus is essential for the formation of episodic memories, which are new memories about previously encountered incidents. This theory has been supported and expanded upon over decades, with seminal work by Scoville and Milner (1957) identifying the critical role of the hippocampus in memory formation, further elaborated by Squire (1992) in his discussions on memory and brain systems and reaffirmed by recent studies such as Moscovitch et al. (2016) among many other research. The hippocampus, a critical structure for episodic memory, includes two main regions: the hippocampus proper (including CA1, CA2, CA3 regions) and the dentate gyrus (DG) (Lorente De Nó, 1934; Amaral & Witter, 1989; Ding & Van Hoesen, 2015; Knierim, 2015). Pattern separation is essential for episodic memory as it minimizes the overlap between neural activities representing similar experiences (Marr, 1971; McNaughton & Morris, 1987; Norman & O'Reilly, 2003; Yassa & Stark, 2011). This cognitive process enables the formation of unique and non-overlapping memory representations, allowing individuals to distinguish between similar but distinct experiences or memories (Yassa & Stark, 2011). Pattern separation is hypothesized to occur predominantly in the DG of the hippocampus (Yassa & Stark, 2011). The DG is responsible for transforming similar input patterns into distinct output patterns, thereby minimizing interference and confusion among memories. Granule cells within the DG play a role in this process, as they help encode distinct aspects of experiences, facilitating the storage of non-overlapping memory traces (McClelland et al., 1995; Norman & O'Reilly, 2003).

Pattern Separation and Its Relation to Episodic Memory

Pattern separation is a neural process that helps differentiate between similar but distinct inputs by transforming overlapping representations into non-overlapping ones. Pattern separation

allows the brain to minimize confusion between memories that share similar features or contexts. By creating distinct neural representations for similar experiences, pattern separation prevents interference between memories and enables the accurate recall of specific events (Yassa & Stark, 2011). For example, consider two similar experiences, such as visiting two different restaurants that have a similar layout. Without pattern separation, these experiences might become conflated, leading to difficulties in recalling specific details about each visit. However, pattern separation ensures that each event is encoded with distinct neural representations, allowing for accurate recall of the individual experiences. This process is especially important when dealing with highly similar inputs, such as distinguishing between two objects that look alike or recalling the details of events that took place in the same location but at different times. Pattern separation ensures that these experiences are stored as unique and non-overlapping memories.

Pattern separation is necessary for episodic memory because it allows the brain to create distinct neural representations for each event, even when events are similar. This ensures that memories remain unique and do not interfere with one another. For example, two similar experiences that occur in the same location on different days can be remembered as distinct episodes because of pattern separation. Without this process, memories of similar experiences could overlap, leading to confusion and difficulty recalling specific details.

In rodents, episodic memory is often modeled through tasks that assess the ability to remember specific locations, objects, or sequences of events. These tasks rely on the same underlying neural mechanisms as human episodic memory, including pattern separation in the DG (Eichenbaum, 2000; Kesner & Rolls, 2015). For instance, tasks like the two-trial spatial Y-maze or novel object recognition, test the rodent's ability to distinguish between different spatial locations or objects, which directly relates to pattern separation and episodic memory. The two-

choice Y-maze task involves training the rodent to choose between two arms of the maze with or without reinforcement. This task evaluates the animal's ability to remember and discriminate between different locations based on previous experiences, thus providing insight into spatial memory and pattern separation (Cleal et al., 2021). Similarly, the novel object recognition task tests the rodent's memory for object identity and spatial context by measuring its preference for exploring a novel object over a familiar one. This task is commonly used to assess recognition memory, a key component of episodic memory (Antunes & Biala, 2012).

Pattern Separation and Its Neural Mechanisms

The DG plays a critical role in the hippocampal memory network by performing pattern separation. The DG receives sensory and contextual information primarily through inputs from the entorhinal cortex (EC), specifically the perforant path, which conveys highly processed multimodal sensory information from cortical areas (Amaral & Witter, 1989; Knierim et al., 2014).

Inputs to the DG: The primary input to the DG comes from Layer II of the medial and lateral EC via the perforant path. This input carries spatial, temporal, and contextual information, which is essential for the formation of episodic memories (Dolorfo & Amaral, 1998; van Groen & Wyss, 1990). The medial entorhinal cortex (MEC) provides spatial information through grid cells, head direction cells, and border cells, while the lateral entorhinal cortex (LEC) contributes non-spatial, object-related information (Hafting et al., 2005; Sargolini et al., 2006). This convergence of inputs allows the DG to integrate spatial and non-spatial information, a pivotal process for pattern separation (Knierim, 2015; Neunuebel & Knierim, 2014).

Outputs from Granule Cells: Granule cells in the DG are the principal excitatory neurons and their axons, called mossy fibers, project to the CA3 region of the hippocampus. The CA3 region is critical for associative memory and pattern completion processes (Kalil, 1981; Rolls,

2007). The sparse and specific activation of granule cells ensures that similar input patterns are transformed into distinct output patterns, reducing overlap and allowing the hippocampus to create unique memory representations. This sparse coding is thought to be one of the key mechanisms by which the DG performs pattern separation (Nakashiba et al., 2012).

Organization within the Hippocampal Network: The DG is the first stage of the trisynaptic circuit in the hippocampus, which includes the DG, CA3, and CA1 subfields (Amaral & Witter, 1989). After receiving input from the EC, the DG processes this information and sends it to the CA3 region via mossy fiber projections (Kalil, 1981). The CA3 region is heavily interconnected and capable of pattern completion, which allows it to retrieve entire memories based on partial or degraded cues (Rolls, 2007). The information is then passed from CA3 to CA1 through the Schaffer collaterals, and from CA1, it is transmitted back to the EC or to other cortical areas for long-term storage (Rolls, 2013). The DG's ability to perform pattern separation is crucial for the proper functioning of this entire network, as it helps to reduce interference between similar memories.

Age Differences Based on Human Studies

Pattern separation is a cognitive process that shows significant variation across different stages of life, from childhood to older adulthood. Studies using the mnemonic similarity task (MST) have provided valuable insights into how these abilities change over time. The MST typically involves participants viewing a series of images and later identifying whether they have seen an exact image before "old", have not seen it before "new", or have seen a similar but not identical image "lure". The ability to correctly identify similar images as "lures" rather than confusing them with previously seen images is a key measure of pattern separation.

Children: Pattern separation skills are still developing in children, typically improving with age. For instance, children aged 4-6 years often struggle with distinguishing between similar items or experiences, leading to more overlap in their memories. As children grow older, their ability to separate patterns generally improves (Canada et al., 2019; Ngo et al., 2018). For example, Ngo et al. (2018) found that younger children tend to have higher false recognition rates for similar items, indicating less effective pattern separation. However, one potential confound in these studies is the role of language or categorization skills, which are still maturing in younger children and may affect their performance on tasks like the MST. Younger children might not fully grasp the subtle distinctions required to perform well on the task, or they may have difficulty with the verbal instructions, which could lead to underestimation of their true pattern separation abilities. Canada et al. (2019) also found that children aged 7-12 years performed better on pattern separation tasks than younger children suggesting that cognitive and linguistic development plays a role in improving performance. While these studies highlight the developmental trajectory of pattern separation, it is important to consider that the MST may not perfectly align with the non-verbal, spatial tasks typically used in rodent studies, like the Simultaneous Location Recognition (SLR) task.

Adolescents: Adolescents (13-18 years), tend to have better pattern separation abilities compared to younger children. During adolescence, the brain undergoes significant structural and functional changes, particularly in the hippocampus, which contribute to improved memory discrimination (Rollins & Cloude, 2018). Adolescents showed improved performance on the MST compared to children, with lower false alarm rates for similar lures, indicating enhanced pattern separation abilities. This improvement is attributed to the continued maturation of the hippocampus and associated neural circuits during adolescence (Rollins & Cloude, 2018).

However, similar to studies with children, potential confounds such as developmental differences in attention, motivation, and categorization skills should be considered.

Young Adults: Pattern separation abilities tend to peak during young adulthood, roughly between the ages of 18-30 years. This period is characterized by optimal cognitive abilities, including memory function (Stark et al., 2010; Riphagen et al., 2020; Youm & Moscovitch, 2021). Riphagen et al. (2020) reported that young adults performed best on pattern separation tasks, such as the MST, compared to other age groups. Young adults typically show the lowest rates of false recognition for similar items, reflecting highly effective pattern separation. Stark et al. (2010) and Youm and Moscovitch (2021) also found that young adults exhibit robust hippocampal activity compared to older adults during these tasks, further supporting their superior pattern separation capabilities.

Middle-Aged and Older Adults: With aging, there is often a decline in pattern separation abilities, starting around middle age (40-60 years) and becoming more pronounced in older adults (65+ years) (Holden et al., 2012; Stark et al., 2013; Leal et al., 2017). Holden et al. (2012) employed a spatial pattern separation task designed to assess participants' ability to distinguish between objects placed in different spatial locations. In their task, objects were presented at varying degrees of spatial separation, challenging participants to recognize whether an object had been moved or remained in the same position. This spatial memory task is particularly relevant to studies on pattern separation because it closely parallels how the brain differentiates between similar spatial inputs—a core function of the DG in the hippocampus. This task is conceptually similar to the SLR task used in our study, where rats must distinguish between familiar and novel object locations. Both tasks require subjects to engage in pattern separation by differentiating between spatial configurations, making them suitable for assessing hippocampal-dependent memory

processes. Holden et al. (2012) found that middle-aged adults (aged 40-60) began to show increased difficulty with spatial pattern separation tasks compared to younger adults (aged 18-30). This decline was particularly evident when objects were placed in spatially similar locations, indicating that the ability to resolve fine spatial distinctions diminishes with age. These difficulties became more pronounced in older adults (65+), who showed significant impairments in distinguishing between similar spatial locations. The decline in spatial memory and pattern separation abilities observed by Holden et al. is consistent with research showing age-related impairments in hippocampal function, particularly in the DG. For example, studies by Stark et al. (2013) and Leal et al. (2017) revealed older adults show higher false alarm rates on the MST and reduced activation in the hippocampus during these tasks. The decline in pattern separation abilities with age is often attributed to structural and functional changes in the DG. These changes include reduced neurogenesis (the process by which new neurons are formed), synaptic plasticity (the ability of synapses to strengthen or weaken over time) impairments, and overall hippocampal atrophy, all of which contribute to decreased cognitive performance in tasks requiring memory discrimination (Leal et al., 2017).

Sex Differences Based on Human Studies

In addition to age-related changes, sex differences in pattern separation abilities have also been observed in some studies. For instance, Saucier et al. (2007) found that females tended to outperform males on object location memory tasks, which rely heavily on spatial discrimination. This finding aligns with other research suggesting that hormonal differences, such as the influence of estrogen, may contribute to sex differences in hippocampal function and memory performance (Galea et al., 2008). However, these sex differences may vary depending on the specific task and context. In the MST, some studies have shown that females may perform better on certain memory

discrimination tasks, particularly those involving verbal or object-based memories, while males might excel in more spatially oriented tasks (Andreano & Cahill, 2009). These differences are likely driven by both hormonal influences and structural differences in the hippocampus between the sexes. There is evidence that suggests differences between males and females in cognitive abilities and hippocampal plasticity (Yagi & Galea, 2019), but note that these differences are not uniform across all individuals and can be influenced by various factors such as genetics, and individual experiences (Moser et al., 1993; Sweatt, 2016). While research on sex differences in pattern separation in humans is limited, some studies have demonstrated distinct cognitive strategies between males and females in spatial memory and problem-solving tasks. For example, Jockwitz et al. (2021) found that females tend to use landmark-based navigation strategies, whereas males more frequently employ cardinal direction strategies. Similarly, Meneghetti et al., (2012) observed that when solving spatial tasks, women prefer route-based strategies and men tend to use survey-based strategies, impacting how each sex processes and recalls spatial information. These differences may result in varying approaches to pattern separation, but they are not necessarily indicative of one sex being better than the other.

To summarize, pattern separation abilities in humans can change across the lifespan, with peak performance typically occurring during young adulthood and declining with age. Sex differences in pattern separation are less well-understood, but there may be variations in cognitive strategies and approaches that are influenced by biological, hormonal, and sociocultural factors.

Age-Related Changes Based on Rodent Studies

In addition to clinical and human studies, research on pattern separation often involves the use of animal models, such as rats, to investigate how this process changes with age and sex. Moreover, rodent studies can reveal the mechanisms underlying these changes. Aging is associated

with cognitive decline in rats, including changes in pattern separation abilities and spatial learning (Gallagher & Nicolle, 1993). The hippocampus undergoes structural and functional changes with age. For instance, older rats show decreased volume in the hippocampus and parahippocampal cortices (Yassa & Stark, 2011). Moreover, a loss of synaptic connections in the perforant pathway and a deficit in the induction and maintenance of plasticity (i.e., long-term potentiation) at DG synapses in aged rats have been reported (Kuhn et al., 1996; Yassa et al., 2011). All of these changes negatively impact cognitive functions and memory formation.

Defining "Young" vs. "Old" in Rats

In rodent research, the terms "young" and "old" are typically defined relative to the lifespan of the species. Sprague-Dawley rats, which were used in this study, generally have a lifespan of around 2 to 3 years under laboratory conditions (Sengupta, 2013). Based on this lifespan, different life stages can be categorized as follows:

Young Adult Rats: Rats that are 4-5 months old are considered young adults. At this stage, rats have reached sexual maturity, and their cognitive and physical development is near its peak. This age group is often used to model young adult humans (roughly equivalent to humans in their 20s or 30s) in research studies (Sengupta, 2013).

Mid Adult Rats: Rats that are 14-15 months old are generally considered middle-aged to early old age(Sengupta, 2013) . While this age represents middle age in the context of their overall lifespan, physiological changes associated with aging, such as cognitive decline and reduced physical activity, often begin to manifest during this period. In human terms, 14-15 months old in Sprague-Dawley rats can be roughly equated to middle adulthood or early old age (equivalent to a human in their 50s or early 60s), depending on various factors such as genetics and living conditions. Although 14-15 months is not "old" in the sense of end-of-life or advanced aging (e.g.,

rats older than 18 months are often categorized as "old" or "geriatric"), this age group still exhibits significant age-related changes. Therefore, these rats serve as a model for studying the onset of aging-related processes, including cognitive decline, which makes them relevant for research on early-stage aging and memory deficits.

In present study, 14-15 months was chosen as the "old" age group to capture the onset of age-related cognitive decline, which can manifest before the rats reach the later stages of their life. Previous research has shown that cognitive deficits, such as declines in spatial memory and pattern separation abilities, often begin to appear during this middle-aged period in rats (Gallagher et al., 2015; Holden & Gilbert, 2012). Thus, this age group serves as a suitable model for investigating the early effects of aging on memory and other cognitive functions.

By using these age groups, the study aims to explore not just the advanced effects of aging but also the early changes that occur as the rats' transition from young adulthood to middle age, which parallels the human experience of aging.

Sex-Related Changes Based on Rodent Studies

Some studies in rodents showed that there might be sex differences in cognitive functions, including pattern separation. Meta-analyses of sex differences in rodents indicate that males tend to excel over females in tasks reliant on the hippocampus (Jonasson, 2005). There are a number of factors influencing cognitive performance in males and females. For instance, the morphology and electrophysiological properties of hippocampal neurons differ in male and female rats. Fitch et al. (1989) showed male rats had a greater number of dendritic intersections in granule cell layers of DG compared to females. In other studies, male rats demonstrated larger early and late long-term potentiation (LTP) in comparison to females when a high frequency stimulus inserted to the different regions of the hippocampus (Monfort et al., 2015; Yang et al., 2004). Moreover,

hormonal fluctuations, especially related to the estrous cycle in females, influence a number of parameters such as cell proliferation (Rummel et al., 2010), neurogenesis (Tanapat et al., 1999), hippocampal volumes (Qiu et al., 2013) synaptic plasticity (Warren et al., 1995), and glutamatergic receptors (Tada et al., 2015). Female Sprague-Dawley rats during proestrus exhibit greater cell proliferation compared to non-proestrus females and males (Tanapat et al., 1999). Adult male Wistar rats have more immature neurons in DG compared to females (Hillerer et al., 2013). Also, for the first time, Qi et al. (2016) showed that proestrus female Sprague-Dawley rats demonstrate greater magnitude of early-LTP in the perforant path compared to diestrus females. They also found that the composition of AMPA/NMDA receptors of CA1 pyramidal neurons is different between males and females, with females showing greater AMPA/NMDA ratio than males.

Neurobiological Mechanisms

Hormonal differences between males and females, especially during adolescence and adulthood, can influence neurogenesis and cognitive functioning (Kight & McCarthy, 2020). Estrogen, a primary female sex hormone, can significantly impact memory processes, including pattern separation, given that there are notable sex differences in the receptor characteristics of the hippocampus (Yagi & Galea, 2019). Estrogen receptors (ERα and ERβ) are abundantly expressed in the hippocampus and play a crucial role in modulating synaptic plasticity and neurogenesis (Frick, 2009). Studies have shown that estrogen enhances dendritic spine density and synaptic connectivity in the hippocampus, which are essential for the formation of distinct memory representations, a process critical for effective pattern separation. These enhancements improve the brain's ability to discriminate between similar experiences by reducing overlap in neural activity patterns (Tuscher et al., 2018). The hormone's neuroprotective effects also contribute to maintaining hippocampal function and reducing cognitive decline (Brinton, 2009).

Testosterone, the primary male sex hormone, also influences cognitive functioning and hippocampal plasticity. Testosterone and its metabolites, such as dihydrotestosterone (DHT), interact with androgen receptors in the brain, including the hippocampus. These interactions can modulate synaptic plasticity, neurogenesis, and the expression of neurotrophic factors (Galea et al., 2008; Hampson et al., 2014). Testosterone has been associated with improved spatial memory and cognitive performance in males (Hampson et al., 2014; Janowsky, 2006). However, the exact mechanisms by which testosterone affects hippocampal function and pattern separation are less well understood compared to estrogen (Galea et al., 2006). The interplay between estrogen and testosterone and their respective receptors highlights the complexity of sex differences in cognitive functions and hippocampal plasticity. While these hormones contribute to differential cognitive strategies and memory processes between males and females, it is important to recognize that these effects are not absolute and can vary widely among individuals.

Research has shown that synaptic plasticity is crucial for learning and memory including pattern separation. Age-related declines in synaptic plasticity can impair these cognitive processes (Fitch et al., 1989; Warren et al., 1995). Neurotransmitter systems, particularly those involving glutamate and gamma-aminobutyric acid (GABA), also play a critical role in pattern separation. Glutamate, the primary excitatory neurotransmitter in the brain, is essential for synaptic transmission and plasticity in the hippocampus. GABA, the main inhibitory neurotransmitter, helps balance excitatory signals and prevent excessive neural activity. Age-related changes in these neurotransmitter systems can affect the efficiency of synaptic transmission and plasticity, thereby impacting pattern separation abilities (Fitch et al., 1989). Alterations in neurogenesis in the DG, are also implicated in age and sex differences in pattern separation as Tanapat et al. (1999)

demonstrated that neurogenesis declines with age, which is predicted to contribute to cognitive deficits.

Sex may influence age-related neurobiological changes, further complicating the landscape of cognitive aging. Studies indicate that females experience different trajectories of cognitive decline compared to males, potentially due to hormonal differences, such as the decrease in estrogen levels during menopause (Brinton, 2009). This decline in estrogen can negatively impact hippocampal function and exacerbate age-related reductions in synaptic plasticity and neurogenesis. Considering these factors, it is essential to include female subjects in preclinical and clinical studies to fully understand the sex-specific mechanisms underlying cognitive aging and the vulnerability to neurodegenerative disorders. Moreover, more than half of the elderly population vulnerable to neurodegenerative disorders, such as Alzheimer's disease, are females. This highlights the need for gender-specific research to develop targeted interventions and treatments. Including female subjects in studies ensures that findings are more representative and applicable to the general population, thereby improving the effectiveness of therapeutic strategies.

Behavioural Tasks

Researchers commonly employ behavioural tests to assess pattern separation abilities in humans and rats. These tasks involve the discrimination of spatial or visual cues. A range of behavioural tasks have been used to study memory dependent on pattern separation in rodents: appetitive delayed match-to-sample task (Gilbert et al., 1998), automated touchscreen tests such as the location discrimination task (McTighe et al., 2009) and trial-unique nonmatching-to-location (TUNL) (Talpos et al., 2010; Oomen et al., 2013), contextual fear conditioning (Tronel et al., 2012), naturalistic tasks such as Y-maze recognition task (Dellu et al., 1992) and spontaneous location recognition (SLR) task (Reichelt et al., 2021). The premise behind these behavioural tasks

is that the ability to distinguish between similar contexts, locations, or events relies on the formation of memory representations following effective pattern separation. However, utilizing naturalistic tasks that leverage rodents' inherent preference for novelty helps circumvent potential issues related to changes in motivation or aversive learning (Reichelt et al., 2021). Moreover, naturalistic tasks do not need extensive training as they do not involve conditioned learning. While tasks such as TUNL allow for precise manipulation of similarity between locations, the Y-maze task also offers a way to vary "overlap" between the spatial locations to test pattern separation. Specifically, the Y-maze can be adjusted by altering the degree of novelty associated with each arm. In essence, the Y-maze task tests the rodent's ability to separate overlapping spatial representations, which is a core function of the DG. The flexibility of the task allows for different levels of spatial or temporal overlap to be introduced, depending on the specific experimental design. Conrad et al. (1996) used the Y-maze for assessing spatial memory and showed its sensitivity to region specific hippocampal damage. In their study, Conrad and colleagues demonstrated that chronic stress impaired spatial memory performance in the Y-maze, highlighting the task's sensitivity to alterations in hippocampal function, particularly in the DG.

Similarly, Walling et al. (2016) examined the effects of prolonged administration of norepinephrine reuptake inhibitors (NRIs) on hippocampal function, particularly in the DG, and showed how these changes impacted spatial and object recognition memory tasks. Their study found that prolonged NRI administration impaired LTP in the DG, which is a critical mechanism underlying memory formation. This impairment was associated with deficits in spatial memory tasks, including the Y-maze. Walling et al.'s findings reinforce the idea that the Y-maze is a reliable test for detecting changes in hippocampal function, particularly those affecting the DG and processes like pattern separation.

Other Factors Affecting Behavioural Performance

Factors such as genetic differences among different strains of rats, context of behavioural tasks, environmental enrichment, and housing conditions could contribute to variations in cognitive performance in behavioural tasks (Reichelt et al., 2021). As evidence, in the SLR task, female Sprague-Dawley rats show reduced performance in the similar SLR task compared with male Sprague-Dawley rats; while female Long Evans rats perform at a level similar to that of males (Buyukata et al., 2018). In addition, age-related cognitive decline in Wistar rats depends on the sex of the animal as well as on the context of the behavioural task (Zhvania et al., 2021).

Considering findings of previous studies, the objective of the current study was to examine 1- The potential interaction effects of age and sex in pattern separation tests, 2- How age and sex influence behavioural performance during spatial separation tasks in rats and 3- How these factors may be impacted by spatial properties of the testing environment. We assessed performance of male and female rats at two different ages (4-5 months versus 14-15 months) on a series of pattern separation tasks. We hypothesized that there will be a difference between behavioural performance of male and female Sprague-Dawley rats in young and old ages.

Material and Methods

This study was carried out in accordance with the recommendations of the Canadian Council on Animal Care. The protocol was approved by Memorial University's Animal Care Committee (19-01-SW).

Subjects

Fifty young (4-5 months) and aged (14-15months) male and female Sprague-Dawley rats were housed at the animal care facility of the Psychology department. They were given limited access (20 mg for male and 15 mg for female rats per day) to laboratory animal feed (Teklad

Global 18% Protein, Rodent Diet) and ad libitum water. Food restriction to 75% of normal free feed consumption was used to promote healthy weight maintenance in aging rats and to encourage exploration and locomotion in subjects. Each rat was housed individually in transparent individually ventilated cages filled with corncob bedding under controlled environmental conditions, with a temperature of 22˚C and a reversed 12:12 h light-dark cycle (lights off from 7:00 am to 7:00 pm) to align their active periods with testing times. Rats were divided into four groups based on age and sex—old male, old female, young male, young female—with 13 animals in each old group and 12 in each young group. To acclimate them to handling and experimental procedures, all subjects were handled daily by the experimenter for two weeks before behavioural testing began. All testing occurred under dim illumination between 9:00 am and 3:00 pm during the dark phase of their light-dark schedule.

Apparatus and Procedure

All rats were tested on three pattern separation tasks that involved discriminating between similar spatial or visual cues: the Y maze spatial task, the SLR task in a circular arena and the SLR task in a square arena.

Y-maze Spatial Task

The first experimental test comprised a Y-maze, with three arms of equal length positioned at 120-degree angles to each other. The apparatus was made of black plastic, each arm was 50 cm long and 17 cm wide and 32 cm high. The floor of the maze was covered with blue children's play sand. After each trial, the sand was mixed, and the maze was cleaned in order to reduce tracking of olfactory stimuli. The maze was placed in a quiet room under dim illumination with low-level background music. Four monochromatic visual cues were placed on the white curtain surrounding the maze and were kept constant during behavioural testing. All rats were transferred to the test

room at least half an hour earlier to habituate to the room prior to testing. The Y maze spatial test consisted of two trials. In the exploration trial (10 min), the access to one of the three arms was restricted by a removable barrier. The arms were counterbalanced such that each of the three arms were used as start, familiar and novel. The direction of the novel arm was at the left of the start arm for half of animals and at the right for the other half.

During the exploration trial rats were placed in start arm with their head oriented in the opposite direction to the center of the maze, and they were allowed to visit the two open arms. During the test trial, performed 24 hours later, the rats were returned to the maze for 5 min and all three arms were accessible to explore. EthoVision XT 14 video tracking software (Noldus, USA) was used to track and analyze the duration and frequency of visits to each arm every minute. One young and one old male animal were excluded from the study because they did not leave the start arm during the first two minutes of the test. For analysis, an entry into an arm was defined as the center of the animal's body crossing a line 15 cm into the arm. Multiple measures were analyzed for the first three min of the test trial including distance, percent entries to each arm, percent time spent in each arm, first choice arm and discrimination ratio (% time spent in novel arm/total time spent in novel and familiar arms).

Established research suggests that rodents' exploratory behavior, including their preference for novel arms or objects, tends to be most robust during the initial phases of a test session (Ennaceur & Delacour, 1988). In our study, the first three minutes of the test trial were chosen for analysis to capture the period when the animals are most actively engaged in exploratory behavior. By focusing on this initial period, we aimed to minimize the effects of habituation or fatigue that can occur if the test is extended beyond this point. Additionally, previous work, including studies using Y-maze and spontaneous location recognition (SLR) tasks, often focuses on the initial

minutes of testing to ensure that data reflect the rats' natural exploratory tendencies and their immediate responses to novelty (Dellu et al., 1992; Reichelt et al., 2021). Moreover, the first three minutes allow us to capture the peak period of decision-making and memory retrieval processes in the task, providing a more accurate measure of pattern separation abilities without confounding effects that could emerge later in the trial.

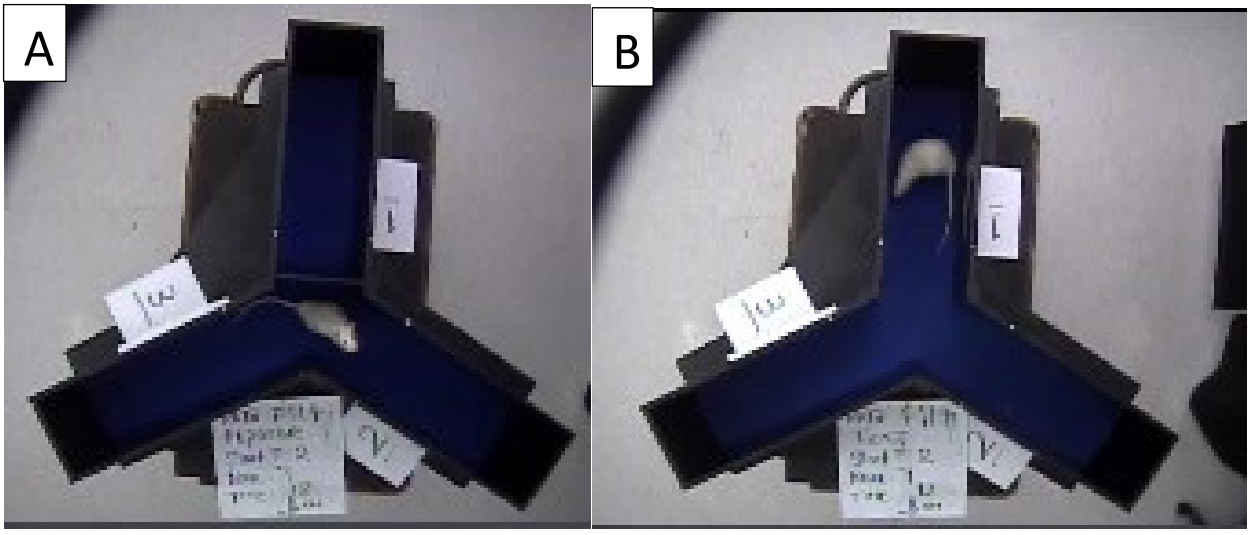

Figure 1. Y-maze apparatus. This picture shows one of subjects exploring arms in exposure (A) and test (B) trials. In exposure trial novel arm (arm 1) is closed.

Spontaneous Location recognition (SLR) Task

The second experimental test comprised the spontaneous location recognition (SLR) task which was conducted using both dissimilar (DSLR) and similar (SSLR) configurations. This memory task involved two phases and evaluates the ability of animals to distinguish and recall the locations of objects introduced in the exposure phase. By employing two task configurations, the degree of similarity in the locations to be remembered can be systematically adjusted. This is achieved by changing the spatial positions of objects, creating scenarios with varying levels of dissimilarity or similarity, thereby altering the demand on pattern separation. A circular open field

arena with dimensions of 90 cm diameter and 45 cm high walls, constructed of black plastic was used in the first test. The arena was divided into 36 equal segments separated by a 10° angle. The floor of the arena was covered with corncob bedding to hide the location labels marked on the arena floor. The protocol used here was previously documented (Bekinschtein et al., 2013; Kent et al., 2015). Here, as the results of the SLR tests conducted in the circular arena did not replicate the results reported in Bekinschtein's paper, both the DSLR and SSLR tests were repeated in a square open field arena with minor modification, e.g. corncob bedding was not used on the floor of the arena, instead the maze was cleaned with 10% ethanol between subjects, and objects used during these tests differed from those used during the circular maze tests. The black, plastic square arena dimensions were 100 cm x 100 cm and wall heights of 35 cm.

Procedure: All subjects were habituated to the testing room for 30 min to reduce stress prior to undergoing behavioural assessments. The testing space containing the open field arena was surrounded by black and white curtains and four large high-contrast spatial cues were attached to the curtains in a location high enough to be seen above the maze walls. Low-level background music was played to reduce the impact of uncontrollable noises.

Habituation: Rats were habituated to the SLR arena for 10 min on three consecutive days.

Sample phase: During the sample phase, rats explored the arena containing identical landmarks in three locations arranged in a triangular formation for 10 minutes. The objects included tall glass bottles filled with green sand, tall 720 ml plastic sprayer bottles filled with purple sand, tall glass candlesticks filled with blue sand and 355 ml Pepsi cola cans.

Test trial: In the test phase, conducted 24 h after sample phase, only two of the three previously explored items were placed back into the arena 1) one object was placed in the same location as the sample phase (Object A) and 2) one object (Object B) was placed in the location

that would be the midway between the positions of Object B and Object C during the sample phase. (see Figure 2 for example of DSLR and SSLR configurations).

Using two configurations of the task, the similarity of the to-be-remembered locations was manipulated by altering the spatial positions of objects. The DSLR refers to dissimilar configuration in which the objects B and C are maximally (120˚ angle from the centre of arena) separated. The SSLR task, which was conducted 72 h after DSLR, refers to similar configuration of the task in which the objects B and C had a smaller separation (50˚ angle from the centre of arena). The novel locations of objects used in each test were counterbalanced for animals to reduce potential biases due to directional preferences or subtle changes in maze characteristics (lighting or sound). Four different set of three identical objects were used for each test configuration to keep the rats interested in the objects across multiple tests. Between each round of testing for individual animals, one scoop of the bedding in the arena was taken out and replaced with a fresh scoop of clean corncob to spread out any odors and avoid olfactory traces. In addition, after completing the day's testing for males, half of the corncob was removed and replaced with clean bedding to test females. Also, between each animal's test, both the arena's walls and objects were cleaned using paper towels and a 10% ethanol solution. All walls were cleaned with a 50% ethanol solution between males and females' tests. In the square arena, where bedding was not utilized, both the walls and the arena floor were cleaned with a 10% ethanol solution and thoroughly dried between testing sessions for each animal.

The behavioural tests were video recorded by a camera installed on top of arenas, and the data scored using BORIS v.713.7 software (Friard & Gamba, 2016). Total sample exploration and total test exploration were calculated. A discrimination index or D-ratio, which is the proportion of total exploration time spent exploring the novel object for each pair of objects, was calculated

using this formula: time (novel) – time (familiar) / time (novel + familiar). Two male rats that did not reach the criterion for exploring objects were excluded from the study (one old and one young). These criteria encompass animals that either interacted with just one or two objects during the sample phase or refrained from exploring objects altogether, instead staying at the periphery of the arena during both sample and test phases.

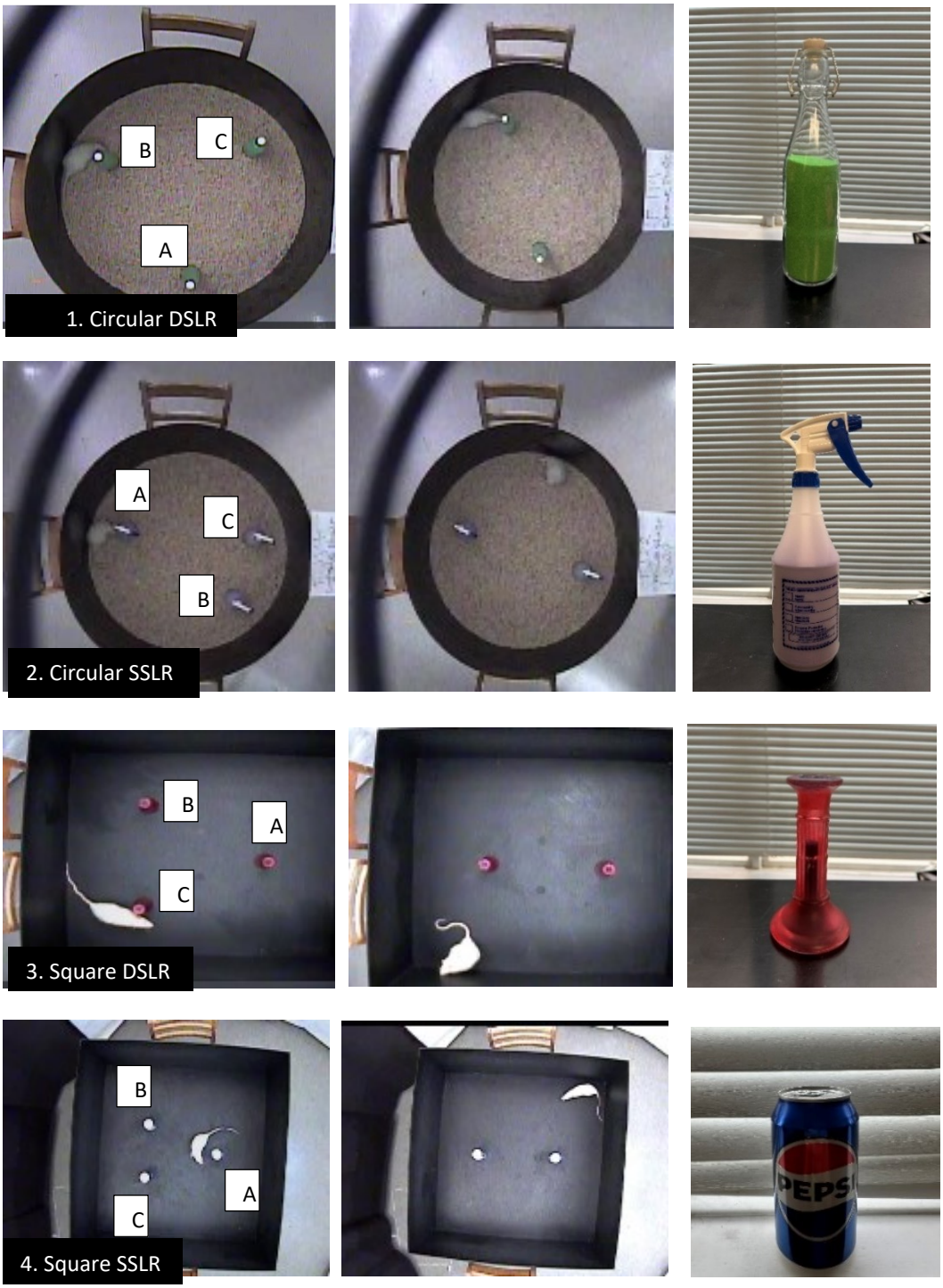

Figure 2. SLR Task apparatus. 1. A subject exploring circular arena containing three similar objects in sample phase and two objects in test phase of DSLR task. Objects B and C are separated 120˚ angle from the centre of arena and 15 cm from the arena's wall 2. A subject exploring circular arena containing three similar objects in sample phase and two objects in test phase of SSLR task. Objects B and C are separated 50˚ angle from the centre of arena and 15 cm from the arena's wall 3. One of subjects exploring square arena containing three similar objects in sample phase and two objects in test phase of DSLR task. Objects B and C are around 50 cm apart from each other and 25 cm from the arena's wall 4. One of subjects exploring square arena containing three similar objects in sample phase and two objects in test phase of SSLR task. Objects B and C are around 30 cm apart from each other and 35 cm from the arena's wall.

Data Analysis: The statistical analysis was conducted using GraphPad Prism 9.4.0 and Jamovi 2.3.2 software. The data were subjected to factorial and repeated measures analysis of variance (ANOVA). If a significant difference was found, pair-wise comparisons were done using the Tukey post-hoc test. The data are presented as the mean ± standard error of the mean (SEM), and statistical significance was determined at $p < 0.05$.

Results

Assessment of the Effects of Age and Sex in the Two-Trial Spatial Y-maze Task

Five variables including total distance, percent entries to the arms, percent time spent in each arm and the discrimination ratios were calculated and analyzed (ANOVA). The distance variable revealed that although there was no overall main effect for age and no interaction effect, there was a main effect for sex ($F(1,44) = 13.98$, $p < .001$). Tukey post hoc analysis showed females were more active based on the total distance travelled in the maze regardless of age (Fig 3A). There were no overall main and interaction effect for age and sex in the percent of entries to the arms during the test trial. However, percent of entries to the novel arm were significantly higher than familiar arm in all groups ($F(1,44) = 37.12$, $p < .001$) (Fig 3 B). Regarding the time spent in each arm, there were an interaction effect for arm and sex ($F(1,44) = 11.44$, $p = .002$) and a main effect for age ($F(1,44) = 14.42$, $p < .001$). The post hoc analysis revealed that female rats spent significantly more time in the novel arm compared to familiar arm and compared to the time males spent in the novel arm. Also, young rats (mean=33.93±9.361) spent more time in the novel arm than old rats (mean=29.32±10.350) (Fig 3 C). No significant difference was found between groups in the D-ratio ($F(1,44) = 7.897, 2.488, 0.557$ for sex, age and interaction effect respectively, $p > 0.05$) (Fig 3 D). The data are presented as the mean ± SEM, and statistical significance was determined at $p < 0.05$.

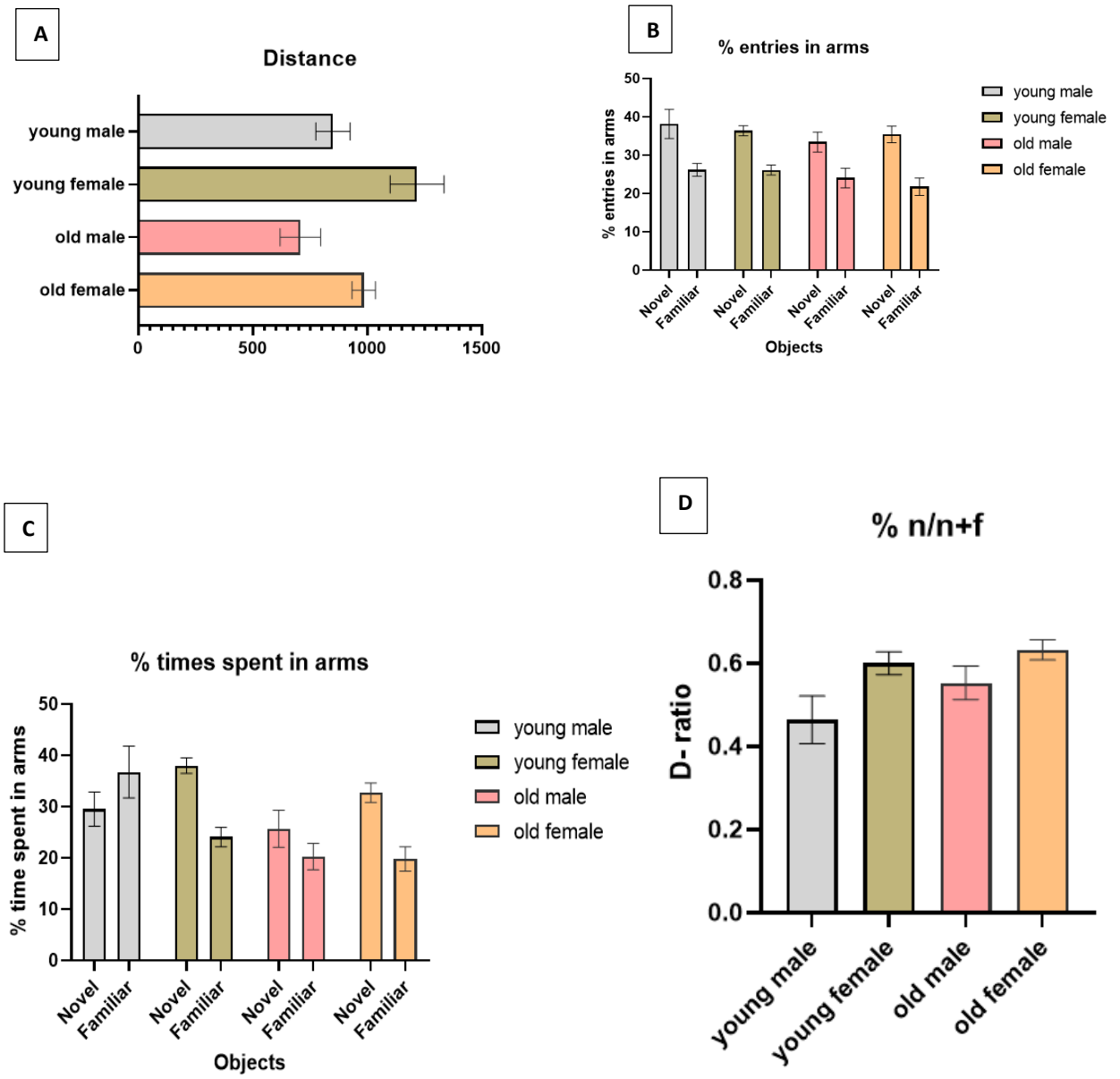

Figure 3. Y-maze task. We conducted a 2(sex) X 2(age) X 2(arm) ANOVA to test if there were any effects of these factors on the rats' function in Y-maze. A. Although there was no overall main effect for age and no interaction of age and sex, there was an effect for sex. Tukey post hoc analysis showed females travelled a greater distance than males regardless of age. B. There were no overall main and interaction effect for age and sex in the percent of entries to the arms during the test trial. Percent of entries to the novel arm were significantly higher than familiar arm in all groups. C. There were a main effect for age and an interaction effect for arm and sex. Female rats spent significantly more time in the novel arm compared to familiar arm and compared to the time males spent in the novel arm. Also, young rats spent more time in the novel arm than old rats. % time spent in start arm have not been shown. D. there was no significant difference between groups in the D-ratio. The data are presented as the mean ± SEM, and statistical significance was determined at $p < 0.05$. Number of animals per group: young male (n=11), young female (n=12), old male (n=12), old female (n=13).

Assessment of the Effects of Age and Sex in the Circular SLR Task

The SLR task was performed in two distinct arenas (circular and square) and two configurations (dissimilar and similar), equalling four, two-trial tests. In each SLR task, two parameters of exploration time and discrimination performance were calculated and analyzed. Exploration time refers to the time spent exploring during both the sample and test phases. In the sample phase, exploration times for each of the three objects are denoted as object '1', '2', and '3'. During the test phase, exploration times for the familiar and novel object locations are labeled 'familiar' and 'novel' respectively. Key variables derived from these exploration times include total sample exploration, total test exploration, and the discrimination ratio.

In Circular SLR task, we conducted a 2(sex) X 2(age) X 3(object) ANOVA to test effects of these factors on the rats' exploration in the sample phase of SLR task. During the sample phase of DSLR and SSLR task, there were no overall main and interaction effect for objects, age and sex. Animals showed equal exploration of each of the objects and no significant age and sex differences were found ($p > 0.05$) (Fig 4 A and C). During the test phase, discrimination ratios were not significantly different where none of the groups demonstrated a preference for the object in the novel or familiar locations in either the DSLR or SSLR task on circular open field arenas ($p > 0.05$) (Fig 4 B & D). However, the p values for main effect of age and interaction effect of sex and age (0.08 and 0.059 respectively) showed a marginal and trending values toward the significant. The data are presented as the mean ± SEM, and statistical significance was determined at $p < 0.05$.

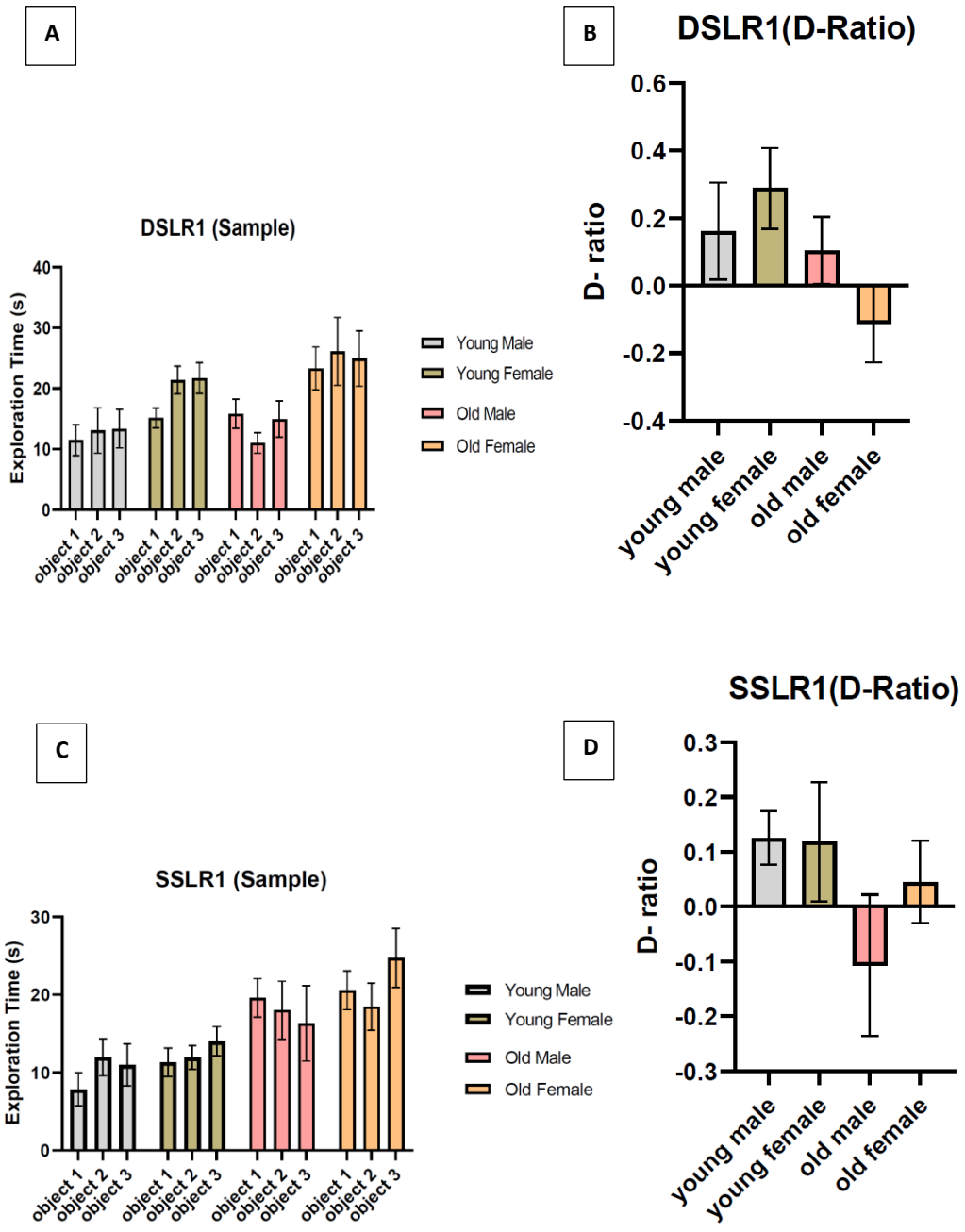

Figure 4. Circular SLR task. A & C. We conducted a 2(sex) X 2(age) X 3(object) ANOVA to test if there were any effects of these factors on the rats' exploration in sample phase of SLR task. There were no overall main and interaction effect for objects, age and sex. During the sample phase of DSLR and SSLR task, animals showed equal exploration of each of the objects and no significant age and sex difference were found. B & D. During the test phase, discrimination ratios were not significantly different and did not show any preference for novel or familiar location in either the DSLR or SSLR task on circular open field arenas. The data are presented as the mean ± SEM, and statistical significance was determined at p <0.05. Number of animals per group: young male (n=12), young female (n=12), old male (n=11), old female (n=12).

Assessment of the Effects of Age and Sex in the Square SLR Task

In the square SLR task, we conducted a 2(sex) X 2(age) X 3(object) ANOVA to determine if there were any effects of these factors on the rats' exploration of objects in sample phase of SLR task. During the sample phase of DSLR and SSLR task, there were no overall main and interaction effect for objects, age and sex. Animals showed equal exploration of each of the objects and no significant age and sex differences were found ($p > 0.05$) (Fig 5A and 5C). In the test phase of DSLR, we conducted a 2(sex) X 2(age) ANOVA to determine if there were any effects of these factors on the rats' discrimination ratios in test phase of SLR task. We found a main effect for age ($F(1,40)= 27.27$, $p<.001$) and sex ($F(1,40)=4.97$, $p=.03$). However, there was no significant interaction effect for these two factors ($p > 0.05$; see Fig. 5B). Post hoc analyses revealed that in the DSLR the D-ratio for the young rats was higher than that for the old rats (Mean: 0.333±0.226, and -0.240±0.513 respectively, $p<.001$). Female rats also had comparatively higher D-ratios than male rats (Mean: 0.177±0.416, and -0.042±0.521 respectively, $p=.03$). We conducted the same analysis in the test phase of SSLR and found that Discrimination ratios were not significantly different in test phase and did not show any preference for novel or familiar location in SSLR task on square open field arenas ($p > 0.05$) (Fig 5 D).

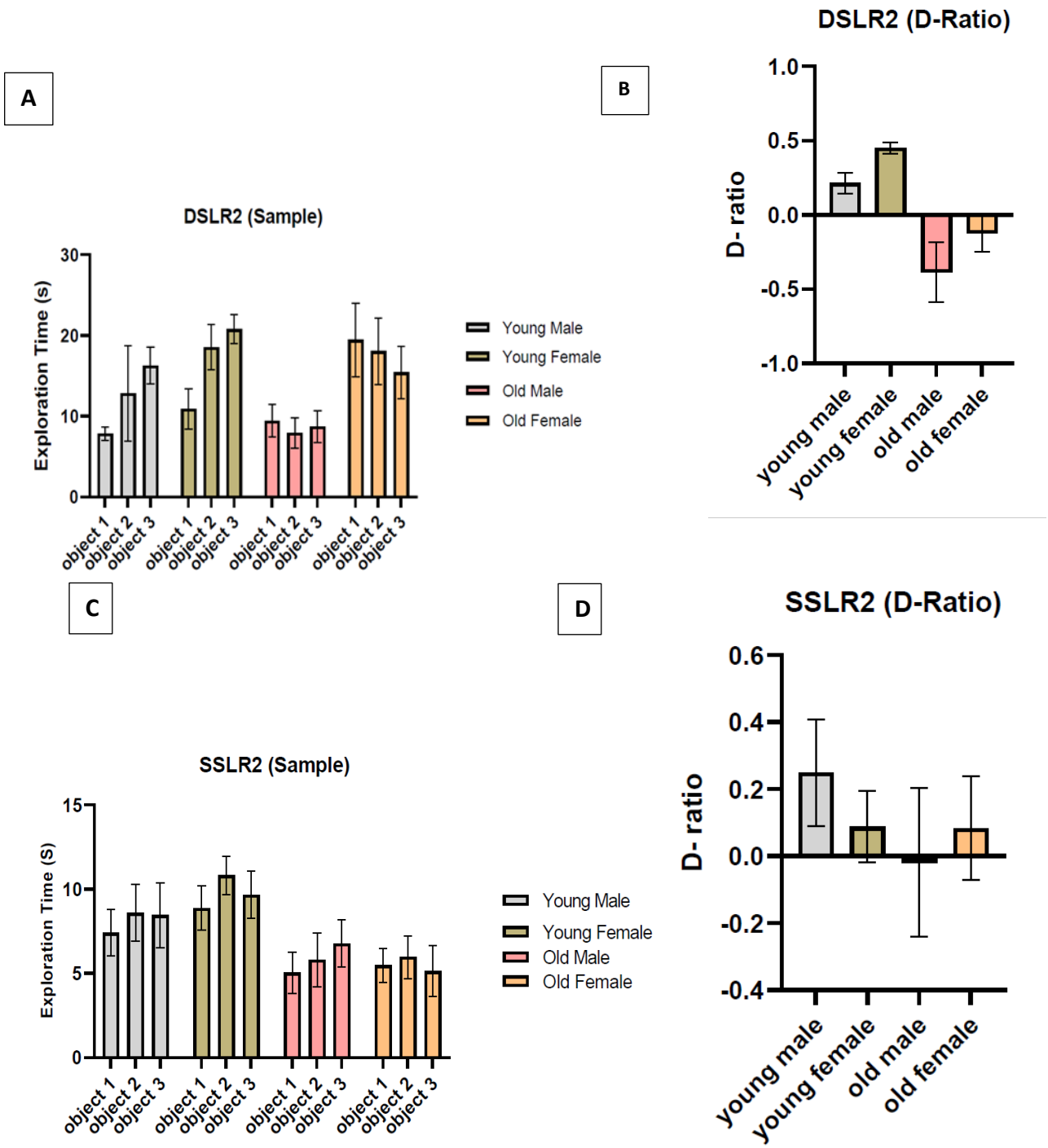

Figure 5. Square SLR task. A & C. A 2(sex) X 2(age) X 3(object) ANOVA was used to assess the effects of these factors on the rats' exploration in sample phase of SLR task. There were no overall main and interaction effect for objects, age and sex. During the sample phase of DSLR task, animals showed equal exploration of each of the objects and no significant age and sex difference were found. B. A 2(sex) X 2(age) ANOVA was done to determine if there were any effects of these factors on the rats' Discrimination ratios in test phase of SLR task. There was a main effect for age ($p<.001$) and sex ($p=.03$). However, there was no significant interaction effect for these 2 factors ($p > 0.05$). The young rats had significantly higher D-ratios in comparison to old rats. Moreover, female rats had higher D-ratios in

contrast to the males. D. The same analysis in the test phase of SSLR showed that Discrimination ratios were not significantly different in test phase and did not show any preference for novel or familiar location in SSLR task on square open field arenas ($p > 0.05$). The data are presented as the mean ± SEM, and statistical significance was determined at $p < 0.05$. Number of animals per group: young male (n=12), young female (n=12), old male (n=11), old female (n=12).

Discussion

The primary objective of this study was to investigate age and sex differences in the pattern separation abilities of Sprague Dawley rats using two two-trial spatial memory tests of pattern separation: the Y-maze and the SLR tasks. Young (4-5 months) and relatively old (14 -15 months) male and female rats were subjected to behavioural tests. Although significant age and sex differences were found in some parameters, the results did not fully support our initial hypotheses in either task or across the different experimental setups. Specifically, the data supported the hypothesis that female rats exhibit higher activity levels and a preference for the novel arm in the Y-maze. However, contrary to our expectations, there was no significant age or sex related decline in pattern separation abilities as measured by the discrimination ratios in the Y-maze task. Moreover, the anticipated sex and age differences in pattern separation were not observed in SLR tasks except in square DSLR test in which young rats and female rats had higher D-ratios. Moreover we did not find any interaction effect of age and sex in pattern separation abilities as measured by the discrimination ratios in either tasks.

Effects of Age and Sex on Memory for a Two-Trial Non-Aversive Spatial Y-Maze

In the spatial Y-maze test, female rats demonstrated increased activity and a preference for the novel arm, a finding consistent with earlier research by (Conrad et al., 2003), who reported sex differences in spatial and non-spatial Y-maze performance after chronic stress in male and female Sprague–Dawley rats, suggesting a sex-dependent variation in novelty-seeking behavior. Additionally, Dellu et al. (1992) found that spatial memory can be influenced by the inter-trial

interval and age. They used two-month-old and eighteen-month-old male Sprague-Dawley rats in a two-trial Y-maze memory task with five different retention times (30 min, 2, 4, 6 and 24 h) and found that in 2-month-old rats, total number and duration of visits to the novel arm were significantly higher for the first 4 inter-trial intervals while at the 24 h interval, there was no longer any difference between the number and duration of visits to the novel arm and the other arms. Impaired recognition was also detected in 18-months-old rats so that there was no significant difference between the duration of visits to three arms for all intervals and the number of novel arm visits was higher only in 30 min interval. Our study found no age-related differences in the discrimination ratio parameter among the rats. This discrepancy may be attributed to differences in the number of subjects or age ranges which were not identical to those in the Dellu et al. study or long inter-trial interval which was reported by Dellu et al (1992).

Effects of Age and Sex on Memory for Two Versions of the SLR

In the initial SLR tests conducted in circular arenas, discrimination ratios did not significantly differ, and rats showed no clear preference for novel or familiar object locations in either the DSLR or SSLR task. Considering the results of DSLR in circular arena was marginal and trending toward significant and for further investigation, we replicated the study in square arenas as reported by Omoluabi et al. (2021). In square arena, young female and male rats (combined) exhibited higher discrimination ratios in the DSLR task, which involved a dissimilar configuration of the test. However, similar to the circular arenas, discrimination ratios in the SSLR task remained unremarkable, showing no preference for novel or familiar locations.

Canatelli-Mallat et al. (2022) evaluated the ability of young, middle-aged, and senile female Sprague-Dawley rats to retain 24 h long-term recognition memory. In the SLR task, there was a markedly diminished novel discrimination capacity in the middle- and old- age rats

compared with the young ones. Saucier et al. (2007) also investigated Long-Evans rats' performance on a task of object location memory and in a spatial water maze task. Rats were housed in either complex environments or in standard shoebox housing. The results indicated that females outperform males on the object location recognition task and males outperform females on the Morris water maze regardless of housing environment. These findings suggest that sex differences in memory tasks are task-specific. Additionally, the study found that environmental enrichment played a significant role in enhancing memory for object location for both sexes, although it did not alter the relative performance differences between males and females on the Morris water maze task. On the other hand, the improvement in the DSLR task in square arenas might reflect the influence of environmental geometry on spatial memory and discrimination. Qi et al. (2015) found that environmental factors, such as the shape and size of the testing arena, could significantly influence spatial memory outcomes. Specifically, they noted that environmental geometry could affect how animals encode and retrieve spatial information. In square arenas, the uniformity and symmetry may provide consistent spatial cues that enhance the ability to discriminate between different locations, thereby improving pattern separation performance. Moreover, the use of a square arena might reduce the complexity of spatial cues compared to irregularly shaped environments, making it easier for rats to form distinct and non-overlapping memory representations. This could explain why rats perform better on spatial tasks in such controlled settings. The findings by Qi et al. (2015) emphasize the importance of considering environmental geometry in experimental design, as it can profoundly impact the interpretation of cognitive abilities such as spatial memory and pattern separation.

Factors to consider when interpreting results

The absence of significant age effects in the performance of the rats suggests that pattern separation abilities may not undergo substantial changes throughout the lifespan of Sprague-Dawley rats, at least within the ages examined in this study. This finding contradicts previous studies which have reported age-related declines in cognitive functions in rodents, including aspects of spatial memory and pattern separation (Gallagher & Nicolle, 1993; Kuhn et al., 1996). It is possible that the age range selected for this study did not capture the critical period of cognitive decline, or that other factors not investigated here may contribute to age-related changes in pattern separation abilities. Gallagher and Nicolle, (1993) utilized rats aged 24 months, which represents a more advanced stage of aging compared to the 14-15 months old rats in our study. Their research highlighted declines in hippocampal-dependent tasks, implicating more severe aging effects that might not be evident in the younger old rats used in our study. Kuhn et al. (1996) focused on a variety of behavioural tasks, including the Morris water maze, which might stress different aspects of cognitive function than the Y-maze and SLR tasks employed in our research. Their study also examined neurobiological markers of aging, such as decreased hippocampal neurogenesis and increased synaptic dysfunction, which are factors that could significantly affect cognitive performance and might not have been fully manifest in the age groups we examined.

Our study employed the Y-maze and SLR tasks, designed to assess spatial memory and the ability to distinguish between similar locations. These tasks test the cognitive abilities without the stress of aversive stimuli or the motivation for reward which can affect performance and might explain some discrepancies between studies that use stress- or reward- inducing methods. Moreover, some other key factors might explain why our findings differ from previous studies. Several methodological factors should be considered when interpreting the results of this study.

Firstly, while Y-maze and SLR tasks are commonly used to assess spatial memory and pattern separation abilities in rodents, they may not fully capture the complexity of these cognitive processes such as higher-order decision making, long-term memory retention, and associative learning. For example, the Y-maze primarily tests the ability to remember and distinguish between different arms based on spatial cues, which might not involve complex problem-solving or the integration of varied sensory inputs that animals encounter in more naturalistic settings. Similarly, SLR tasks focus on the ability to recognize changes in the location of objects, which does not necessarily engage cognitive domains such as emotional memory or tasks that require the synthesis of contextual information. Additionally, variations in the configuration and size of the arenas, type of objects, number of subjects and their ages used in this study may have influenced the mean rats' performance, and future research could systematically investigate the impact of such factors on pattern separation abilities. For example, the objects used in the SLR tasks in this study may not be distinctive enough to achieve significant results. Additionally, optimal intervals should allow for the consolidation of memory without leading to significant forgetting, typically ranging from several hours to a day, depending on the complexity of the task (Barker & Warburton, 2011).

Variations in environmental enrichment, diet, and overall laboratory conditions can influence cognitive function and neuronal health. Studies have shown that rodents housed in enriched environments exhibit enhanced neurogenesis and improved performance in memory tasks, suggesting that environmental complexity can positively influence brain plasticity and cognitive abilities (e.g., Kempermann et al., 1997).

Moreover, different strains of rats (e.g. Sprague-Dawley) may exhibit varying degrees of cognitive resilience or decline, which might not have been accounted for in comparative studies. Genetic variability can significantly influence the cognitive trajectory of an organism. Owen et al.

(2005) have demonstrated that certain gene polymorphisms are associated with variations in spatial memory and hippocampal structure in mice, suggesting a genetic predisposition to different cognitive outcomes.

Our study did not directly assess cellular and molecular changes such as changes in hippocampal structure and function, neuronal loss and alterations in synaptic plasticity which might explain why we observed no significant age effects within the parameters tested. Future work could benefit from integrating cellular and molecular analyses to better understand the underlying mechanisms that support or impair pattern separation in different ages and sexes.

Furthermore, we did not investigate hormonal levels and potential changes in hormonal receptors. The role of hormones such as estrogen and testosterone in cognitive functions has been explored by Galea et al. (2008), who found that fluctuations in hormone levels affect hippocampal-dependent memory tasks differently in male and female rodents, impacting their spatial memory and pattern separation abilities.

While the Y-maze and SLR tasks are generally adequate for studying pattern separation, the specific configurations of these tasks in terms of object selection and testing intervals need careful consideration to accurately assess age and sex differences in pattern separation abilities. The differences in findings between our study and previous research may stem from variations in methodology, test conditions, and the specific ages and biological conditions of the subjects. A comprehensive approach that includes both behavioural assessments and cellular-level investigations might provide a more complete picture of how and why cognitive functions change with age/sex differencs in Sprague-Dawley rats.

Implications and Future Directions

The findings of this study contribute to our relative understanding of the factors influencing pattern separation abilities in Sprauge Dawley rats. Future research could explore additional factors that may modulate cognitive functions in rodents, such as environmental enrichment, hormonal influences, or genetic factors. Moreover, investigating the neural substrates underlying pattern separation abilities using techniques such as neuroimaging or neurophysiological recordings in both humans and rodents could provide further insights into the mechanisms governing these cognitive processes.

Extending stimuli beyond the visual or spatial domain to other dimensions (e.g., odor for rodents or verbal stimuli for humans) will provide greater insights into information processing both within and outside the hippocampal regions. Also, tests such as the Continuous Recognition Memory (CRM) task, which has been adapted for rodents to assess their ability to distinguish between similar and identical objects over continuous trials, could be insightful. This test, as modified by Barker & and Warburton (2011), can more directly measure pattern separation by challenging the animals' memory across a gradient of similarity.

Future studies using targeted behavioural tests of pattern separation coupled with imaging or electrophysiological techniques will elucidate important links between DG and pattern separation. To better understand the physiological changes that occur in the brain during aging or between sexes, it would be beneficial to integrate longitudinal studies that track hormonal levels, synaptic plasticity, and neural circuitry changes over time.

Finally, it is vital to understand which types of treatments may be more beneficial for men versus women in combating neurodegenerative diseases and age-related cognitive decline. So, it

is important to study the age and sex differences in pattern separation abilities in the context of neurodegenerative models and experimental manipulations.

Conclusion

In conclusion, this study did not find significant age or sex differences in the pattern separation abilities of Sprauge-Dawley rats across various experimental conditions. While these results may challenge prevailing assumptions about age and sex effects on cognitive functions in rodents, they underscore the complexity of pattern separation processes and highlight the need for further research to elucidate the factors influencing these abilities.

Summary

The overall findings of this work underscore that while significant age and sex effects on pattern separation were not observed within the studied parameters, this does not negate the possibility that such influences exist but may be modulated by external factors like environment, hormonal levels, and genetic makeup. This suggests that cognitive functions are complex and influenced by a myriad of factors that require a multifaceted approach to fully understand. Future research should thus not only replicate and expand on these findings but also incorporate broader biological and environmental variables to paint a more complete picture of cognitive aging and sex differences.

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