An associative analysis of recognition memory

Benjamin Keep

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Abstract

Recognition memory is a fundamental cognitive process which is often impaired in conditions such as Alzheimer's disease. In rodents, recognition memory is often studied using spontaneous object recognition tasks (SOR) in which objects that differ in terms of their novelty, recency, or prior location, are explored by animals. The theoretical basis and explanations of performance in these tasks remain controversial, often based on theories of familiarity and recollection. Sometimes opponent process (SOP) offers an alternative explanation and postulates that two priming processes underlie recognition memory. Self-generated priming occurs when a current stimulus has been recently experienced, and retrieval-generated priming arises when an object is predicted by another stimulus through prior association. In this thesis, I examined specific predictions of SOP using SOR task variants in mice. I explored associative and recency-based processes defined by SOP which may occur during object recognition memory. I used variants of the object in context task, which map onto retrieval-generated priming, to explore blocking and indirect object recognition effects, and used variants of the relative recency task, which map onto self-generated priming, to investigate distractor effects upon recency discrimination performance. I provided some evidence to support the use of SOP to study recognition memory during association-based and recency-based memory tasks. Further work is required to validate and develop these findings to establish this method as a suitable general framework for studying recognition memory.

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Chapter 1: Introduction

1.1 Recognition memory

1.1.1 What is recognition memory?

Recognition memory is a process which allows organisms to discriminate between previously encountered and novel stimuli (Warburton & Brown, 2015). In simpler terms, it is an essential process which allows humans to recognise people and items that they encounter during their day to day lives and allows the retrieval of information about an item, and similarly allows animals to recognise conspecifics and other important things within their environment, such as resources (e.g., food or shelter) and dangers (e.g., predators or alarm calls). The process is versatile and allows various judgements using different types of information such as the relative familiarity of stimuli or locations, the recency of the last encounter with a stimulus, or whether an item has a previous association with another item, location or context (Warburton & Brown, 2010, 2015). There are several different definitions of recognition memory that use different approaches to define the process, some assume unobservable mental representations, whereas some instead use observable changes in behaviour.

1.1.2 Why is it important to study?

1.1.2.1 Recognition memory decline in dementia

Recognition memory declines with age (Koen & Yonelinas, 2014) and further deteriorates in dementias such as Alzheimer's disease (Hajilou & Done, 2007; Irle, Kessler, Markowitsch, & Hofmann, 1987; Laatu, Revonsuo, Jäykkä, Portin, & Rinne, 2003). Dementia is characterised by deficits in cognitive abilities across two or more cognitive domains, such as memory, language, or learning (Arvanitakis, Shah, & Bennett, 2019), and these severe cognitive deficits significantly impact upon the activities of daily life (Gauthier et al., 2006). Dementia is a global health concern with a worldwide incidence estimated at approximately 55.2 million people in 2019, with 14.1 million residing within the

European region, predicted to increase to 78 million by 2030 and 139 million by 2050 (Organization, 2021). Furthermore, deficits in recognition memory may be an early indicator of Alzheimer's disease (Didic et al., 2010; Zola, Manzanares, Clopton, Lah, & Levey, 2013).

1.1.2.2 Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease and the most common form of dementia within the elderly which accounts for an estimated 60% to 80% of dementia cases (Association, 2018; W. W. Barker et al., 2002). People associated with caring for those afflicted with AD suffer a great social and psychological burden which is difficult to quantify (Fiandaca, Mapstone, Cheema, & Federoff, 2014) and the global economic burden of AD is astounding. For example, AD related healthcare costs in the United States including long-term care and hospice care were estimated at $277 billion in 2018 and were projected to increase to $1.1 trillion by 2050 (Association, 2018). If interventions were available which could delay the onset of AD by 5 years it is estimated that this could reduce both the number of people afflicted by the disease and the costs associated with this by approximately 50% (Sperling et al., 2011b).

1.1.2.3 Alzheimer's disease treatments

There are two categories of approved drugs for the treatment of AD (Calabrò, Rinaldi, Santoro, & Crisafulli, 2021), partial N-methyl D-aspartate (NMDA) antagonists and cholinesterase inhibitors which only temporarily ameliorate symptoms (Bond et al., 2012; Howard et al., 2015; Molinuevo, Berthier, & Rami, 2011; N. Zhang, Wei, Du, Shi, & Cheng, 2015) and which have variable levels of efficiency (Takeda et al., 2006). Two new treatments have recently been approved, one in November 2019 in China (sodium oligomannate (GV-971); Syed, 2020), and one in the USA in June 2021 (amyloid-targeting human monoclonal antibody aducanumab (ADUHELM); FDA, 2021). However, these both remain controversial and have not yet been approved in other global regions (Yeo-Teh & Tang, 2023).

Some neurodegenerative disease specialists have suggested that after the onset of clinical symptoms the therapeutic pharmacological agents currently available to treat the condition may not be effective (Fiandaca et al., 2014). Furthermore, early intervention during the long preclinical period of AD appears to be a critical aspect to slow down the progression of the disease (X.-X. Zhang et al., 2021). This highlights the importance of establishing a better understanding of the preclinical (asymptomatic) stages of the condition so that therapeutic interventions can be administered at an earlier stage, before the onset of symptoms, when the brain is less compromised and where they may provide far more effective treatment (Breijyeh & Karaman, 2020; Sperling et al., 2011a; X.-X. Zhang et al., 2021; Zola et al., 2013).

1.1.2.4 Preclinical stages of Alzheimer's disease

Due to major advances in neuroimaging, cerebrospinal fluid (CSF), peripheral blood, and other biomarkers (Fiandaca et al., 2014; Sperling et al., 2011b) the preclinical state of AD can now be identified. This has led to a critical need to gain a better understanding of these biomarkers and the cognitive changes that occur during the preclinical stages of the condition (Fiandaca et al., 2014; Sperling et al., 2011b). The preclinical stage of AD can last for several years and is characterised by mild memory loss and some pathological changes in the brain, which can be identified through imaging or CSF/blood biomarkers (Sperling et al., 2011a), with no clinical symptoms of AD and no functional impairment in daily life activities (Breijyeh & Karaman, 2020). Studies in humans have identified deficits in visual recognition memory as an early potential predictor of AD (Didic et al., 2010; Zola et al., 2013). Therefore, there is a need to better understand the preclinical stages of the condition and a need to develop diagnostic tools and therapeutic strategies which could be used during these asymptomatic periods to delay or prevent the progression to clinical symptoms.

1.1.2.5 Summary

Due to the global prevalence of dementia, particularly of the Alzheimer's type, and the associated economic and social burden, it is critical that we develop new methods to delay or prevent this condition. Because recognition memory deterioration appears to be predictive of developing AD, it is crucial that we gain a deeper understanding of recognition memory, and the mechanisms and processes which underly its decline, and how we might be able to delay or prevent this in the future.

1.2 Recognition memory in humans

1.2.1 Theories of recognition memory

Early work exploring human amnesia identified that amnesic patients had deficits in recognition memory (Warrington & Weiskrantz, 1970). This led to the development of different theories to explain recognition memory. One of the early theories was a dual process account that was based on familiarity and recollection (Mandler, 1980). Mandler described these processes using the example "*Consider seeing a man on a bus whom you are sure that you have seen before; you "know" him in that sense. Such a recognition is usually followed by a search process asking, in effect, Where could I know him from? Who is he? The search process generates likely contexts (Do I know him from work; is he a movie star, a TV commentator, the milkman?). Eventually the search may end with the insight, That's the butcher from the supermarket*". In this view, two separate processes underlie recognition memory, the recollection of specific contextual information related to a previous item or event, and the assessment of general familiarity of an item or event (e.g., Mandler, 1980; Yonelinas, 2002). Theories from neuropsychological, cognitive, and neuroimaging studies of human memory have continued to primarily focus on familiarity and recollection (For a review see: Yonelinas, 2002; Yonelinas, Aly, Wang, & Koen, 2010), although there are other dual-process theories using alternative explanations (e.g., Sanderson & Bannerman, 2011). Dual-process theories of familiarity and recollection assume

that the two processes operate independently and may occur in sequence or in parallel (Yonelinas, 2002; Yonelinas et al., 2010). An alternative view is that recognition memory is a single process and many of these accounts have often been based on signal-detection theory (Green & Swets, 1966). Signal-detection theory suggests that both recollection and familiarity recognition memory decisions are based on the strength of a memory trace for an item in relation to a decision criterion, and if the strength of the memory trace exceeds the criterion, then it is specified as old, and if not, then it is specified as new (Wixted, 2007). In this single process view, the familiarity of the item could be what underlies the strength of the memory trace (Wixted, 2007). Therefore, single-process theories suggest that the differences between familiarity and recollection can be accounted for by distinctions between strong (i.e., recollection) and weak (i.e., familiarity) memory traces (Dunn, 2004; Squire, Wixted, & Clark, 2007).

1.2.2 Measurements of human recognition memory

Early studies of human recognition memory were based on introspection and required phenomenological judgements from participants, related to their state of awareness, associated with past experiences, and their feelings towards items that they may or may not have seen before (Tulving, 1985). For example, in the remember/know paradigm (Tulving, 1985) participants first study word lists and then are tested with a series of items, some of which they have previously studied and some which are new. They are then required to respond ‘remember’ if they can recall specific details about an item and are required to respond ‘know’ if they do not recall any such details, but an item provokes a sense of being familiar that leads them to believe that they have previously studied it. These types of measurements, based on subjective experience, have continued to be used often in more recent studies which require ‘remember’, ‘know’, ‘old’ or ‘new’ judgements (e.g., Bellana, Ladyka-Wojcik, Lahan, Moscovitch, & Grady, 2023; Berry, Henson, & Shanks, 2006; Espinosa-García, Vaquero, Milliken, & Tudela, 2017).

An alternative approach which attempts to separate the processes of familiarity and recollection has been the application of signal-detection theory and the

analysis of receiver operating characteristics (ROCs) (Yonelinas, 1994, 2002; Yonelinas et al., 2010; Yonelinas & Parks, 2007), which provide estimates for the contribution of familiarity and recollection during the memory tasks used. The typical task this procedure is used for requires participants to first study lists of words, and then in the test that follows, judge if words are old or new. The data obtained are then plotted as true positive response rates against false positive response rates as a function of response confidence. In a more recent version of the task, participants were required to give subjective confidence-based recognition responses to a series of previously seen and new images, the responses were 'recollect', 'definitely old', 'probably old', 'unsure', 'probably new', or 'definitely new' (Duarte, Ghetti, & Geng, 2023). The participants were given instructions related to the different responses and instructed to only select "recollect" if they were sure that they had seen an item before and that they could recollect some qualitative information about the event where they had experienced it, such as their feelings about the item or what they thought about when they initially saw it.

1.2.3 Issues with these types of measurements

Because these measures of recognition often require verbal judgements, based on subjective psychological experience, they may not be appropriate for use with animal models, dementia patients, young children, or non-native speakers. Animal models are essential for studying the neural basis of recognition memory and for testing therapeutic strategies and treatments for its decline. Many of these types of studies are not possible using humans, thus animal models are necessary. For example, research to establish the safety, efficacy, and specific targets for certain drugs, before they are approved for human use, must be carried out using animal models. Thus, it is essential that recognition memory tasks, and the measurements used, are translational from humans to animals and vice versa. Furthermore, using these types of measurements have led to definitions of recognition memory that are not very useful for translation to animal work. For example, it has been suggested that familiarity involves a feeling that an item has been previously experienced, which if sufficiently strong can lead to an old judgement, and that

recollection involves the item engendering an attempt to retrieve a memory, related to that item (e.g., Medina, 2008; Wilding & Rugg, 1996).

1.3 Recognition memory in animals

1.3.1 Measurements of recognition memory in animals

To explore recognition memory in animals, behavioural tasks were developed that were thought to be good analogues to the tasks used in humans (e.g., the remember/know paradigm, Tulving, 1985). Initially, procedures were developed to examine recognition memory in primates, and these were known as the delayed matching-to-sample (DMTS; Gaffan, 1974) and delayed non-matching-to-sample (DNMTS; Mishkin & Delacour, 1975) tasks. Variants of these tasks have continued to be used in more recent years in primates (e.g., M. Eacott, Gaffan, & Murray, 1994; Rodriguez, Zörcher, Bartlett, Nathanielsz, & Nijland, 2011; Suzuki, Miller, & Desimone, 1997; Turchi, Saunders, & Mishkin, 2005; Zola et al., 2000) and other species including rats (e.g., Mumby, Pinel, & Wood, 1990; Rothblat & Hayes, 1987), birds (M. Good & Macphail, 1994; Healy, 1995), and dogs (Davila, 2023). In a typical DMTS task animals are presented with a sample stimulus for a short time (usually seconds) and then the stimulus is removed. Following a delay, the animal is presented with one stimulus that is identical to the sample and one that is different. The animals are reinforced for selecting the stimulus that matches the sample. The DNMTS task is typically identical except that the animal receives reinforcement for selecting the stimulus that does not match the sample.

Another task for assessing recognition memory in animals is the serial recognition task (Fahy, Riches, & Brown, 1993; F. A. W. Wilson & Rolls, 1993). Here, animals are shown stimuli, first as ‘novel’, and then following several other intervening items, shown secondly as ‘familiar’. The subject is differentially reinforced to respond to the items as being either ‘novel’ or ‘familiar’ throughout the experiment.

1.3.2 Issues with these types of measurements

These measurements of recognition memory all require extensive training over multiple trials. Unfortunately, this makes them less translatable to humans because human memory is generally not tested under regimes of reinforcement or elevated hunger levels. However, an alternative approach, which does not require training or reinforcement, has been widely used in rodents (the spontaneous object recognition task) which may offer more translational value than these more traditional methods. This approach is discussed below.

1.4 Recognition memory in rodents

1.4.1 Spontaneous object recognition (SOR) tasks

Variants of the SOR task (For a review see: Dere, Huston, & Silva, 2007) have been widely used to investigate the neural substrates of recognition memory (For a review see: Winters, Saksida, & Bussey, 2008). SOR tasks rely on rodents' propensity to explore novelty and measure object recognition through assessing differences in exploration time between novel and familiar objects (Ennaceur & Delacour, 1988). In the simplest version of this task rodents are exposed to two copies of a junk object and then, following an interval, tested with a copy of the familiar object and a novel object. Rodents generally explore the novel object more than the familiar object during the test. Almost all normal rats will demonstrate a preference for novel objects which far exceeds the level of random variations of the exploration behaviour (Ennaceur & Delacour, 1988), a phenomenon which is also evident in mice (Dere, Huston, & Silva, 2005; Dodart, Mathis, & Ungerer, 1997; Messier, 1997). This preference for novelty must be spontaneous, it cannot be based on instructions, and it is short-lived (Ennaceur, 2010) thus SOR tasks usually have test durations of 3 minutes for rats and 3-10 minutes for mice (Dere, Huston, & Silva, 2007; Dix & Aggleton, 1999). The SOR task has many variants that have been used to explore different aspects of memory and its underlying neural mechanisms (e.g., G. R. Barker, Bird, Alexander, & Warburton, 2007; Bonardi, Pardon, & Armstrong, 2016, 2021; M. A. Good,

Barnes, Staal, McGregor, & Honey, 2007; Hannesson, Howland, & Phillips, 2004; Mitchell & Laiacona, 1998; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002; Nelson, Cooper, Thur, Marsden, & Cassaday, 2011; Norman & Eacott, 2005; Sanderson et al., 2011; Sep, Vellinga, Sarabdjitsingh, & Joëls, 2021; Spanswick & Dyck, 2012; Spanswick & Sutherland, 2010; Tam, Bonardi, & Robinson, 2015; Tam, Robinson, Jennings, & Bonardi, 2014; D. I. Wilson et al., 2013). For example, the concept of episodic memory divides memories for events that have been experienced into, "what" happened, "where" it happened, and "when" it happened (Tulving, 1983) and different variants of the SOR task have been used to address each of these components of memory separately. Four examples of commonly used SOR tasks are shown in Figure 1 and described below.

1.4.1.1 Novel object recognition task

The spontaneous object recognition task is widely used and often referred to as the novel object recognition task (Ennaceur & Delacour, 1988; Figure 1A) and is considered to be a suitable test to assess the "what" component of memory (Chao, de Souza Silva, Yang, & Huston, 2020). In this task an animal, typically a rat or a mouse, is exposed to two copies of object A. Following a delay interval (minutes, hours, or days) (Dere et al., 2007), the animal is exposed to a copy of A and a novel object B. Typically, greater exploration of B compared to A is observed and interpreted as recognition by the animal that object B is novel.

1.4.1.2 Relative recency task

Another variation of the SOR task is the relative recency task (Mitchell & Laiacona, 1998; Figure 1B) which has been used to assess the "when" component of memory (Chao et al., 2020). For example, it has been used to investigate memory for the temporal order or the recency in which items were experienced (G. R. Barker et al., 2007; Bonardi et al., 2016, 2021; M. A. Good et al., 2007; Hannesson et al., 2004; Hatakeyama, Sugita, Yamada, & Ichitani, 2018; Mitchell & Laiacona, 1998; Nelson et al., 2011; Sanderson et al., 2011; Tam et al., 2015; Tam et al., 2014)(e.g., Mitchell & Laiacona, 1998; Tam et al., 2013; Hatakeyama

et al., 2018). This task consists of two different objects sequentially presented over two sample phases (e.g., two copies of object A and following a delay interval, two copies of object B) followed by a test with both objects (e.g., one copy of object A and one copy of object B). Rodents generally explore the less recent object (e.g., object A) more during the test, which is interpreted as recognition by the animal that one of the objects has been experienced relatively more recently than the other object (Mitchell & Laiacona, 1998). Relative recency effects can also occur in object-in-place and object-in-context tasks (e.g., Tam et al., 2015).

1.4.1.3 Object-in-place task

Another commonly used variation of the SOR task is the object-in-place task (Dix & Aggleton, 1999; Figure 1C) which can be used to assess the "where" component of memory (Chao et al., 2020), in relation to the location in which the item was previously experienced. This task allows location learning to be explored with less reliance on navigational skills that may be required for other tasks (e.g., the Morris water maze) (Aggleton & Nelson, 2020). Here, animals are exposed to junk objects (e.g., objects A, B, C and D) placed in different locations for a set amount of time. Following a delay interval, animals are exposed to copies of the original junk objects (e.g., objects A, B, C and D) placed in the positions previously used; however, two of the objects are in the same location as before and the remaining two objects have switched to the opposite locations (e.g., D, B, C and A). Animals generally explore the two switched objects more than the two objects which have remained in their previous locations. This is interpreted as recognition by the animal that some of the objects have changed place and are now in locations that may have been previously explored but have not been experienced with that object. There are many variants of this task such as a two object version where animals are presented with A and B and then tested with two copies of A, placed in the two locations previously used (e.g., Bonardi et al., 2016, 2021). Animals generally explore the copy of A that has been placed in the location that previously contained B.

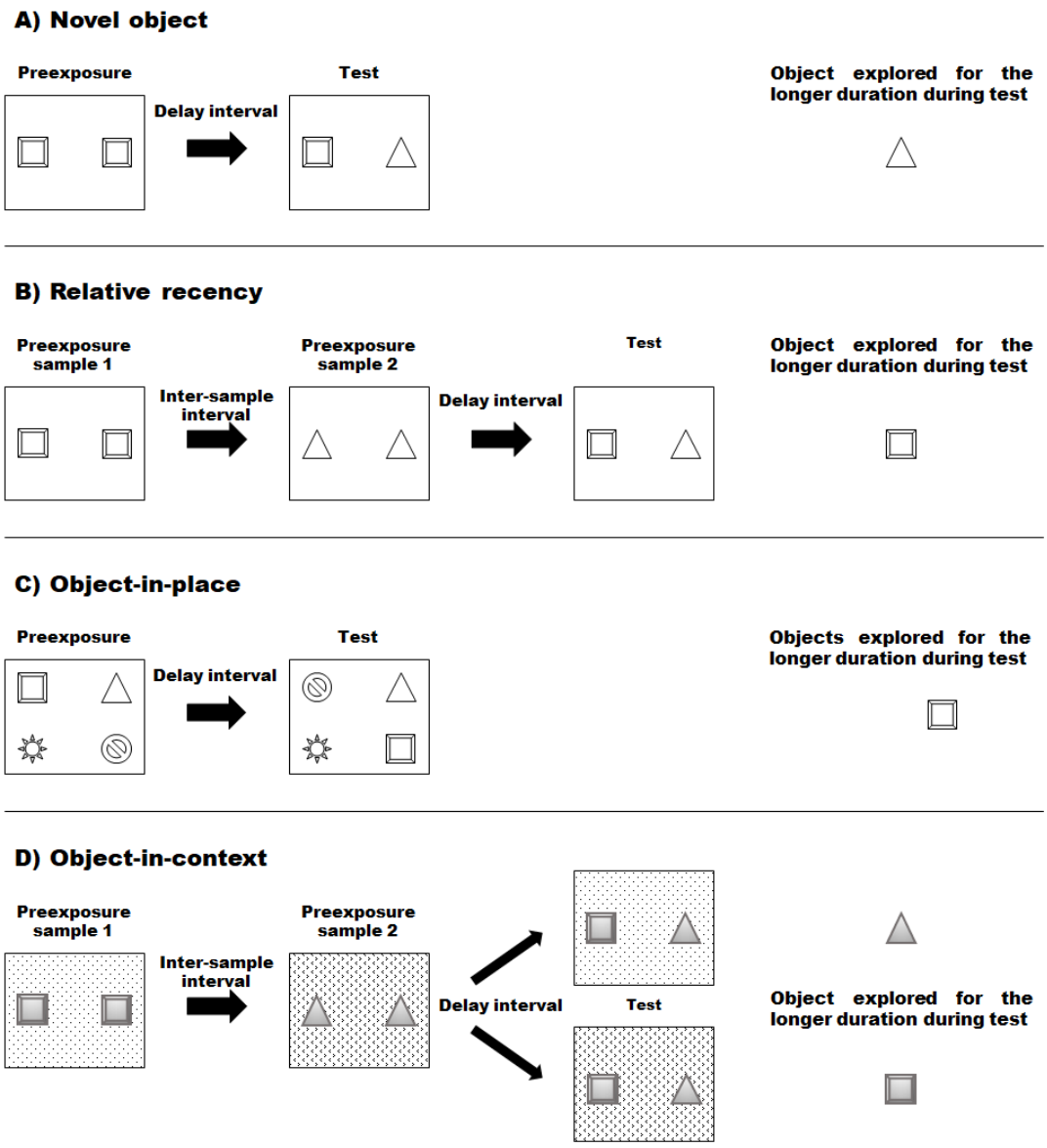


Figure 1. Schematic examples of experimental protocols used for variations of the spontaneous object recognition (SOR) task within recognition memory research.

1.4.1.4 Object-in-context task

Another popular version of the SOR task is the object-in-context task (Figure 1D) which can also be used to assess the "where" component of memory (Chao et al., 2020), in relation to the context in which the item was previously experienced. This task is considered to be a test of association-based memory (For a review see: Sep et al., 2021) and has been carried out using different experimental designs (e.g., Balderas et al., 2008; Barsegyan, McGaugh, & Roozendaal, 2014; Dix & Aggleton, 1999; M. J. Eacott & Norman, 2004). The design used by Barsegyan et al. (2014) is illustrated in Figure 1D. Here, animals are exposed to two copies of object A in context X and following a delay interval are exposed to two copies of object B in context Y. After another delay interval, animals are tested either in context X or Y with one copy of both A and B. Context X and Y and context object combinations during training are counterbalanced as well as context object combinations during test. During the test, healthy animals generally explore the object that is presented in a different context more than the object that is presented in the same context, relative to the sample phases.

1.4.2 Issues with the data obtained from SOR tasks

Because SOR tasks have been extensively used to investigate the neural substrates of recognition memory (e.g., Warburton & Brown, 2010, 2015; Winters et al., 2008), this has led to recognition memory often being defined by the neural mechanisms thought to underlie various processes, such as familiarity and recollection or task complexity. For example, lesion studies in rodents have suggested that the perirhinal cortex is a crucial component during familiarity judgements and that recollection is largely hippocampus dependent (Warburton & Brown, 2010, 2015). Other authors have suggested that functional dissociations are instead related to task complexity rather than familiarity and recollection. For example, for simpler memory tasks (e.g., the novel object recognition task) the perirhinal cortex appears to be required, while during more complex tasks (e.g., the object-in-place task), additional areas such as the medial prefrontal cortex, the nucleus reuniens and the medial dorsal nucleus need to be recruited (Aggleton &

Nelson, 2020). However, other authors disagree with the evidence supporting these functional dissociations which has led to controversy and debate surrounding these types of definition (e.g., Squire et al., 2007).

1.4.3 Advantages of SOR tasks

SOR tasks have advantages over other memory tasks, such as reinforced DMTS and DNMTS tasks, because they do not include food deprivation, reinforcing stimuli (e.g., food or footshock), or learning and retaining response-reward associations, making them relatively less stressful and arousing (Dere et al., 2007). Moreover, they require a low cognitive demand, due to the use of readily discriminable objects as opposed to complex discriminations (Warburton & Brown, 2010). The absence of training and the reduction in stress and arousal mean that these tasks are more analogous to the conditions in which human recognition memory is typically measured and also more similar to recognition in the wild (Ennaceur & Delacour, 1988), making them a powerful tool for pharmacological and neurological memory research using rodents (Dere et al., 2007; Ennaceur & Delacour, 1988).

1.4.4 Translation of SOR tasks to humans

SOR tasks are conceptually translatable to humans because, similarly to rodents directing more exploration towards novel items compared with familiar ones, humans direct more of their gaze towards novel items compared with familiar ones. For example, the visual paired-comparison test is a nonverbal test of recognition memory used in humans, in which infants are shown pairs of visual stimuli and then view novel stimuli paired with familiar ones (VPC; Fagan, 1970). Using this task, human infants have been shown to gaze longer at novel visual targets than familiar ones (For a review see: Burbacher & Grant, 2012). In recent years, VPC task study designs have utilised automatic eye-tracking with gaze-contingent presentation to avoid the potential error and bias that may arise using more traditional methods, which have relied on trained observers to record the visual fixations of the infants (Burbacher & Grant, 2012; Horváth, Hannon, Ujma,

Gombos, & Plunkett, 2018). VPC using eye-tracking has been successfully used in adult human adults to explore differences in recognition memory between normal elderly, elderly diagnosed with mild cognitive impairment, and elderly with Parkinson's disease (Crutcher et al., 2009). Furthermore, the method has the potential to screen for and to track the progression of neurodegenerative diseases, such as AD, because it can be used equally well in both healthy adults and those with compromised verbal and motor functions (For a review see: Bueno, Sato, & Hornberger, 2019).

To summarise, SOR tasks and VPC tasks, using eye-tracking, are conceptually identical in that neither of them require training, verbal instructions, or verbal judgements and instead they both rely on the propensity of the participants to direct more attention towards novel than familiar items. Therefore, data from experiments using SOR tasks in rodents should be very translatable to humans' studies using VPC tasks and eye-tracking.

1.5 An alternative approach to study recognition memory

1.5.1 Traditional methods to study recognition memory

As discussed above, recognition memory has been traditionally explored using human theories of memory (e.g., familiarity and recollection, Mandler, 1980; Yonelinas, 2002) and measurements that typically involve familiarisation to word lists and the identification of words from those lists, during tests which include old and new words (e.g., Bellana et al., 2023; Berry et al., 2006; Duarte et al., 2023; Espinosa-García et al., 2017). These types of tasks and measurements used for humans are not necessarily suitable for use with certain groups of people, such as dementia patients, infants, or non-native speakers. They are also not suitable for animals and so different types of tasks have often been adopted to measure recognition in animals (e.g., DMTS and DNMTS tasks, Davila, 2023; Healy, 1995; Mumby et al., 1990; Rodriguez et al., 2011); in these tasks, in place of the verbal instructions that humans are usually given, animals instead receive intensive training. This crucial difference limits the translational value of the tasks

used which could be problematic, as animal models are essential for investigating the neural mechanisms of recognition memory and for testing drugs that may delay or prevent its deterioration.

1.5.2 A behavioural approach to study recognition memory

Instead of adapting human research for use in animals we propose using an alternative approach that starts with animals and then can later be used for humans. Our approach uses an associative learning framework to explain recognition (Brandon, Vogel, & Wagner, 2003; Wagner, 1981). Associative learning involves one event predicting another through past experiences (Wasserman & Miller, 1997) and is widespread across animals ranging from protozoa to humans. Furthermore, it is fundamental to adaptive behaviour and in developing environmental knowledge (J. Pearce, 1997; Pontes, Mobley, Ofria, Adami, & Dyer, 2020). Thus, recognition of past events that signal important positive or negative consequences are critical for learning to occur. Therefore, it is likely that recognition memory is a behavioural phenomenon that is also widespread across animals. If this is the case, then a theory of behaviour may be more suitable to investigate and explain recognition memory.

Here, we investigate the suitability of using a well-established model of associative learning which also provides a theory of memory to explain recognition memory. We will use SOP (Sometimes opponent process; Brandon et al., 2003; Mazur & Wagner, 1982; Vogel, Ponce, & Wagner, 2019; Wagner, 1981) which is a model of associative learning and memory that has been successfully used by several authors to explore recognition memory (e.g., Honey & Good, 2000a; Robinson & Bonardi, 2015; Tam et al., 2014; Whitt, Haselgrove, & Robinson, 2012; Whitt & Robinson, 2013). Using the combination of SOP and SOR task variants, we will measure recognition as a reduction in behavioural responses to previously encountered stimuli compared to novel ones. This approach side steps many of the complexities associated with defining familiarity and recollection and their underlying neural mechanisms. Furthermore, because the same approach can be used for animals and humans (i.e., SOR and VPC eye-

tracking tasks), it circumvents many of the translational issues related to using the different measurements traditionally used for studies in humans and animals.

1.6 Sometimes Opponent Process (SOP)

1.6.1 Stimulus representation

Recent work has suggested that recognition memory and performance during SOR tasks can be accounted for using a model of associative learning and memory (Honey & Good, 2000a; Robinson & Bonardi, 2015; Tam et al., 2014; Whitt et al., 2012; Whitt & Robinson, 2013); originally referred to as standard operating procedures (SOP; Mazur & Wagner, 1982; Wagner, 1981) but more recently termed sometimes opponent process (Brandon et al., 2003; Vogel et al., 2019). SOP is a comprehensive theory of learning and memory proposed for non-human animals that is also applicable to humans and other organisms (Brandon et al., 2003; Mazur & Wagner, 1982; Vogel et al., 2019; Wagner, 1981). It is based on the assumption that every stimulus is represented in memory by a large but finite number of elements (each element is a stimulus feature such as size, shape, colour, odour or texture) and that each of these elements resides in one of three different states (Figure 2). Initial presentation of a novel stimulus moves elements of that stimulus into a primary activation state termed A1, equivalent to the centre of attention, which produces a strong response. A1 has limited capacity and elements will quickly decay into a second active state termed A2, equivalent to the periphery field of attention, which elicits a relatively weaker response - A1 thus commands more behavioural responding than A2. A2 has a much greater capacity than A1 and elements from A2 slowly decay back to an inactive state, which can be thought of as long-term memory. Elements must complete the cycle, thus cannot move from A2 to A1. Different elements of a stimulus can occupy different states (e.g., a proportion of elements could be in A1, a proportion in A2 and a proportion in inactive simultaneously), but each single element can only occupy one state at any given time.

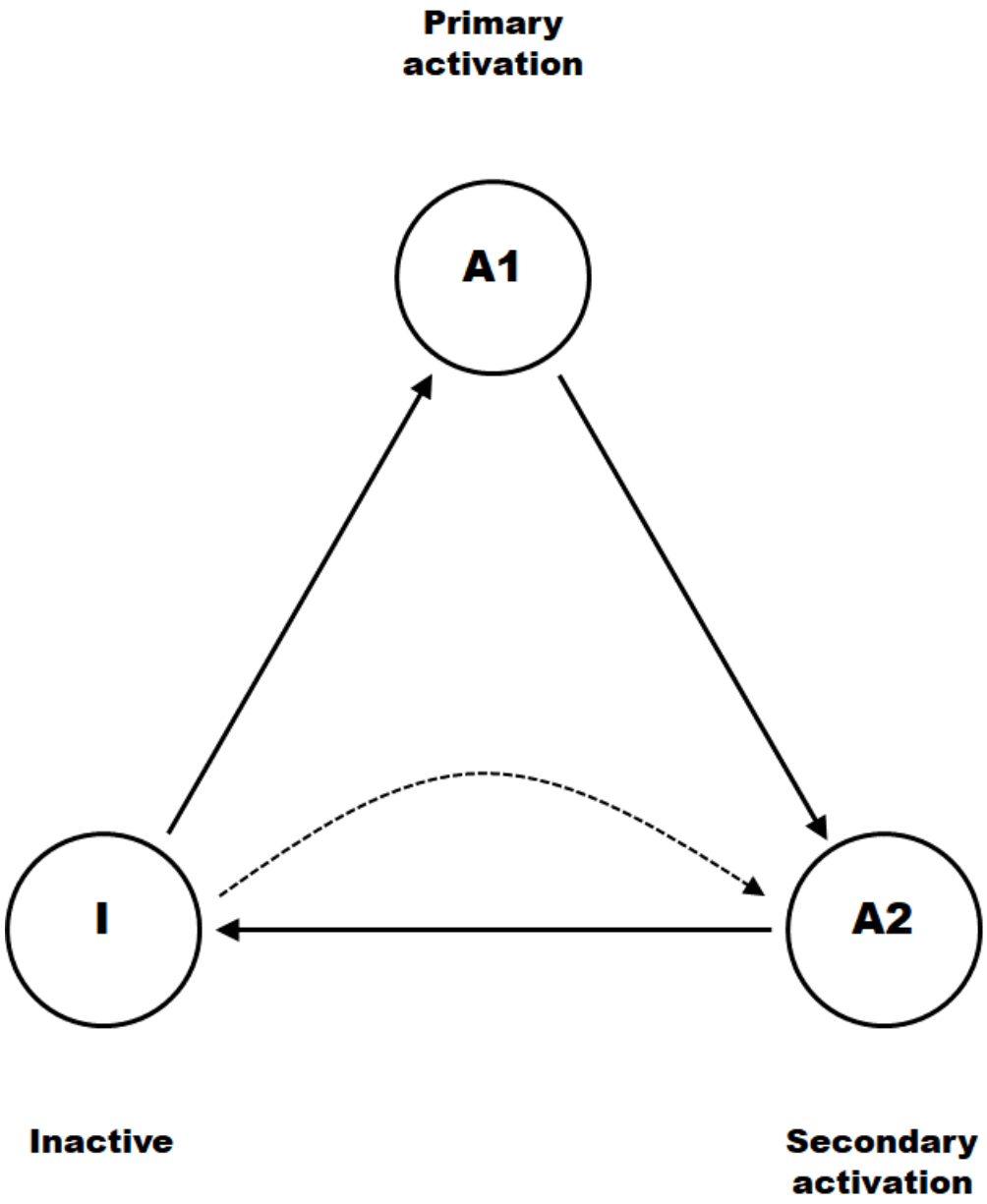


Figure 2. Sometimes opponent process (SOP) model of associative learning and memory. Elements of a stimulus can be excited from the inactive state to the A1 state following a corresponding stimulus presentation. Elements enter the A2 state as a result of decay from the A1 state or directly from the inactive state following presentation of a previously associated stimulus (Adapted from: J. M. Pearce, 2013).

The number of elements which initially enter A1, and the decay rate of these elements between activation states, can be different for individual stimuli. This is because SOP is a probabilistic model which can be expressed in mathematical terms (Brandon et al., 2003; Mazur & Wagner, 1982; Vogel et al., 2019; Wagner, 1981). For example, the assumed momentary probability of elements moving from inactive to A1 (termed $p1$), during exposure to the corresponding stimulus, is a function of stimulus intensity (e.g., stimulus salience or exposure time) such that as stimulus intensity increases, proportionally more elements will enter A1 provided they are available and residing in the inactive state. Elements passively decay from A1 into A2 with the momentary probability pd1, and then decay from there back to inactive with a momentary probability of pd2. These rules for momentary changes in element distribution across the three states are as follows:

1. $PA1 = p1(\mathrm{PI}) - \mathrm{pd1}(PA1)$
2. $PA2 = pd1(PA1) - pd2(PA2)$
3. $PI = pd2(PA2)$

$PA1$, $PA2$, and PI refer to the proportion of elements that reside in the A1, A2, and inactive states respectively (For further information on mathematical decay parameters and simulations of the model see: Brandon et al., 2003; Vogel et al., 2019).

SOP postulates that reduced behaviour directed towards an item indicates recognition of that item, and that this recognition process originates from a reduction in stimulus processing produced by two independent priming processes: self-generated priming, which occurs when a current stimulus has been recently experienced, and retrieval-generated priming, in which an object is predicted by another stimulus through prior association.

1.6.2 Self-generated priming

A weaker response to presentation of a stimulus will be elicited over time (i.e., habituation) compared with the initial presentation of the stimulus, if the same stimulus is presented multiple times in quick succession. The SOP model suggests

that this is because during consecutive presentations an increasing number of the stimulus elements will occupy A2 because they have not had sufficient time to decay back to the inactive state. Because most elements from the stimulus are now in A2 and will take time to decay back to the inactive state, there are successively less of them available in the inactive state which can re-enter A1. Self-generated priming is a process of recency-based memory and has been suggested as the process underlying short-term habituation (Sanderson & Bannerman, 2011).

Self-generated priming can be studied using relative recency tasks (Figure 1B). As mentioned above, animals are first exposed to object A and subsequently to a different object B. After a delay they are tested with objects A and B simultaneously. Empirically, animals generally direct more exploration towards A than B. According to the SOP model, this has been suggested to reflect (e.g., Tam et al., 2014) that during exposure to A, elements of that object are activated into A1 and then begin to decay into A2. During exposure to B, elements of that stimulus will enter A1 and then begin to decay into A2. During test, more of the elements from A will have returned to inactive and be available to reenter A1 relative to B, which will have relatively more elements still occupying A2 due to its more recent presentation. Therefore, responding (exploration time) will be greater towards the less recent A than the more recent B. During this task, retrieval-generated priming (described below) is relatively equated because the context in which both objects have been presented in is the same during both sample phases. Thus, both objects would be predicted equally well by the context during the test.

1.6.2 Retrieval-generated priming

SOP explains associative learning by postulating that if elements from two stimuli occupy A1 simultaneously an excitatory association is formed between them. When one of these stimuli is next encountered, elements from the other stimulus move directly from inactive into A2 (Figure 2). Retrieval-generated priming is a process of association-based memory and has been suggested as the process underlying long-term habituation (Sanderson & Bannerman, 2011). Retrieval-

generated priming can be studied using OIP tasks (Figure 1C) described earlier. For example, SOP theorises that during the preexposure period, elements from each of the objects (e.g., objects A, B, C and D) will enter the A1 state as each stimulus is explored. Simultaneously, elements from items in the surrounding area of each stimulus (contextual cues) will also enter the A1 state, and an association between each object and its corresponding contextual cues may be formed. During the test, when two objects switch location (e.g., D, B, C and A), the two objects in their original locations (e.g., B and C) will have elements enter straight into the A2 state because of their association with their surrounding contextual cues. The two switched objects (e.g., D and A) however will have relatively more elements move into the A1 state as there is no longer an association with their surrounding contexts. Therefore, responding is greater to the switched objects than the objects which have remained in their preexposure locations. During this task, self-generated priming is equated because all the objects have been presented equally recently. Thus, reductions in responding towards objects due to self-generated priming effects would be similar for all objects.

Performance on the basic SOR task, which can be viewed as a classic test of recognition, can be explained in terms of a combination of self-generated and retrieval generated priming. For example, during the sample phase both copies of object A are repeatedly explored thus would have elements in A2 via self-generated priming. Therefore, during the test object B would have relatively more elements available to enter A1 and generate stronger responding than object A. During the sample phase both copies of object A may also become associated with their surrounding contextual cues thus during the test object A would have elements moved directly into A2 via retrieval-generated whereas object B would not. Therefore, object B would again have relatively more elements available to enter A1 and command stronger responding than object A.

1.6.3 SOP's account of learning

One of the key strengths of SOP is that it is already a well-established theory of learning that has been used to describe many learning phenomena such as

instances of contextual learning (Vogel, Ponce, & Brandon, 2020), long-term habituation (Sanderson & Bannerman, 2011; Uribe-Bahamonde, Becerra, Ponce, & Vogel, 2019), and cue competition effects such as overshadowing and blocking (Brandon et al., 2003; Vogel et al., 2019).

1.6.3.1 Pavlovian conditioning

During Pavlovian conditioning, food (unconditioned stimulus; US) is often paired with a tone (conditioned stimulus; CS) and following pairings of the food with the tone, an excitatory association forms between them, which SOP suggests is because both stimuli (repeatedly) have elements in A1 simultaneously. This results in the tone (CS) later provoking elements of the food (US) directly into A2, which results in a response such as salivation (conditioned response; CR); the extent of the conditioned response, in this case salivation, is a function of the strength of the association between the tone (CS) and the food (US) (Brandon et al., 2003).

1.6.3.2 Inhibitory learning and extinction

Extinction is a learning phenomenon that SOP can explain by suggesting that when the CS and US concurrently have elements in either A1 (A1/A1) or in A1 and A2 (A1/A2) excitation or inhibition occur respectively. Therefore, when the CS has many elements in A1 and the US has many elements in A2, an inhibitory association is formed between them. The inhibitory association results in further presentation of the tone (CS) now inhibiting elements of the food (US) from moving directly into the A2 state, which consequently reduces the conditioned response such as salivation. In other words, when the tone (CS) is presented without food (US) many times, the animal salivates (CR) less with subsequent presentations of the tone (CS), and extinction of the salivation response (CR) is eventually observed.

An inhibitory association can also form such as that observed during conditioned inhibition. Here, A is typically paired with reinforcement (A+) to establish a CR and once established, A is paired with stimulus X and no reinforcement is given (AX-). X becomes a conditioned inhibitor (CI), which is a stimulus that predicts

the absence of an otherwise expected outcome (e.g., reinforcement) and consequently decreases the CR; therefore, when X is presented with A, the CR is reduced. In this instance the A+ association will be formed identically to the Pavlovian conditioning process explained above. However, when A (e.g. tone) is presented with X (e.g. a light), elements from both A and X will occupy A1 and elements of the US (e.g. food) will occupy A2. SOP postulates that because elements of the US are in A2 and elements of X are in A1 simultaneously a negative association is formed between them. This means that when X is next presented it inhibits elements of the US moving from I into A2 thus becomes a CI. Therefore, if A is presented alone there will be a strong CR whereas if A and X are presented together the CR will be much weaker and more generally the inhibitor counteracts the effects of any excitor.

1.6.3.3 Cue competition

1.6.3.3.1 Background

Cue competition occurs during Pavlovian conditioning when more than one stimulus is paired with the same unconditioned stimulus (US), and consequently each individual stimulus acquires differential control over later behaviour (e.g., Angulo, Bustamante, Estades, Ramírez, & Jorquera, 2020; Kamin, 1969; Mackintosh, 1975; J. M. Pearce, Graham, Good, Jones, & McGregor, 2006; Richards & Krauter, 1999; Urushihara & Miller, 2009). For example, when a cue is trained in compound with another cue (e.g., AX→food) it typically acquires less control over behaviour than when it is conditioned alone; this is referred to as overshadowing (Urcelay & Miller, 2009; Urushihara & Miller, 2009). Another well-studied cue competition effect is blocking (which will be described in more detail in a later chapter) which can occur when a single stimulus is paired with an unconditioned stimulus (US) during initial trials (e.g., a tone paired with a foot shock; A+), and then the same stimulus is presented in compound with a second stimulus and paired with the same US (e.g., a tone + a light paired with a foot shock; AB+). Following these trials, tests with B (e.g., a light), in the absence of the US (e.g., a foot shock), generally produce a weak CR (e.g., freezing behaviour)

relative to controls (e.g., a group that had the same amount of AB+ training but no prior A+ trials).

1.6.3.3.2 SOP's account of blocking

Rescorla Wagner explained blocking by defining rules such that for a predicted US no learning would occur (Rescorla, 1972). The learning rules of SOP use the same principles in that if the US is predicted, as it is during compound training of blocking, then both inhibitory and excitatory learning occur. This is because presentation of the US results in some A1 activity and the presence of the predictor also produces A2 activity, and these counteract each other which makes it appear that little learning has occurred; learning has been 'blocked' (Brandon et al., 2003).

1.7 Experimental tests of SOP as an account of recognition memory

1.7.1 Introduction

SOP explains learning and memory using the same theoretical framework (Brandon et al., 2003; Wagner, 1981) which may allow it to accommodate much of the existing knowledge related to rodents object recognition (Robinson & Bonardi, 2015). It can be used to generate specific testable predictions which can be applied to object recognition studies with rodents. Previous work testing such predictions has provided experimental support for use of SOP to explain object recognition (Bonardi et al., 2016, 2021; Honey & Good, 2000b; Sanderson & Bannerman, 2011; Sanderson et al., 2009; Sanderson et al., 2011; Tam et al., 2015; Tam et al., 2014; Whitt et al., 2012; Whitt & Robinson, 2013). Some of this work will be described in this section.

1.7.2 A dual-process account of habituation

Early work by Davis (1970) suggested that the process of habituation may be dissociable into short-term and long-term forms and that the interstimulus interval, that is the interval duration between stimulus exposures, can determine effects on

short-term and long-term startle response habituation in rats. In this study, rats were exposed to tones during habituation training that were separated by interstimulus intervals of either 2s (massed training) or 16s (spaced training). Massed training resulted in a greater decrement in startle responding than spaced training. When the rats were tested 1-minute or 24-hours later the reverse effect was observed. That is, there was a greater decrement in the startle response for spaced training than for massed training. Therefore, longer interstimulus intervals produced a long-term form of habituation that persisted over a longer time whereas shorter intervals, between stimulus exposures, resulted in a strong short-term form of habituation that dissipated more rapidly, and these effects are differentially affected by the retention interval delay (the delay between training and test).

SOP offers an explanation for these data and suggests that short-term habituation may be produced by self-generated priming and that long-term habituation may occur through retrieval-generated priming (Brandon et al., 2003; Mazur & Wagner, 1982; Wagner, 1981). That is that, during short interstimulus interval training, self-generated priming would be increased through multiple stimulus exposures in short secession which would result in many elements of the stimulus accumulating in A2, thus would reduce responding towards the stimulus. This would also reduce retrieval-generated priming because there would be fewer elements of the stimulus available to enter A1 concurrently with elements of the contextual surroundings. Therefore, during the test, massed training would result in relatively less habituation because fewer elements of the stimulus would be provoked into A2, via retrieval-generated priming. The opposite effect would occur during long interstimulus interval training. Self-generated priming would be reduced because the spaced training would allow more time for elements to continue to decay from A2 back to inactive. Therefore, responding to the stimulus would be greater than with massed training because there would be more elements of the stimulus available to enter A1 and elicit strong responding. Furthermore, this would also result in more opportunities for elements of the stimulus to form excitatory associations with the elements of the surrounding context. During the test, spaced training would result in relatively more habituation because more of the stimulus'

elements would be activated into A2, via retrieval-generated priming, which would consequently reduce responding.

The SOP account of short-term and long-term habituation has been tested and consistent with this dual-process account of memory, further evidence has been provided demonstrating that, depending on its duration, the interval between stimulus exposures either impairs or enhances habituation (Sanderson & Bannerman, 2011; Sanderson et al., 2009; Sanderson et al., 2011). For example, Sanderson et al. (2009) tested knockout mice ($GluA1^{-/-}$) and wild type controls on short-term and long-term spatial habituation. They exposed mice to one arm of a Y-maze repeatedly and then tested the mice for their preference to explore the unvisited arm of the maze over the familiar arm. They found that, if the intervals between the trials and prior to the test were short (1-minute apart), then the knockout mice exhibited impaired spatial memory relative to the wild type controls, but when these intervals were long (24-hours apart), they displayed enhanced spatial memory relative to control mice. The authors suggested that GluA1 deletion interferes with hippocampal synaptic plasticity and at the same time prevents short-term spatial memory which is hippocampal dependent. In terms of SOP this refers to GluA1 deletion resulting in a selective deficit in self-generated priming.

In further work, Sanderson et al. (2011) demonstrated that the same knockout mice were impaired in short-term visual memory but not in long-term visual habituation. The authors considered their results and those of Sanderson et al. (2009) and suggested that these data indicated that short-term and long-term recognition memory are governed by separate processes, and that these processes can be accounted for by SOP (Wagner, 1981). Therefore, because their knockout mice were impaired in recency-based recognition memory but not in association-based memory, this may reflect that these mice had a selective deficit in self-generated priming. This reduction in self-generated priming may have implications for retrieval-generated priming because if less elements were active in A2, then

consequently more elements would be available for A1 activity where they could form associations.

Sanderson and Bannerman (2011) further explored the hypothesis that short-term and long-term recognition memory are governed by separate processes using C57BL/6J/Ola mice and a spatial novelty preference paradigm. They repeatedly exposed mice to familiar spatial locations (two arms of a Y-maze, defined as start and familiar) and then gave them a novelty preference test (a third novel arm was also made available). They used both short and long intervals between the training trials and between training and test and varied the number of exposure training trials. They observed greater habituation when tested after a long (24-hour interval) interval than a short interval (1-minute interval) and greater habituation for massed training (1-minute intervals) than spaced training (24-hour intervals). These results demonstrated that both short-term and long-term processes likely contribute to habituation. In a final experiment, the authors tested the prediction that an associative process contributes to long-term spatial habituation using a plus-maze. They repeatedly exposed mice to two pairs of arms of the maze during training. For example, if the four arms of the maze were labelled clockwise as A, B, C, and D, then the mice would be exposed to A and B in some trials and C and D in other trials. The rationale was that A→B and C→D associations would form. Mice were then tested in probe trials where they were given access to A, B, and D but not C. If A predicted B (A→B), then D would be explored more during probe trials because it would be more surprising (Rescorla, 1972). In terms of SOP (Wagner, 1981), B would be primed by A, thus have elements provoked directly into A2, and consequently would suffer a reduction in exploration whereas D would be unprimed and have all its elements available to enter A1 and elicit stronger responding. This is exactly what was observed: D was explored significantly more than B. This procedure has been successfully replicated in an experiment in which handling cues were matched across stimulus spacing treatments (Whitt & Robinson, 2013). This was to remove a possible confound which may have arisen because animals were manually removed and replaced, to and from the arena, during the study by Sanderson and Bannerman (2011). This

may have led to the differences observed in test performance being due to differences in handling leading to nonspecific changes in arousal rather than due to the influence of stimulus spacing during preexposure (Whitt & Robinson, 2013). However, Whitt and Robinson (2013) also reported that spaced training produced better recognition memory than massed training.

Taken together, the results of these studies (Sanderson & Bannerman, 2011; Sanderson et al., 2009; Sanderson et al., 2011) provide evidence of a dual-process account of habituation and suggests that short-term habituation is recency-dependent, and that long-term habituation depends on incrementally strengthened associative memory processes.

1.7.3 A dual-process account of object recognition memory

As discussed above, it has been shown that GluA1 knockout (GluA1−/−) mice are impaired in short-term, but not long-term, spatial recognition memory and visual recognition memory (Sanderson et al., 2009; Sanderson et al., 2011). Sanderson et al. (2011) tested whether these mice would also be impaired in non-spatial object recognition memory using three different SOR task variants: were novel object (Figure 1A), relative recency (Figure 1B), and object-in-context (Figure 1D). The knockout mice displayed impaired performance on the novel object and relative recency tasks but not during the object-in-context task. Therefore, their impairment in recency-based memory also extends to non-spatial object recognition memory and adds further support to dual process theories of memory that may reflect non-associative short-term and associative long-term priming processes (Wagner, 1981).

Tam et al. (2014) continued investigations into this apparent dissociation in object recognition memory processes. They tested rats with and without neurotoxic lesions to the dorsal hippocampus on object recognition, using three different SOR task variants: novel object (Figure 1A), relative recency (Figure 1B), and object-in-place (Figure 1C). Two retention intervals were tested during the novel object and object-in-place tasks (5-minutes and 2-hours between sample and test phases);

for the relative recency task the interval between the two sample phases was also tested at 5-minutes and 2-hours. They found that, with a longer delay, normal rats' performance worsened in the novel object task but improved in the relative recency task. In the object-in-place task the delay had no effect on performance. However, the lesioned rats showed a selective deficit in the object-in-place task at the longer delay.

These data were consistent with SOP's priming processes account of recognition memory (Wagner, 1981). SOP asserts that during the test of the novel object task, the first sampled object A would have elements residing in A2, via self-generated priming because of the exploration of this object by the rats during the sample phase, which would have reduced responding towards A. Therefore, object B would be explored more than A because B would have all its elements available to enter A1 and elicit stronger responding. When the retention interval was increased to 2-hours, many of A's elements would have decayed from A2 back to inactive, relative to the shorter retention interval, which would have resulted in A having more elements available to enter A1, thus increasing responding towards A. Therefore, it predicts that a longer retention interval will eliminate self-generated priming and reduce performance.

In the test of the relative recency task, both objects would have had elements in A2, via self-generated priming, because of exploration of the objects by the rats during the sample phases. However, the first sampled object A would be explored more than second sampled object B, because A would have had more time for its elements to decay back from A2 to inactive, relative to B, resulting in more elements available for A1 activation and stronger responding. Finally, SOP postulates that retrieval-generated priming underlies performance in the object-in-place task. Therefore, it predicts that retrieval-generated priming depends on associations that should not significantly weaken over longer delays thus varying the delay should have no effect on performance in this task.

1.7.4 Competition between priming processes

SOP states that self-generated priming and retrieval-generated priming are separate processes that are sometimes in opposition thus may compete and interact with one another (Sanderson & Bannerman, 2011; Tam et al., 2015; Wagner, 1981). SOP suggests that these priming mechanisms may underlie object recognition task performance. For example, self-generated priming may underly relative recency task performance because retrieval-generated priming is equated during this task, that is, both objects have equal opportunity to form associations with the surrounding cues. In contrast, retrieval-generated priming may underly object-in-place task performance because self-generated priming is equated during this task, that is, all objects have been equally recently experienced. Association-based memory is thought to underly object-in-context task performance (Ainge, Van Der Meer, Langston, & Wood, 2007; M. A. Good et al., 2007; Mumby et al., 2002; Norman & Eacott, 2005; Sep et al., 2021; Spanswick & Dyck, 2012; Spanswick & Sutherland, 2010; D. I. Wilson et al., 2013). However, Tam et al. (2015) suggested that recency-based memory may also play a role in reported object-in-context task performance because the recency of the test objects is not typically equated (e.g., Figure 1D, Ainge et al., 2007; Mumby et al., 2002; Norman & Eacott, 2005; D. I. Wilson et al., 2013). In a typical object-in-context task object A is presented in context x and object B in context y across two sequential sample trials. This is followed by a test with both objects in one of the contexts (e.g., Ax, Bx or Ay, By). If the test is in context x then recency could reduce object-in-context performance and enhance it when the test is in context y (Tam et al., 2015).

This hypothesis was explored using a modified object-in-context task in rats (Tam et al., 2015, Experiment 1). Two groups of animals received exposure to object A in context x during the first sample phase, and to object B in context y in the second. This was followed by a test with A and B, in context x for group x, and in context y for group y. Group x performed worse than group y, and they suggested that this was because recency facilitated performance in group y but opposed it in

group x. That is, for the group tested in x B would be more surprising than A as it would have no prior association with x; however, A would be more surprising than B because it was experienced less recently. Therefore, the two processes may have counteracted each other, reducing discrimination between A and B. In contrast, for group y A would be more surprising than B as it would have no prior association with y, and A would *also* be more surprising than B because it was experienced less recently. Therefore, the two processes may have both functioned to increase exploration of A and consequently enhance discrimination between A and B. The results were consistent with these SOP predictions.

Nitka, Bonardi, and Robinson (2020, Experiment 1) further investigated the interactions between recency-based and association-based effects in human variants (using eye-tracking) of rodent object recognition tasks, which combined aspects of the relative recency and object-in-context tasks. They exposed participants to image A in context x and image B in context y over two sequential sample phases (xA – interval - yB). This was followed by a test with A and B presented in either x or y. Recency discrimination was stronger when the objects were tested in y than in x. Similarly, to Tam et al. (2015), performance was greater in the arrangement in which SOP would suggest that self-generated priming and retrieval-generated priming would complement one another and worse in the arrangement where they would compete. That is, in the test with y, both priming processes would have functioned to reduce exploration of B whereas in the test with x, self-generated priming would have reduced responding towards B and retrieval-generated priming would have reduced responding towards A. Therefore, exploration of A would have been greater in y than in x, consistent with the data provided.

1.7.5 Association-based object recognition memory

A study by Whitt et al. (2012) demonstrated indirect object recognition which has previously been demonstrated by other authors (e.g., Dellu, Fauchey, Le Moal, & Simon, 1997; Dix & Aggleton, 1999; M. Eacott & Gaffan, 2005). Indirect object recognition comprises of presentation of object P with stimulus X and of object Q

with stimulus Y. In a test that follows, stimulus X is presented with P and Q and animals' generally direct more exploration towards Q than P. Whitt et al. (2012) used a modified version of this task which included an additional phase where X was presented alone prior to the test (PX and QY, X, P and Q; where P and Q were junk objects, and X and Y were different patterned arena wall coverings). This modification was to reduce the possibility that the data obtained could be explained by generalisation decrement – that is, that the animals may be responding to a novel object-context combination, as opposed to associative memory processes in which an object memory may be activated by a context with which it has a prior association. They found that using this modified procedure also resulted in rats directing more exploration towards Q than P. They interpreted their results based on the SOP model (Brandon et al., 2003; Mazur & Wagner, 1982; Vogel et al., 2019; Wagner, 1981). According to their SOP interpretation, associations formed between the objects and contexts (x→P, y→Q) during the initial phases and then when rats were exposed to context x with no objects present, elements of P entered A2 via retrieval-generated priming due to its association with x (x→P). Therefore, during the test exploration of P was reduced by A2 activity relative to exploration of Q. This suggests that arena→object associations may underlie performance during association-based object recognition memory tasks.

1.7.6 Evidence of a dissociation between object memory tasks in a transgenic mouse

As discussed above, GluA1 knockout (GluA1−/−) mice exhibit a selective deficit in recency-based tasks (Sanderson et al., 2009; Sanderson et al., 2011), which SOP suggests may reflect that these mice have an impairment in self-generated priming but not in retrieval-generated priming. The opposite effect has been reported in a transgenic mouse model of Alzheimer's disease which displayed a selective deficit in association-based tasks but not in recency-based tasks (Bonardi et al., 2016, 2021). In these studies, 5-month-old $APP_{swe}/PS1_{\Delta e9}$ transgenic mice and their wild type littermates completed three SOR task variants, the novel object, relative

recency, and object-in-place tasks. At this age these mice typically do not exhibit cognitive deficits in the widely used novel object SOR task variant (Bonardi, de Pulford, Jennings, & Pardon, 2011; Jardanhazi-Kurutz et al., 2010; Kelly et al., 2017). The data reported by both studies were consistent with this and showed no difference in discrimination performance during the novel object task between the transgenic mice and their wild type controls (Bonardi et al., 2016, 2021). Similarly, both studies also reported no deficits in the transgenic mice, compared with their wild type littermates, during the relative recency task. If self-generated priming underlies performance in these two tasks, as previously suggested (Sanderson et al., 2011), then this priming process remained intact in these transgenic animals. However, during the object-in-place task, which is considered by some to be a test of associative recognition (Aggleton & Nelson, 2020), the transgenic mice displayed a selective deficit in performance compared with the control mice. If retrieval-generated priming underlies performance in this task, then this priming process was likely impaired in these transgenic mice. The authors interpreted their results in terms of SOP (Wagner, 1981) and suggested that the transgenic mice had a selective deficit in retrieval-generated priming which disrupted their performance in the object-in-place task but not the other two tasks in which self-generated priming was sufficient to maintain performance (Bonardi et al., 2016, 2021). These results were consistent with previous work (Sanderson et al., 2009; Sanderson et al., 2011) and add further support to the possibility of a dissociation between recency-based and association-based object recognition memory.

1.7.7 Summary

In summary, the SOP model (Brandon et al., 2003; Mazur & Wagner, 1982; Wagner, 1981) allows us to make very specific, testable predictions, based on two priming processes within the same theoretical framework. Using this approach, the evidence presented above suggests that there may be a dissociation between recency-based and association-based object recognition memory, consistent with SOP. Furthermore, it suggests that associative memory processes may function

similarly to associative learning – that is, that arena→object associations may underlie performance during association-based object recognition memory tasks, such as object-in-context (e.g., Dellu et al., 1997; Dix & Aggleton, 1999) and its variants (e.g., Whitt et al., 2012). Therefore, because associative learning can be susceptible to cue competition effects (e.g., blocking and overshadowing, Kamin, 1969; Pavlov, 1927), object recognition memory may also be subject to these phenomena. Moreover, the evidence above also identified two genetically modified mouse models as suitable models for exploring further predictions of SOP, based on its two priming processes and how they may interact and compete with one another. Thus, GluA1 knockout (GluA1−/−) mice and APP/PS1 ($APP_{swe}/PS1_{\Delta e9}$) mice both serve as useful tools for exploring predictions related to these processes, as respectively, one exhibits a selective deficit in recency-based tasks (Sanderson et al., 2009; Sanderson et al., 2011) and the other an impairment in association-based tasks (Bonardi et al., 2016, 2021). Therefore, predictions based on SOP would assume that these mice either have impaired self-generated priming (GluA1−/−) or retrieval-generated priming ($APP_{swe}/PS1_{\Delta e9}$). For example, SOP would predict that object memory during object-in-context tasks would be subject to blocking effects, in healthy mice, because retrieval-generated priming would be susceptible to such an effect. If an effect of blocking was demonstrated in healthy mice, then SOP would also predict that this effect would be absent in APP/PS1 mice because of their impairment in retrieval-generated priming.

Therefore, for this thesis we explored further predictions of SOP related to object recognition memory. We first tested hypotheses using healthy C57BL/6J mice and then used APP/PS1 ($APP_{swe}/PS1_{\Delta e9}$) mice as a tool to further explore these predictions based on their performance deficit during association-based tasks. We chose APP/PS1 ($APP_{swe}/PS1_{\Delta e9}$) mice because we had access to these mice, because of the previous work carried out in relation to SOP (Bonardi et al., 2016, 2021) and because the pathology and cognitive impairments for this model have been well characterised across the lifespan of this mouse (Arendash et al., 2001; Barbero-Camps, Fernández, Martínez, Fernández-Checa, & Colell, 2013; Bonardi et al., 2011; Calvo-Rodriguez et al., 2020; Cheng, Low, Logge, Garner, & Karl,

2014; Dong et al., 2020; Donkin et al., 2010; Garcia-Alloza et al., 2006; Hong et al., 2016; Jardanhazi-Kurutz et al., 2010; Li et al., 2014; Mei et al., 2020; Pedrós et al., 2014; Ruan, Kang, Pei, & Le, 2009; Savonenko et al., 2005; Yan et al., 2013; Yoshiike et al., 2008; Zhu et al., 2017) The next section describes this model in detail.

1.8 Transgenic mouse model of Alzheimer's disease

1.8.1 Alzheimer's disease background

AD occurs due to damaged or destroyed nerve cells related to cognitive function in various parts of the brain, including areas related to basic bodily functions, such as walking and swallowing (Association, 2018). The underlying cause of pathological changes which are present in AD (e.g., accumulation of amyloid-β protein, intracellular neurofibrillary tangles, and misfolded tau protein) currently remain unknown (Breijyeh & Karaman, 2020). Symptoms of AD include an irreversible deterioration in memory, intellect, behaviour, and cognition (Balducci & Forloni, 2011). There are two forms of AD; the most common is late onset AD (LOAD) in which the initial symptoms occur after the age of 65, and the other is early onset AD (EOAD) which accounts for approximately 5% of all cases and in which the initial symptoms usually occur between 30 and 65 years of age (Dorszewska, Prendecki, Oczkowska, Dezor, & Kozubski, 2016; Piaceri, Nacmias, & Sorbi, 2013). AD is generally divided into familial (genetic inheritance, FAD) and sporadic (no familial aggregation, SAD) cases where familial instances are largely early onset, and sporadic cases are mostly late onset and account for more than 90% of AD patients (Dorszewska et al., 2016; Piaceri et al., 2013).

1.8.2 Alzheimer's disease pathology in humans

AD pathology can be defined by the accumulation of extracellular amyloid-β protein (Aβ) which forms Aβ plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated and misfolded tau protein, and its deposition results in neuroinflammation marked by astrocytic and microglial activation

(Association, 2018; Jankowsky & Zheng, 2017). The deposition of Aβ has been identified as a major pathological feature (Sperling et al., 2009) and its overproduction has been proposed as a trigger for AD prior to the deposition of tau proteins (Götz, Schild, Hoerndli, & Pennanen, 2004). It has been postulated that excess Aβ initiates a pathological cascade which leads to AD – the amyloid cascade hypothesis (Jankowsky et al., 2005). Alternative hypotheses have also been proposed, such as the cholinergic hypothesis where the absence of sufficient acetylcholine (ACh) in the neuronal and neuro-muscular regions is postulated as the cause of AD, and the tau hypothesis where the principle causative substance underlying the development and progression of AD is assumed to be tau not Aβ (Kametani & Hasegawa, 2018; Srivastava, Ahmad, & Khare, 2021).

Using Pittsburgh Compound B (PiB) positron emission tomography (PET) imaging, subtle amyloid deposition can be identified by low levels of PiB retention in clinically unimpaired amyloid-positive elderly persons and it is relatively easy to distinguish this type of amyloid deposition from that seen in patients with AD, which show a much higher amount of PiB retention (Aizenstein et al., 2008). It has been suggested that elevated levels of Aβ accumulation in cognitively normal elderly is a detectable early stage in the continuum of AD (Sperling et al., 2009). Furthermore, there is evidence to suggest that soluble oligomers of Aβ are responsible for synaptic and cognitive dysfunction in AD patients (Hardy & Selkoe, 2002). As levels of soluble oligomers of Aβ increase prior to plaque formation, they may underly the cognitive deterioration during the early stages of AD. Support for the amyloid cascade hypothesis has primarily come from transgenic mice which overproduce Aβ and have repeatedly shown cognitive deficits in learning and memory (Jankowsky et al., 2005). They have also shown cognitive deficits and synaptic dysfunction prior to detectable plaque deposition, supporting the theory that soluble oligomers of Aβ play a key role in the early stages of AD (Balducci & Forloni, 2011; Mucke et al., 2000). However, more recently many other mechanisms have been proposed to contribute to AD including neuroinflammation, defects in energy metabolism, oxidative stress, and autophagy failure (For a review see: Calabrò et al., 2021). At present there appears

to be no singular cause of AD; instead an inter-correlation of all AD pathogenesis act to enhance the severity of the disease (Srivastava et al., 2021).

1.8.3 Amyloid precursor protein (APP) and presenilin-1 (PS1) mice

Mice which express the human familial mutations of AD (FAD) have been a fundamental tool for investigation into the pathological mechanisms, progression, and cognitive impairments of AD (Balducci & Forloni, 2011; Mucke et al., 2000). These models are based on the discovery of mutations in the human genes encoding amyloid precursor protein (APP) and subunits of the γ-secretase complex containing FAD mutations (e.g., presenilin-1 (PS1) or presenilin-2 (PS2); Stanga et al., 2018; Tai, Weng, LaDu, & Brady, 2021) and animals harboring combinations of these mutations reproduce many key features of AD pathology including the accumulation of Aβ plaques and oligomers (Balducci & Forloni, 2011; Lee et al., 1997; L. Liu et al., 2002; Richner, Bach, & West, 2009). The APP gene is involved in the production of Aβ (Chartier-Harlin et al., 1991) and the PS1 and PS2 genes are involved in the release of Aβ from APP and in the production of Aβ peptides (Lee et al., 1997). These mutations all increase Aβ deposition and in transgenic mice which carry two of these mutations (e.g., APP and PS1), Aβ deposition is increased at a much faster rate than single transgenic mice which hold only a single mutation (e.g., APP or PS1; Balducci & Forloni, 2011).

1.8.4 Double-transgenic mice ($APP_{swe}/PS1_{\Delta e9}$)

Double-transgenic mice were created for the purposes of accelerated Aβ deposition and a more comparable AD-like pathology including substantial cognitive impairment, extracellular Aβ deposition and neurodegeneration relative to single-transgenic mice (Balducci & Forloni, 2011). These features make them a more suitable model for the study of AD as they more closely parallel the pathology of AD in humans, and the accelerated progression allows studies to be conducted over relatively shorter time periods, using younger animals as opposed to very old fragile animals, which significantly reduces costs (Garcia-Alloza et al., 2006). A widely used double-transgenic model that co-expresses both the Swedish

APP (APP_{swe}) and exon-9-deleted variant PS1 ($PS1_{\Delta e9}$) mutant transgenes is the $APP_{swe}/PS1_{\Delta e9}$ mouse (hereafter APP/PS1). These mice exhibit a progressive neurodegenerative pathology (Szapacs, Numis, & Andrews, 2004) which mirrors many aspects of human AD pathology and is very widely characterised.

1.8.5 Advantages and limitations

The main advantage with using the APP/PS1 mouse as a model of AD is its mirroring of Aβ pathology found in human AD patients which produces alterations in neuroinflammation, neuronal function and behaviour, making it a well-suited model for research investigating Aβ pathology associated behavioural changes (Tai et al., 2021).

The main limitations with the aforementioned transgenic mice is that they lack intracellular neurofibrillary tangles, a pathological maker used for AD diagnosis, despite the presence of hyperphosphorylated tau protein (Balducci & Forloni, 2011). Furthermore, neurodegeneration is not as widespread as that which occurs in human AD, and they lack regional brain atrophy (Balducci & Forloni, 2011). As they exhibit an exaggerated Aβ pathology compared to humans, which is more representative of familial AD (Balducci & Forloni, 2011; Kalkan, Akkaya, Inal-Gültekin, & Sanchez-Perez, 2022), and display behavioural impairments at a young age (generally from around 6 months old e.g., Gao et al., 2015; Kilgore et al., 2010; Sierksma et al., 2013), they fail to incorporate age-related factors which occur later such as mid-life hypertension (Balducci & Forloni, 2011). Thus, they are not a useful model for research studying tau pathology or certain age-related risk factors.

1.8.6 Alzheimer's disease-like pathology in double-transgenic mice ($APP_{swe}/PS1_{\Delta e9}$)

At 3-4 months APP/PS1 mice exhibit alterations in insulin and IGF1 in the brain and mitochondrial dysfunction, tau hyperphosphorylation and synapse loss in the hippocampus (Hong et al., 2016; Pedrós et al., 2014). Soluble and insoluble levels of Aβ40 and Aβ42 are detectable (Garcia-Alloza et al., 2006; Pedrós et al., 2014)

as well as Aβ plaques in limited numbers in the cortex and hippocampus (Garcia-Alloza et al., 2006; Zhu et al., 2017). There is astrocyte activation in the frontal cortex and activated microglia in the cortex and hippocampus (Ruan et al., 2009; Zhu et al., 2017). By 6 months-old, levels of soluble Aβ42 and insoluble Aβ40 and Aβ42 have significantly increased and Aβ plaques are easily detectable and are largely confined to the hippocampus and cortex (Garcia-Alloza et al., 2006; Mei et al., 2020; Pedrós et al., 2014; van Groen, Kiliaan, & Kadish, 2006; Zhu et al., 2017). The number and area faction of these plaques continues to increase up to 22 months-old in a near linear manner (Ruan et al., 2009; Zhu et al., 2017). Brain ratio of insoluble Aβ 40:42 shifts to favour Aβ42 (Garcia-Alloza et al., 2006) and mice now have impaired glucose and insulin tolerance (Pedrós et al., 2014). Microglia and astrocytes, which are critical actors of the neuroinflammatory response (Di Benedetto et al., 2022), generally closely associated with amyloid plaques, have increased and clusters of activated astrocytes are now detectable in hippocampus (Ruan et al., 2009; Zhu et al., 2017). Astrocytes and microglia continue to increase almost linearly up to 12 months of age. At 8-10 months, Aβ accumulation in the brain causes mitochondrial Ca^{2+} overload which leads to neuronal loss which is substantial in the hippocampus (Calvo-Rodriguez et al., 2020; Dong et al., 2020). Significant plaques remain in hippocampus and cortex and are now present in thalamus and cerebellum (Dong et al., 2020; van Groen et al., 2006). In the vicinity of plaques there is a loss in neuronal function and associated neuritic abnormalities (Garcia-Alloza et al., 2006; Meyer-Luehmann et al., 2009). Expression of TNF-α, IL-1β, IL-6 and MCP-1 by activated microglia and astrocytes are now detectable and these proinflammatory cytokines increase with age (Ruan et al., 2009).

1.8.7 Cognitive and memory impairments

APP/PS1 mice show progressive age-related cognitive impairments in many cognitive tasks compared with wild type mice of the same age (Arendash et al., 2001). In rodents, deficits in spatial learning and memory are often assessed using variants of the Morris Water Maze task which typically involves a large pool of

opaque water surrounded by spatial cues and an escape platform which can be moved to various locations (For a review see: D'Hooge & De Deyn, 2001). Generally, over multiple training trials in which animals are placed in different random locations at the start of each trial, the animals learn to find a hidden/submerged escape platform by using spatial cues. Learning is typically measured as a decrease in latency across trials to locate the platform. Memory can then be assessed at any chosen time point after training using probe trials in which the escape platform is removed. The time spent by the animal in areas proximal to where the platform was located is taken as greater memory for the information learned during training (Tucker, Velosky, & McCabe, 2018). Deficits in learning and memory have been identified in the APP/PS1 mouse model that we used, using variants of the aforementioned task, at 6 months-old (Gao et al., 2015; Izco et al., 2014), but not in all cases (e.g., Mao et al., 2016), at 7 months-old (Barbero-Camps et al., 2013; Jardanhazi-Kurutz et al., 2010; Li et al., 2014; Shen et al., 2017), with further deterioration occurring as the mice age (Arendash et al., 2001; Donkin et al., 2010; Du et al., 2016; He et al., 2020; Izco et al., 2014; Janus, Flores, Xu, & Borchelt, 2015; Jardanhazi-Kurutz et al., 2010; Savonenko et al., 2005; Wei et al., 2020; Yoshiike et al., 2008; W Zhang et al., 2011; Wenjun Zhang et al., 2012).

Associative learning is often evaluated in rodents using fear conditioning (passive avoidance) tasks in which a context and/or cue (CS) is paired with an US (generally a foot shock). Future exposure to the CS in isolation generally produces a freezing response (CR) behaviour which is taken as evidence that the animal has learned the trained association (Curzon, Rustay, & Browman, 2011; VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). In APP/PS1 mice, deficits in variants of fear conditioning tasks are not evident at 4 months-old (Kilgore et al., 2010), they can be detected between 6 and 7 months-old using some specific experimental parameters but not others (Cheng et al., 2014; Kilgore et al., 2010; Shen et al., 2017), and are easily detectable by 8-9 months-old (Gong et al., 2020; He et al., 2020; Janus et al., 2015; Wei et al., 2020; W Zhang et al., 2011).

Deficits in other tasks have also been reported. For example, tests of spatial memory using the Y-maze spontaneous alternation test have reported impairments at 6-9 months-old (Gong et al., 2020; Srivastava et al., 2021) but not in all cases (Arendash et al., 2001; Yan et al., 2013) and open field tests for anxiety-like behaviour have demonstrated no apparent changes at 4-5 months-old (Jardanhazi-Kurutz et al., 2010; Kelly et al., 2017) with increased anxiety-like behaviour occurring between 6 and 9 months-old in some experiments (He et al., 2020; Jardanhazi-Kurutz et al., 2010; Mao et al., 2016) but not others (Gong et al., 2020).

1.8.8 Recognition memory impairments

Deficits in visual recognition memory have been identified as an early potential predictor of AD in humans (Didic et al., 2010; Zola et al., 2013) and Aβ accumulation has been identified as a major pathological feature of the disease (Sperling et al., 2009). Therefore, we may expect APP/PS1 mice to show a deficit in recognition memory at around 4-5 months of age when Aβ related pathological changes have begun to occur in the brain (Hong et al., 2016; Pedrós et al., 2014; Ruan et al., 2009; Zhu et al., 2017), Aβ accrual is detectable in addition to Aβ plaques in limited numbers (Garcia-Alloza et al., 2006; Pedrós et al., 2014; Zhu et al., 2017), and other cognitive deficits in learning and behaviour are not yet evident (Kelly et al., 2017; Kilgore et al., 2010). However, SOR tasks using APP/PS1 mice (typically the novel object recognition task) have only provided strong evidence of impaired recognition memory in older mice of 9-months-old or older (Donkin et al., 2010; Jardanhazi-Kurutz et al., 2010; Li et al., 2014; Yan et al., 2013; Yoshiike et al., 2008). At younger ages between 6 and 7-months-old, deficits in these tasks become unreliable (Barbero-Camps et al., 2013; Cheng et al., 2014; Jardanhazi-Kurutz et al., 2010; Pedrós et al., 2014; Shen et al., 2017).

For example, Barbero-Camps et al. (2013) presented 7-month-old APP/PS1 mice, and their wild type littermates, with two copies of an object and allowed them to explore the objects for 10-minutes. Following a 1-hour retention interval (interval between sample and test), mice were presented with a copy of the original object

and a novel object. Both APP/PS1 and wild type control mice explored the novel object significantly more than the familiar object. Similarly, Jardanhazi-Kurutz et al. (2010) presented 6.5-month-old APP/PS1 mice, and their wild type littermates, with two copies of an object and allowed them to explore the objects for 15-minutes. Following a 1-hour retention interval, mice were presented with a copy of the original object and a novel object. Again, APP/PS1 and wild type control mice performed equally well and explored the novel object more than the familiar one. Both tasks indicated that there was no detectable deficit in the APP/PS1 mice, during this task at this age. Shen et al. (2017) used 7-month-old APP/PS1 mice but instead of wild type littermates they used C57BL6/J mice as their control. They presented mice with two identical objects and allowed them to explore the objects for 10 minutes. After a 1-hour or a 24-hour retention interval, mice were tested for their exploration of a copy of the original object and a novel object of similar size. The authors reported that the control mice directed significantly more exploration towards the novel object than the familiar object compared with the APP/PS1 mice, at the 1-hour but not the 24-hour retention interval. Therefore, this study indicated that the APP/PS1 mice may have had a slight impairment in performance during this task at this age. Cheng et al. (2014) tested 6-month-old APP/PS1 mice, and their wild type littermates, also using the novel object task with a 10-minute sample phase and a 1-hour retention interval. The authors reported a significant difference in test performance between the APP/PS1 and control mice. The wild type mice directed more exploration towards the novel than the familiar object whereas the APP/PS1 mice explored both objects almost equally, indicating a deficit in the APP/PS1 mice during this task at this age. Finally, Pedrós et al. (2014) tested 6-month-old APP/PS1 mice, and used C57BL6/J mice as control mice, during a novel object recognition task in a Y-maze setup. They presented mice with two copies of an object, one presented in each arm of the maze, during a 10-minute sample phase. Following a 2-hour retention interval, mice were tested with a copy of the original object and a novel object, one placed in each arm of the maze. The APP/PS1 mice failed to discriminate between the novel and familiar objects whereas the control mice did not, indicating an impairment in the APP/PS1

mice during this task at this age. In summary, deficits in APP/PS1 mice during SOR tasks are not reliably detectable at 6-7 months old.

At younger ages of 4-5 months-old, no evidence of deficits have been reported in APP/PS1 mice during SOR tasks (Bonardi et al., 2011), with the exception of the two studies previously discussed which reported no deficits, at 5-months-old, in novel object and relative recency tasks but did report a selective deficit in object-in-place tasks (Bonardi et al., 2016, 2021). Hence, these mice appear to be impaired at this age in association-based object memory tasks (e.g., Aggleton & Nelson, 2020) but not in more recency-based tasks (e.g., relative recency and novel object; Sanderson et al., 2011). SOP suggests that this may indicate that these mice, at this age, have a selective deficit in retrieval-generated priming which impairs performance during association-based tasks, but that self-generated priming remains intact, thus is still able to support performance during recency-based tasks (Bonardi et al., 2016, 2021; Wagner, 1981). Therefore, these mice at 5-months-old serve as a useful tool to further explore predictions of SOP related to object recognition memory based on its theoretical priming processes.

1.8.9 Transgenic mouse model of preclinical Alzheimer's disease

The results of the aforementioned studies (Bonardi et al., 2016, 2021) suggest that recognition memory deficits are present in 4-5 month-old APP/PS1 mice. However, these impairments are subtle and not easily detectable using the novel object recognition test, which is generally employed to test recognition memory in these mice (Barbero-Camps et al., 2013; Gong et al., 2020; Jardanhazi-Kurutz et al., 2010; Kelly et al., 2017; Mao et al., 2016; Pedrós et al., 2014; Shen et al., 2017; Yan et al., 2013; Yoshiike et al., 2008). Although Aβ pathology has commenced by this age, other cognitive and recognition memory deficits are not yet generally observed (Garcia-Alloza et al., 2006; Hong et al., 2016; Kilgore et al., 2010; Pedrós et al., 2014; Ruan et al., 2009; Zhu et al., 2017). By 6 months old, Aβ pathology has significantly increased and is easily detectable but deficits in recognition memory are still not reliably evident (Gao et al., 2015; Garcia-Alloza et al., 2006; Izco et al., 2014; Kilgore et al., 2010; Mei et al., 2020; Pedrós

et al., 2014; Ruan et al., 2009; Sierksma et al., 2013; van Groen et al., 2006; Zhu et al., 2017).

Therefore, these data suggest that APP/PS1 mice at 4-5 months-old may be a suitable model for preclinical AD as they parallel many aspects of this stage of the disease in humans. For example, in humans the preclinical stage can be defined by subtle memory loss, some pathological changes in the brain but no obvious cognitive impairments (Breijyeh & Karaman, 2020; Sperling et al., 2011a). The aforementioned 4-5 month-old APP/PS1 mice (Bonardi et al., 2016, 2021) mirrored this characterisation well as they exhibited subtle memory loss that was not detectable using the novel object recognition test, therefore it was not an obvious impairment, and some Aβ related pathological changes in the brain had likely occurred (Bonardi et al., 2016, 2021). Thus, this mouse model could potentially be used to identify a novel cognitive marker for preclinical AD which could then be used to inform future research which could lead to the development of simplistic early-stage AD diagnostic tests for human participants.

1.9 Aims and experimental chapters

1.9.1 Primary aim

The primary aim of this thesis was to test further, fundamental predictions of SOP to further evaluate its suitability as a theoretical framework for studying recognition memory. We initially used SOP to generate predictions for possible object recognition memory effects (e.g., cue competition, indirect object recognition, or distractor effects) and then used healthy young mice (3- and 5-month-old male C57BL/6J mice) and SOR task variants to test these hypotheses.

1.9.2 Secondary aim

The secondary aim of this thesis was to test specific predictions of how 5-month-old APP/PS1 mice should perform during tasks in which we observed the predicted object recognition effects in C57BL/6J mice. For example, if blocking occurred during an association-based object recognition task using C57BL/6J

mice, which SOP would predict because retrieval-generated priming should be subject to blocking effects, then we would predict that blocking would not occur in the APP/PS1 mice because, according to this hypothesis, they should be impaired in retrieval-generated priming. As discussed above, it is also possible that they may not be able to complete the task in the first place independent of a blocking condition.

1.9.3 Summary of experimental chapters

Our preliminary set of experiments (Chapter 3) explored whether blocking effects could be observed with respect to object recognition memory. A fundamental feature of SOP is how it explains association-based learning and memory which it asserts occurs through retrieval-generated priming (Brandon et al., 2003; Mazur & Wagner, 1982; Wagner, 1981). Because this priming process relies on association formation it should be susceptible to cue competition effects such as blocking (Brandon et al., 2003; Wagner, 1981). Initially, a series of pilot studies were undertaken to test whether mice could discriminate between various objects (structures constructed of plastic building blocks) that would be suitable for use in a proposed blocking design. A series of object recognition memory tasks based on various blocking designs was then completed.

The next set of experiments (Chapter 4) examined indirect object recognition in relation to object recognition memory using experimental designs based on the object-in-context task used by Whitt et al. (2012). Similarly, to blocking, indirect object recognition is consistent with SOP, which suggests that this effect may reflect that presentation of a stimulus (e.g., X), which has a prior association with an object (e.g., PX), can associatively activate the memory of the object (e.g., P) via retrieval-generated priming. In other words, exposure to X prior to a test with objects P and Q will reduce exploration of object P relative to Q because the memory of P has already been associatively activated by X. A sequence of object recognition memory tasks based on Whitt et al. (2012) were undertaken. These included replications of the aforementioned study using 3- and 5-month-old C57BL/6J mice and 5-month-old APP/PS1 mice and their wild type littermates, as

well as variants of the task which included the potential for blocking to occur within the experimental design.

The final set of experiments (Chapter 5) investigated possible distractor effects corresponding to object recognition memory that would be predicted by SOP. These were based on a standard relative recency task (e.g., Bonardi et al., 2016; Tam et al., 2014) with the inclusion of additional distractor objects that were presented either before the target objects, between them during the sample-sample interval, or both. SOP suggests that performance in recency-based recognition memory tasks may reflect that in the test with both objects, the less recent object (A) has had more time for its elements to decay (from A2 to inactive) than the more recent object (B), so that object A is explored more at test. It also proposes that this effect should be enhanced by the inclusion of an intervening distractor stimulus (e.g., C) during the sample-sample delay (e.g., A-interval-C-interval-B). This is because the additional stimulus elements would create competition for memory space in A1 and A2, particularly A1 due to its smaller capacity, and consequently cause rapid decay of existing elements already residing in these activation states (Kaye, Swietalski, & Mackintosh, 1988a; Mazur & Wagner, 1982). That is exposure to object C, during the sample-sample interval, would increase the decay of elements from object A already residing in A1 and A2. Consequently, during the test, object A would have relatively more elements available to enter A1 and elicit stronger responding than it would have had if object C had not been experienced during the sample-sample interval. This sequence of experiments used 3- and 5-month-old C57BL/6J mice as well as 5-month-old APP/PS1 mice and their wild type littermates.

Chapter 2: General methods

2.1 Materials and set-up

2.1.1 Subjects

A sample size of approximately 16 animals per experimental group was initially used. This was based on statistical guidelines for authors submitting to Springer journals (e.g., Learning & Behavior, 2023) and on related previously published work (Bonardi et al., 2016). Initially, an *a priori* power analysis was performed in G*power (not a post hoc power analysis based on prior data), using the *F* tests ANOVA repeated measures, within-between interaction function (Faul, Erdfelder, Lang, & Buchner, 2007) to calculate an estimated sample size for testing our hypotheses. For the power calculation, α was set to 0.05 and power was set at 0.8 to detect a moderate effect size of Cohen's $f = 0.48$, which has been suggested as an appropriate effect size for psychological research (Brysbaert, 2019). The estimated sample size from the calculation was 12. In addition to the power analysis, we considered data from a previous experiment which demonstrated successful object discrimination during a novel object recognition task using a sample size of 15 animals, with an ANOVA test of $p = 0.004$ and an effect size of $\eta_p^2 = 0.48$ (Bonardi et al., 2016). We rounded up our initial sample size to 16 to allow for equal counterbalancing across subjects.

These animals were either experimentally naive male C57BL/6J mice (purchased from Charles River, UK) or experimentally naïve male and female APPswe/PS1dE9 transgenic mice. The transgenic mice were an Alzheimer's disease model based on the genetic background of C57BL/6J mice (For more information see: The Jackson Laboratory, 2023) that were bred in the University of Nottingham's transgenic animal unit from breeding stock obtained from The Jackson laboratory. The mice used were approximately either 3 months-old or 5-months-old at the start of object recognition memory tests. They were housed in a room with controlled temperature (21 ± 1.5ºC), relative humidity (50 ± 8%) and air exchange and were maintained on a 12-hour light/dark cycle (07.00-19.00

hours). Mice were housed in groups of 2-5 in individually ventilated cages (Tecniplast; dimensions (W x D x H) 391 x 199 x 160 mm), which contained substrate, nesting material and enrichment. They had *ab libitum* access to food and water.

2.1.2 Experimental set-up

All experiments were conducted in a quiet, well-lit room that was maintained at a temperature of approximately 21°C. The experimental arenas were two white, translucent plastic containers with walls and floors that measured (length x width x height) 60 x 40 x 45cm. Each arena had a wooden frame suspended above which housed two downward facing LED spotlights, placed 22cm apart, which generated an illumination of 50 lx at floor level. It also housed a downward facing camera that was positioned 90cm directly above the middle of the area which enabled the entire floor and the lower parts of each wall to be viewed. The cameras were connected to a laptop computer, which was situated on a nearby desk, and which ran Anymaze^{TM} (Version 4.5; Stoelting, Wood Dale, Illinois) tracking software. This set-up tracked the trajectory of the mouse's heads while they were in the arenas and allowed several metrics to be recorded. Superimposed square zones with sides of approximately 9cm in length were created as regions of interest (Figure 3), in which objects were centrally placed and interactions with the objects were recorded.

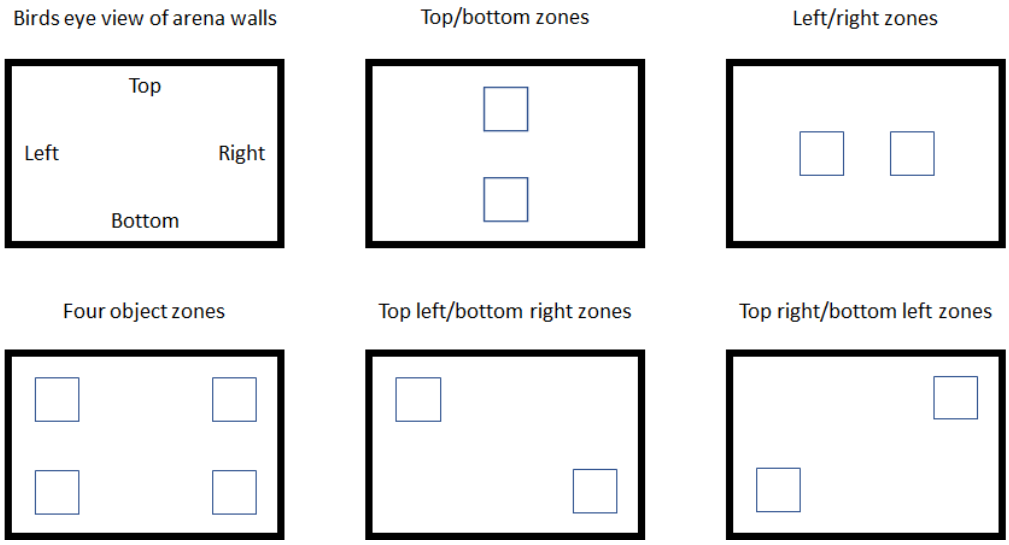


Figure 3. Schematic representation of the experimental arenas used and the configurations of superimposed zones in which objects were placed.

For each task, mice were placed into a cardboard tube within their home cage, which was a home cage tunnel as recommended by the NC3Rs (Gouveia & Hurst, 2013, 2017), and then the tube with the mouse inside was placed into the center of the arena, orientated so that the mouse's head was facing the top wall of the arena (Figure 3). When the task had finished, mice were encouraged to climb into a large plastic 500ml container and returned to their home cage. The arenas were cleaned with alcohol prior to each time that a mouse was placed into them.

2.1.3 Objects

The objects used were a collection of 10 different junk objects, four duplicates of each (total: 40 objects). These ranged in size from 3-8cm wide and 3-13.5cm high and were used for all experiments unless otherwise stated (Figure 4).

Figure 4. Objects used during object recognition memory tasks. Dimensions from left to right, top to bottom (height x width): Egg cup/Christmas bauble: 9 x 5cm, Lego: 3 x 6cm, Plug: 4.5 x 4.5cm, Salt: 8 x 4cm, Football: 7 x 7cm, Silver mill: 8 x 4cm, Star/doorknob: 7 x 8cm, Jar: 5.5 x 5cm, Tabasco: 13.5 x 3cm, Deodorant: 10 x 5cm.

2.1.4 Arena inserts

For some tasks arena inserts were used to enhance the contextual features of the arena. These were constructed of medium density fiberboard which was covered with linoleum that was one of two distinctive patterns (Figure 5). Each insert was 45cm high and covered the right or left wall and half of the top and bottom walls (Figure 3) so that two inserts covered the entire arena. This allowed for all arena walls to be covered by a single pattern or for half of the arena walls to be covered by one pattern and the other half by a different pattern. With the inserts in place the arena floor space was reduced to 42cm x 32cm (length x width).

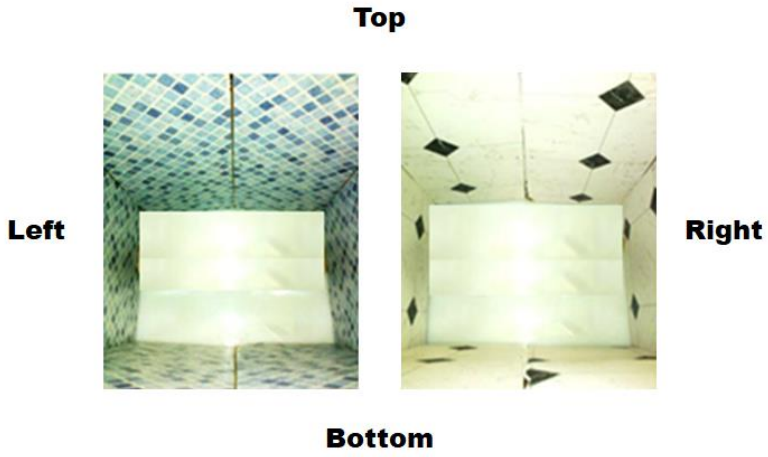


Figure 5. Arena inserts that were used during specific sample and test phases to enhance the contextual features of the arena.

2.1.5 Anymaze compared with manual scoring

Traditionally, animal behaviour has been recorded using manual human scoring (Noldus, Spink, & Tegelenbosch, 2001) which has been subject to many criticisms. These include being labour intensive, time consuming and potentially producing imprecise results due to fatigue, variability introduced by inter-rater inconsistencies, and through the subjective nature of the task (Antunes, Goes, Vígaro, & Teixeira-Silva, 2011; González-Gaspar et al., 2021; Peters, Pothuizen, & Spruijt, 2015). Thus, an automated system can be advantageous as it vastly reduces both the time spent and number of researchers required, it removes scoring variability through standardised tracking parameters, and it can precisely measure metrics such as distance which cannot be accurately estimated by human observers (Noldus et al., 2001). However, automated systems such as Anymaze need to be validated prior to their use to assess their reliability, and this has been previously done via comparison with manual scoring. For example, a study which used a free-exploratory paradigm in rats reported a significant correlation ($p < 0.001$) between three sets of data that were obtained from Anymaze and two human observers (Antunes et al., 2011). Also, a direct comparison of data collected for five different parameters using Anymaze, manual video analysis by researchers, and an

alternative video processing software (Analixity), was completed during an elevated plus maze test in rats and mice (González-Gaspar et al., 2021). The authors reported no statistically significant differences between the three methods used. These data suggest that Anymaze is a suitable, reliable method for collecting behavioural rodent data.

2.2 General procedure

2.2.1 Habituation

All mice were habituated to the experimental set-up prior to undertaking object recognition memory tasks. Each mouse was allowed to move freely around the arena for 10 minutes each day for 6 consecutive days, a procedure which has been recommended for mice (Vogel-Ciernia & Wood, 2014). The distance travelled by the mice during each session was tracked and a reduction in locomotion over time was taken as evidence of habituation to the experimental set-up (Leussis & Bolivar, 2006). Habituation data were not included in the results sections of this report. However, these data are reported in Appendix A.

2.2.2 SOR tasks

Following habituation, mice completed a novel object recognition task to give them some experience of having objects present in the arenas and to ensure that they were directing more exploration towards novel objects than familiar objects. The data from this pretraining task for each batch of mice are reported in Appendix B. For this task, mice were first exposed to two copies of object A for 10 minutes during a sample phase, followed by an interval, and then exposed to a copy of object A and a novel object B for 5 minutes, during a test phase. The interval between the sample and test phases was 2 hours for C57BL/6J mice and 24 hours for transgenic mice. The extended interval for transgenic mice was used so that the data obtained would be directly comparable with previous data from our lab using this task and strain of mice (Bonardi et al., 2016, 2021).

The basic procedure for all variants of the SOR task were identical. Each task involved at least one sample phase, as well as a subsequent test phase. Objects were placed in the arenas, centrally located within superimposed zones in various configurations (Figure 3) depending on the individual task. The objects were secured to the arena floor using Blu Tack®. Mice were always allowed to freely explore the arena and objects for the entirety of all sessions for all tasks. Object identities and locations were counterbalanced across subjects and where applicable, task order, arena inserts, wall inserts, and floor inserts were also counterbalanced across subjects.

2.2.3 Data handling

Raw exploration times were used for analyses. These were defined as the duration of time that the animals' heads were within square superimposed zones that the objects of interest were placed centrally within these data were recorded and generated automatically by Anymaze^{TM} tracking software. The total amount of time spent in each zone in each phase for each mouse (exploration time) was computed in 1-minute time bins. Data were analysed using Student's t-tests, one-sample t-tests, ANOVAs, and mixed ANOVAs. Significant interactions from mixed ANOVAs were explored using simple main effects analyses, the pooled error term for between-subject comparisons was used for interactions involving a between-subjects factor. For ANOVAs post hoc pairwise comparisons using the Holm correction were used for further analyses of data where applicable. During all statistical analyses, whenever Mauchly's test of sphericity was significant, results of the Greenhouse-Geisser correction were reported. The specific tests and test parameters used for each experiment have been reported within the corresponding methods section for each experiment.

Data from the pretraining SOR tasks are reported in Appendix B. These data were reported for all five minutes of test, expressed as mean exploration time of the novel and familiar objects for each minute of test ± standard error of the mean (SEM).

During Chapters 3 and 4, only data from the first three minutes of test were analysed as this is generally the method used for these types of recognition memory tasks in both mice (Bonardi et al., 2016, 2021; Spanswick & Dyck, 2012) and rats (Asiminas et al., 2019; Langston & Wood, 2010; Tam et al., 2014), because object recognition memory effects during such tasks are often transient. These data were reported as the mean percentage of time spent exploring the different experimental classes of objects ± standard error of the mean (SEM).

To measure discrimination during the recency-based distractor tasks used throughout Chapter 5, discrimination index (DI) was calculated and analysed, as this type of independent variable is generally used for similar tasks such as temporal order memory tasks (e.g., G. Barker, Evuarherhe, & Warburton, 2019; Hatakeyama et al., 2018). This was calculated as previously reported in Hatakeyama et al. (2018) from the total exploration of object A and B during the first 2 minutes of test using the formula:

$$\frac{(A-B)}{(A+B)}$$

Data for these tasks were reported as mean discrimination index (DI) ± standard error of the mean (SEM).

For all experimental tests, mice that spent less than 5 seconds exploring objects during any test phase were excluded from those analyses. This method has been recommended and previously applied to object recognition memory tasks using both rats and mice (Vogel-Ciernia & Wood, 2014). Where the total exploration of the objects by the mice were more than two standard deviations away from the mean, during the minutes of the test that would be analysed, then these mice were excluded from the analysis. Where animals were excluded, these were reported during the results sections of the relevant experiments.

Data for all tests are also reported in Appendix C. These data were reported for all five minutes of test, expressed as mean exploration time of each experimental class of object for each minute of test ± SEM.

Chapter 3: Pilot work and initial blocking tasks

3.1 General introduction

In an appetitive conditioning procedure, a tone is often paired with a food outcome. After many pairings of the tone and the food, presentation of the tone results in a conditioned response, typically head entry into the food magazine. SOP explains this learning procedure by assuming that excitatory associations can form between stimuli if elements of those stimuli are concurrently activated into the A1 state (Mazur & Wagner, 1982; Wagner, 1981), such as during the tone and food pairings. Once an association has been formed between two stimuli, future exposure to one of those stimuli activates the other stimulus' elements into the A2 state, referred to as retrieval-generated priming. This associative priming mechanism allows SOP to explain many learning occurrences such as long-term habituation (Sanderson & Bannerman, 2011; Uribe-Bahamonde et al., 2019), instances of contextual learning (Vogel et al., 2020), and cue competition effects such as blocking (Brandon et al., 2003; Vogel et al., 2019).

The associative priming processes described by SOP may also be applicable to the objects used during spontaneous object recognition tasks with rodents, if it is assumed that exploration is the unconditioned response to an object (Robinson & Bonardi, 2015; Tam et al., 2014). Furthermore, it has been argued that SOP can accommodate much of what is known about rodents' object recognition because of its unique feature - that it is a well-established theory of learning that also incorporates an explanation of memory (Robinson & Bonardi, 2015). However, further evidence is required to evaluate its suitability as a model of memory and to support its account of object recognition. Therefore, this experimental chapter explored the possibility that cue competition effects, such as blocking, may occur during object recognition memory tasks. This was based on the SOP prediction that retrieval-generated priming should be susceptible to such effects because retrieval-generated priming uses associative context-dependent information (Brandon et al., 2003; Mazur & Wagner, 1982; Vogel et al., 2020; Wagner, 1981).

Cue competition effects are observed in Pavlovian conditioning when stimuli "compete" for associative strength. That is, multiple cues trained together with an outcome (e.g., food) interact in their control over behaviour, such that the individual cues do not control later behaviour to the same extent (Richards & Krauter, 1999; Urushihara & Miller, 2009). This occurs on trials in which more than one stimulus is paired with the same unconditioned stimulus (US) (e.g., Angulo et al., 2020; Kamin, 1969; Mackintosh, 1975; J. M. Pearce et al., 2006). For example, when two cues are trained together in compound with an outcome (e.g., AX→food), one of the cues typically produces a weaker response (e.g., X) compared to the other cue (e.g., A, usually more salient than X); this is referred to as overshadowing (Urcelay & Miller, 2009; Urushihara & Miller, 2009).

Another well-studied cue competition effect is blocking which has been demonstrated across a range of species (e.g., honeybees, Blaser, Couvillon, & Bitterman, 2004; rats, Jennings & Bonardi, 2017; and humans, Nixon, 2020; snails, Prados et al., 2013; mice, Sanderson, Jones, & Austen, 2016). This effect is generally interpreted using associative models of learning (e.g., J. M. Pearce & Hall, 1980; Rescorla & Wagner, 1972) but can also be explained using alternative models (e.g., probabilistic contrast models, Houwer, Beckers, & Glautier, 2002). Blocking usually occurs when a single stimulus is paired with an unconditioned stimulus (US) during initial trials (e.g., a tone paired with a foot shock; A+). In subsequent trials, the same stimulus is presented in compound with a second stimulus and paired with the same US (e.g., a tone + a light paired with a foot shock; AB+). During subsequent tests with B (e.g., a light), in the absence of the US (e.g., a foot shock), a conditioned response (CR) (e.g., freezing behaviour) is usually very weak relative to controls (e.g., a group that had the same amount of AB+ training but no prior A+ trials). In other words, the number of pairings is not the only crucial element, as when these are equated, there are still observed differences in learning. This is because an unexpected or surprising outcome (US) and contiguity (spatial and temporal closeness) between the cue and the outcome (CS→US), are both required for learning to occur (Balsam, Drew, & Gallistel, 2010; Holland & Schiffino, 2016). This can be explained by various stimulus

processing models of learning (Mackintosh, 1975; J. M. Pearce & Hall, 1980; Rescorla & Wagner, 1972; Wagner, 1981).

Using the above examples and the exemplification of the more general Rescorla-Wagner principle, that the US will not support learning if it is predicted or expected (Rescorla & Wagner, 1972), the associative account for blocking is as follows. During initial trials (A+), the tone forms a strong association with the foot shock, thus the tone now predicts the foot shock. During compound trials (AB+) because the tone is now predictive of the foot shock, the light consequently acquires less associative strength with the foot shock - learning about the light has been "blocked".

Continuing with the same examples, SOP explains blocking by suggesting that during initial training (A+), an association forms between the tone and the foot shock as both stimuli simultaneously have elements in the primary activation state, A1. During the second phase of training (AB+), elements of the foot shock move directly into the secondary activation state, A2, via retrieval-generated priming through their prior association with the tone. Therefore, there are fewer elements of the foot shock available to enter A1 and form an association with elements from the light, which now occupy A1. This results in less associative strength forming between the light and the foot shock relative to the associative strength that developed between the tone and the foot shock during initial training (A+). Therefore, during subsequent exposure to the light, in the absence of the foot shock, very few elements of the foot shock are activated directly into A2 relative to a scenario where the animals had the same amount of exposure to the tone and light paired with the foot shock (AB+) but with no prior exposure to just the tone and foot shock (A+).

To examine SOP's account of object recognition, in which association formation through retrieval-generated priming is a key aspect, we initially aimed to test whether blocking effects occurred during object recognition memory tasks with mice, as SOP would predict. To investigate this, we designed a proposed

experiment that will use a spontaneous object recognition task arranged in a format that could identify a potential blocking effect (Figure 6).

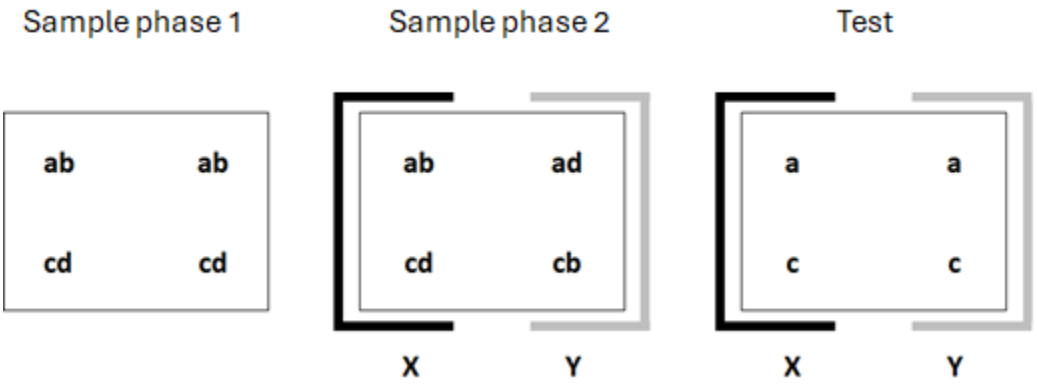


Figure 6. Proposed experimental design that tested whether blocking occurred during a SOR task. Mice were exposed to two compound objects (two copies of ab and two copies of cd) that were composed of separate individual elements (a, b, c, and d) in sample phase 1. In sample phase 2, they were exposed to two compound objects (ab and cd) presented in context X and two novel compound objects (rearranged elements ad and cb) in context Y. During test, two individual elements from the compound objects (two copies of a and two copies of c) were presented in each context, X and Y.

A SOP interpretation of this task is that during the initial phase, associations will form between the elements of each compound object (b→a, d→c). Therefore, b will now predict a and d will now predict c. Consequently, during the second phase, a and c will be retrieved in X, by their prior association with b and d respectively, therefore they will not form associations with X, as X will not be able to associatively predict them. However, the rearranged objects (ad and cb) used during the second phase, will not have prior associations between their elements. Thus, a and c will be surprising and will not be retrieved in Y, and so associations between these elements and context Y will form normally (Y→a and Y→c). Thus, during test, the elements a and c will be explored more in context X than in context Y. This is because a and c will have elements move directly into A2 in

context Y, via retrieval-generated priming through their prior association with Y. This will elicit a relatively weaker response compared with a and c in context X, as these will have all of their elements available to enter A1 and produce a relatively stronger response.

We predicted that blocking would occur during the proposed task based on the assumption of SOP and retrieval-generated priming (Brandon et al., 2003; Mazur & Wagner, 1982; Vogel et al., 2020; Wagner, 1981), on previous studies in mice which has demonstrated blocking effects during both appetitive and fear conditioning (Bonardi, Bartle, Bowles, de Pulford, & Jennings, 2010; But also see: Maes et al., 2016; Sanderson et al., 2016; Yamada, 2010), and on other work that has shown that mice readily associate objects with contexts during spontaneous object recognition memory tasks (Spanswick & Dyck, 2012).

Before we tested the proposed experimental design (Figure 6), we wanted to first confirm that mice could discriminate between the compound objects used (ab, cd, ac, and bd) because the logic of the experimental design depended on them being able to do so.

3.2 Experiment 1

3.2.1 Introduction

Experiment 1 was a pilot study to test whether mice could discriminate between objects that could be used for the proposed object recognition blocking task (Figure 6). We chose to construct the objects using coloured plastic building blocks as mice have generally been shown to be able to discriminate between objects made with these (e.g., Bettis & Jacobs, 2012; Guimarães et al., 2016; Rosa et al., 2003; Simeonovska-Nikolova, 2016; Simeonovska-Nikolova, Avramska, & Georgiev, 2016; Yang et al., 2017). Each compound object (e.g., ab) was composed of two separate elements that could be presented individually (e.g., a and b). Because the design of the proposed experiment was contingent on the individual elements, within each compound object, forming associations with one another, it was essential that mice could recognise and discriminate between these

elements and between the different compound objects used. Therefore, during Experiment 1, mice completed two tasks where they were exposed to two copies of a compound object, during a sample phase, and then tested with either a novel and familiar compound object (Task 1) or a novel and familiar element (Task 2; Table 1).

We predicted that mice would be able to discriminate between the novel and familiar compound objects and individual elemental items. This was because C57BL/6J mice have previously been shown to be able to discriminate between two different objects constructed from coloured plastic building blocks, during novel object recognition tasks (Bettis & Jacobs, 2012; Guimarães et al., 2016). These objects and tasks were similar to the ones used during Experiment 1.

Task	Sample phase	Test
1	AB	AB and CD
2	AB	A and C

Table 1. Experimental design for Experiment 1.

3.2.2 Materials and method

3.2.2.1 Subjects

The subjects were 16 male naïve C57BL/6J mice that were approximately 3 months old at the start of Experiment 1.

3.2.2.2 Objects

The objects used were constructed of coloured plastic building blocks and were pairs of structures that were various colours and ranged in height and width from 4.8-7.68cm and 3.2-4cm respectively (Figure 7). All pairs of structures were secured to identical rectangular (8x7.2cm) grey plastic bases.

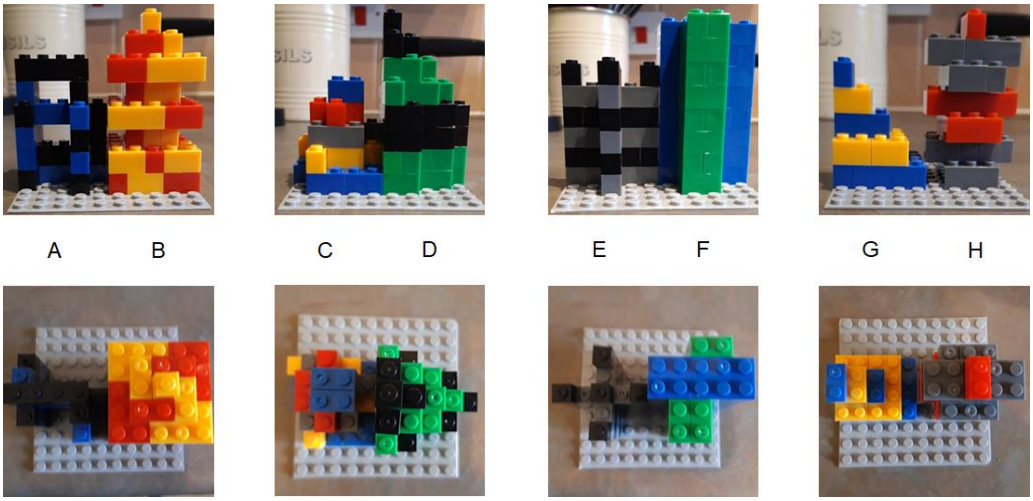


Figure 7. Objects used for Experiment 1. The top panel shows side view, and the bottom panel shows birds-eye view for each of the four compound objects which each consisted of two individual elements.

3.2.2.3 Procedure

This procedure consisted of two tasks that were each composed of a 10-minute sample phase and a 5-minute test, separated by a 24-hour interval (Table 1). The objects used were four compound objects, AB, CD, EF and GH, which could each be separated into their individual elements, A and B, C and D, E and F, and G and H (Figure 7). During an initial sample phase, mice were exposed to two copies of a compound object and were then tested with either one familiar and one novel compound object (Task 1) or a familiar and a novel element (Task 2). During a second sample phase, mice were exposed to two copies of a different compound object to what they had experienced during the initial sample phase. This was followed by a second test. If mice had received compound objects for their initial test, then they received elements for their second test and vice versa. Bottom left/top right zones were used for the initial sample phase and test and top left/bottom right zones were used for the second sample phase and test. Object identities, novel and familiar locations, and task order were counterbalanced across subjects (Table 2).

	Sample 1		Test 1		Sample 2		Test 2	
Animal	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**
1-2	AB	AB	AB	**CD**	EF	EF	E	**G**
3-4	AB	AB	A	**C**	EF	EF	EF	**GH**
5-6	CD	CD	**AB**	CD	GH	GH	**F**	H
7-8	CD	CD	**B**	D	GH	GH	**EF**	GH
9-10	EF	EF	EF	**GH**	AB	AB	A	**C**
11-12	EF	EF	E	**G**	AB	AB	AB	**CD**
13-14	GH	GH	**EF**	GH	CD	CD	**B**	D
15-16	GH	GH	**F**	H	CD	CD	**AB**	CD

Table 2. Counterbalancing of object identities, novel and familiar locations, and task order for Experiment 1. Characters in bold font depict novel objects.

3.2.3 Results and Discussion

During the sample phases of Experiment 1, mice explored the compound objects for similar amounts of time (Table 3). Mean total exploration time (s) of objects during both sample phases were analysed using an ANOVA with sample phase (sample phase 1/sample phase 2) and object location (to be novel/to be familiar) as within-subject factors. There was no main effect of sample phase ($F(1, 15) = .020$, $p = .890$, $MSe = 2.976$, $\eta_p^2 = .001$) or object location ($F(1, 15) = .923$, $p = .352$, $MSe = 143.401$, $\eta_p^2 = .058$) and no interaction between them ($F(1, 15) = 3.048$, $p = .101$, $MSe = 918.090$, $\eta_p^2 = .169$).

Sample phase 1		Sample phase 2	
Novel	Familiar	Novel	Familiar
64.7 ± 22.2	54.1 ± 14.3	56.7 ± 21.0	61.3 ± 15.7

Table 3. Mean (±SD) total exploration time (s) of objects and their locations during both sample phases for Experiment 1. Locations were categorised by whether they would have the novel or familiar object placed there during test.

During test, mice explored the novel and familiar compound and elemental objects for similar amounts of time (Figure 8). Mean percentage of time spent in zones for each object type, for the first three minutes of test, were analysed using an ANOVA with construction (compound/element) and object (novel/familiar) as within-subject factors. There was no main effect of construction ($F(1, 15) = .010$, $p = .921$, $MSe = .090$, $\eta_p^2 = 6.785$) or object ($F(1, 15) = .199$, $p = .662$, $MSe = 1.983$, $\eta_p^2 = .013$) and no interaction between them ($F(1, 15) = .023$, $p = .882$, $MSe = .085$, $\eta_p^2 = .002$).

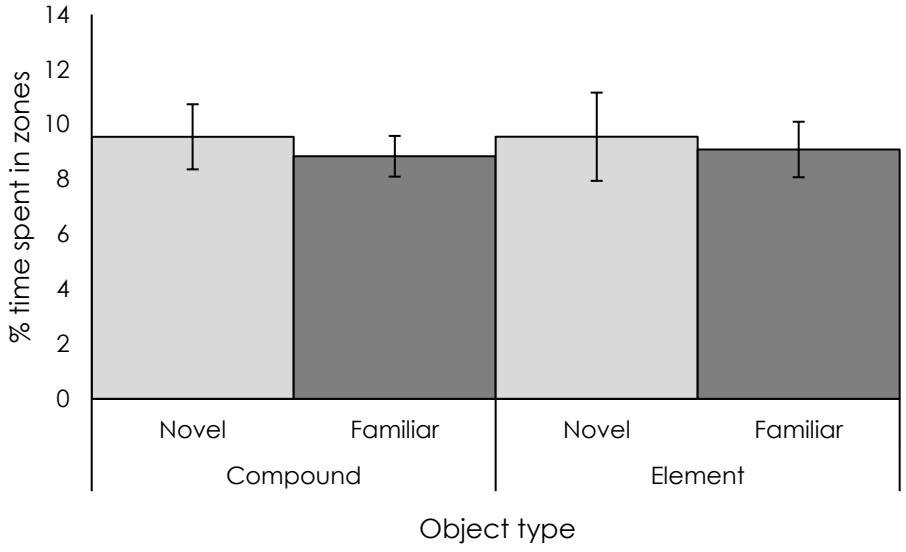


Figure 8. Mean percentage of time (±SEM) spent in each object zone in the first three minutes of test, during Experiment 1. Objects were a novel and a familiar compound object and a novel and familiar element item.

Mice failed to discriminate between novel and familiar compound objects and between novel and familiar elemental objects. This could have been because the objects used were too similar in structure and the mice may have not been able to discriminate between them, or that the objects were composed of colours that the mice may have not been able to differentiate between, or a combination of both. This outcome was surprising as mice have been shown to discriminate between objects constructed from coloured plastic building blocks similar to ones used during Experiment 1 (Bettis & Jacobs, 2012). However, when the objects were constructed to be more similar to one another, male mice were unable to discriminate between them (Bettis & Jacobs, 2012). Additionally, mice have been shown to be capable of learning complex visual discriminations, including complex photographic stimuli (Bartko et al., 2011), when presented as two-dimensional stimuli on a video display unit (Bussey, Saksida, & Rothblat, 2001), computer monitor (Brigman, Bussey, Saksida, & Rothblat, 2005), or touchscreen monitor (Bartko et al., 2011; Horner et al., 2013). However, these mice received reinforcement training over many trials and the stimuli used for these discriminations were white patterns on black backgrounds. In contrast, spontaneous object recognition tasks do not use training and reinforcement and the objects used for Experiment 1 were composed of colours. However, during spontaneous object recognition tasks, rodents have been shown to be able to discriminate between objects constructed using coloured building blocks, although these are generally pairs of objects that are different structures (e.g., a square versus a triangle, Guimarães et al., 2016) and are usually different solid colours (Arriola, Angulo, & Alonso, 2017; Guimarães et al., 2016; M. E. Hopkins & Bucci, 2010) such as yellow versus blue (Arriola et al., 2017) or green versus purple (M. E. Hopkins & Bucci, 2010). Where mixed colours have been used within each object, the object pairs needed to be very different from one another for animals to be able to discriminate between them (Bettis & Jacobs, 2012).

Taken together this suggests that the stimuli used for Experiment 1 may not have been appropriate because they were either too similar in structure to one another, or because of the colours they were composed of, or a combination of both.

3.3 Experiment 2

3.3.1 Introduction

Because mice did not discriminate between objects during Experiment 1, modified versions of those objects were used for this experiment to attempt to increase discriminability. Objects constructed from plastic building blocks that differed in several features have been shown to be discriminable by rodents during spontaneous object discrimination tasks (e.g., Bettis & Jacobs, 2012; Guimarães et al., 2016; Rosa et al., 2003; Simeonovska-Nikolova, 2016; Simeonovska-Nikolova et al., 2016; Yang et al., 2017). For example, Bettis and Jacobs (2012) used a pair of objects that were different heights, colours, and shapes. One of the objects was a small construction that was black, white, and red in colour and consisted of a single tower attached to a thin base (one brick high). The other object was a large construction that was white, yellow, red, and blue in colour and consisted of two towers attached to a thicker base (three bricks high). Mice were able to discriminate between these objects. Angulo et al. (2017) used a yellow cube that measured (Length x Width x Height) 3.2 x 3.2 x 5cm and a blue pyramid measuring 6.4 x 6.4 x 5.2cm and reported that rats readily discriminated between these objects.

Thus, for Experiment 2, we constructed new objects that were structurally more unique from one another, than the previous objects used during Experiment 1, and that were painted solid black or solid white. Black and white were chosen as colour vision in mice is known to be poor. For example, mice have dichromatic vision and cannot discriminate between red and green (Huberman & Niell, 2011) thus colour is most likely not a feature that mice will attend to (Bettis & Jacobs, 2012). Furthermore, objects which humans perceive as differing mostly by colour, may be perceived as identical by mice (Ennaceur, 2010). Moreover, mice have been shown to readily discriminate between complex white patterns on black backgrounds (Bartko et al., 2011; Boehm et al., 1998; Brigman et al., 2005; Horner et al., 2013).

3.3.2 Materials and method

3.3.2.1 Subjects

The subjects were 16 naïve male C57BL/6J mice that were approximately 3 months old at the start of Experiment 2.

3.3.2.2 Objects

The objects were constructed of plastic building blocks and were painted solid black or solid white (Figure 9). They ranged in height and width from 4.8-9.6cm and 3.2-4.8cm respectively. All pairs of structures were secured to grey plastic bases that were equal in size to the paired objects footprints.

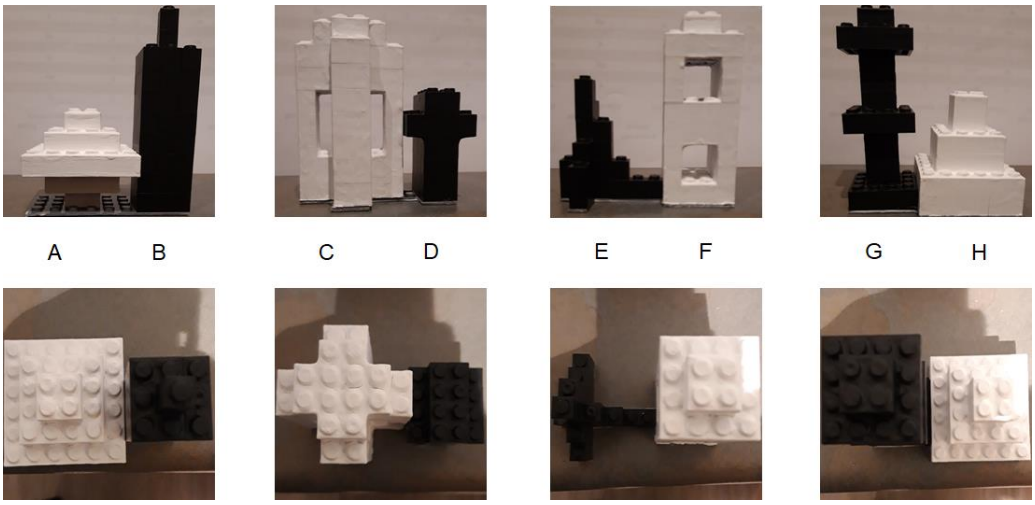


Figure 9. Objects used for Experiment 2. The top panel shows side view, and the bottom panel shows birds-eye view for each of the four compound objects which each consist of two individual elements.

3.3.2.3 Procedure

Experiment 2 was identical to the first stage of Experiment 1 (sample phase 1 and test 1) and included a 10-minute sample phase and a 5-minute test, separated by a 24-hour interval (Table 1). This was run as a between-subjects experiment. Mice were exposed to two copies of a compound object, these were AB, CD, EF and

GH, which could each be separated into their individual elements, A and B, C and D, E and F, and G and H (Figure 9). Half of the mice were tested with one familiar and one novel compound object, and the other half were tested with a familiar and a novel element (Table 1). Bottom left/top right zones were used for this task. Object identities and novel and familiar locations were counterbalanced across subjects identically to the first stage of Experiment 1 (Table 2, sample phase 1 and test 1).

3.3.3 Results and Discussion

The mice spent equal amounts of time exploring the objects during the sample phase of Experiment 2. Differences in mean total exploration times (s) between the two object locations (categorised as above) were explored using a Student's *t*-test which showed no difference between them (mean ±SD, novel: 68.4 ± 20.7, familiar: 64.6 ± 23.4; $t(15) = .548$, $p = .592$).

During test, mice did not explore the novel compound and elemental objects more than the familiar compound and elemental objects (Figure 10). The mean percentage of time spent in object zones, during the first three minutes of test, were analysed using an ANOVA with construction (compound/element) and object (novel/familiar) as within-subject factors. There was no main effect of construction ($F(1, 7) = .375$, $p = .559$, $MSe = 1.784$, $\eta_p^2 = .051$) or object ($F(1, 7) = .456$, $p = .521$, $MSe = 7.454$, $\eta_p^2 = .061$) and no interaction between them ($F(1, 7) = 4.656$, $p = .068$, $MSe = 41.760$, $\eta_p^2 = .399$). Because the interaction between construction and object approached significance, we pooled the data from the first test of Experiment 1 with the data from Experiment 2. These data were analysed using an ANOVA with construction (compound/element) and object (novel/familiar) as within-subject factors and Experiment (1/2) as a between-subjects factor. This revealed no effect of construction ($F(1, 14) = .228$, $p = .640$, $MSe = 4.197$, $\eta_p^2 = .016$), object ($F(1, 14) = 3.946$, $p = .998$, $MSe = 4.824$, $\eta_p^2 = 2.819$), or Experiment ($F(1, 14) = 1.420$, $p = .253$, $MSe = 21.071$, $\eta_p^2 = .092$) and no interactions between these three factors (smallest $p = .079$).

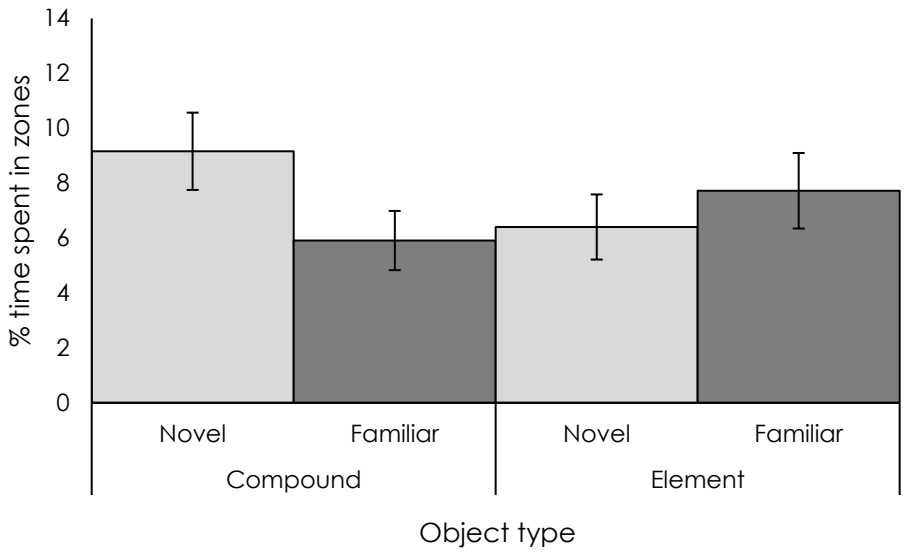


Figure 10. Mean percentage of time (±SEM) spent in the zones of the novel and of the familiar compound and element objects, in the first three minutes of test during Experiment 2.

During Experiment 2, mice did not discriminate between novel and familiar compound or elemental objects. This was surprising as mice have been shown previously to be able to discriminate between both solid black and solid white stimuli and between black and white complex visual patterns (Boehm et al., 1998; Hyde & Denenberg, 1999). It is possible that other task parameters such as sample and test phase durations may have influenced object discrimination during this task. However, the same mice readily discriminated between junk objects during the pretraining spontaneous object recognition task which used the same task parameters. Therefore, it is unlikely that these factors impacted the object discrimination between the compound and component stimuli used during Experiment 2. This would suggest that the issue is more likely to be that the building block stimuli used here were not appropriate for testing our proposed blocking design. It is possible that they were too complex for the mice to discriminate.

Where mice have been shown to discriminate between complex visual patterns (e.g., Bartko et al., 2011; Brigman et al., 2005; Horner et al., 2013), the

experiments required extensive training with reinforcement schedules. For example, Brigman et al. (2005) trained mice to discriminate between two white patterns on a grey background and reported that the mice required approximately 10 sessions, to reach a learning criterion of 80% correct choices across two successive sessions. Each session consisted of 20 differentially reinforced trials, which utilised correction trials where after an incorrect response was made, the same stimulus presentation was repeated until the mouse made the correct choice. Similarly, Bartko et al. (2011) trained mice to discriminate between two white patterns on a black background and reported an average of approximately 16 sessions, in which each session consisted of 30 differentially reinforced trials and a correction procedure, to reach their learning criterion. These tasks illustrate how difficult these types of complex stimuli can be for mice to discriminate between. Moreover, they suggest that during the spontaneous object recognition task used for Experiment 2, exposure to the objects, in the absence of training and reinforcement schedules, was insufficient for mice to acquire enough information about the objects to be able to perform the discrimination between novel and familiar during test.

3.4 Experiment 3

3.4.1 Introduction

SOP describes associative priming processes, in which retrieval-generated priming is a fundamental component, which may also occur during object recognition memory. Therefore, Experiment 3 explored the prediction that retrieval-generated priming should be susceptible to blocking effects, during object recognition memory tasks with mice, because this priming process employs associative context-dependent information (Brandon et al., 2003; Mazur & Wagner, 1982; Vogel et al., 2020; Wagner, 1981). Because the mice were unable to discriminate between the objects during Experiments 1 and 2, for Experiment 3, an alternative experimental design was employed. This utilised a combination of junk objects, which had previously been used during pretraining spontaneous object recognition

tasks (described in Chapter 2; data from these tasks are reported in Appendix B), that mice had successfully discriminated between. The experimental design contained two tasks, a blocking task and a control task, and used a within-subjects design (Table 3).

Task	Sample phase	Compound phase	Test
Blocking	A + p	A + p in X	A + B in X
Control	A + q	A + p in X	A + B in X

Table 3. Experimental design for Experiment 3. A and B represent junk objects, p and q depict rectangular patterned inserts that were placed near the objects, and X denotes distinct patterns that covered the entirety of the arena walls. Task order and identities of A, p, q and X were counterbalanced across subjects.

During the blocking task, mice were first exposed to two copies of junk object A with p (p→A), during a sample phase. Mice were then further exposed to these during a compound phase with the addition of X (pX→A). During the test that followed, mice were exposed to the familiar object A and a novel object B, both with X (XA and XB; Table 3). The control task was identical to the blocking task except that p was replaced by q during the sample phase (sample: q→A, compound: pX→A, Test: XA and XB; Table 3).

The rationale was based on SOP which suggests that during the sample phase, an association between p and A would form in the blocking condition (p→A) and an association between q and A in the control condition (q→A). During the compound phase, an association would not form between X and A in the blocking condition as p would already predict A (sample: p→A, compound: pX→A). Whereas it would form in the control condition as p would not be predictive of A (sample: q→A, compound: pX→A). Therefore, if blocking occurs during object recognition memory tasks as SOP predicts, then during the test we would expect a difference between the two tasks in the exploration of objects A and B. Object A would be susceptible to retrieval-generated priming during the control task because it is predicted by X, via its prior association (X→A), which would result

in relatively less exploration of object A than B. During the blocking task, object A would not be susceptible to retrieval-generated priming, as its association with X would have been 'blocked' during the compound phase due to its prior association with p (pX→A). This would result in relatively more exploration of object A than object B.

The junk objects (A and B) were used in combination with patterned inserts as blocking and control cues (p and q). These were patterned rectangular laminated paper with distinct patterns printed on them, either solid black, solid grey, horizontal black and white stripes, or black spots on a white background, placed in close proximity to the objects. These designs were chosen as they have previously been shown to be discriminable by mice (Hyde & Denenberg, 1999). For the compound phase and test, the arena walls were covered with distinct patterns (X) configured so that all the arena walls were of the same pattern (Figure 11).

3.4.2 Materials and method

3.4.2.1 Subjects

The subjects were the 16 male C57BL/6J mice which had completed Experiment 2.

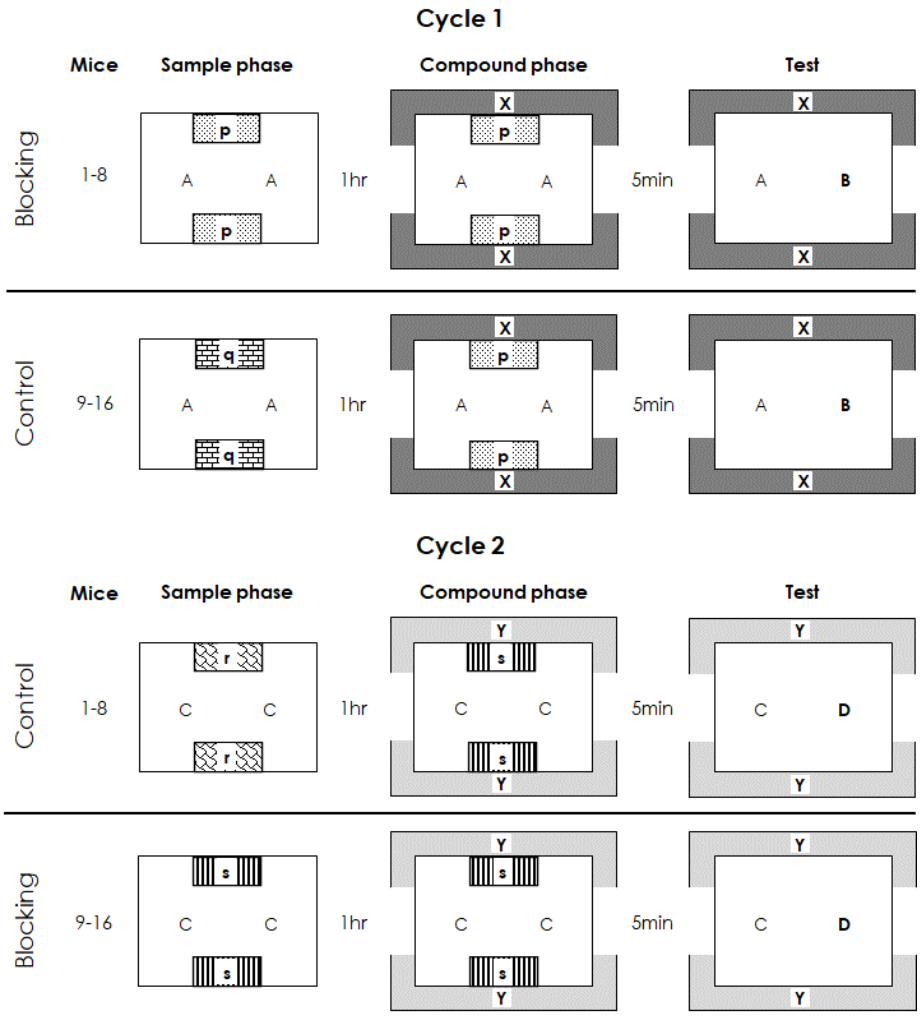


Figure 11. Schematic of the experimental procedure used during Experiment 3 and 4. Target objects are represented by A, B, C, and D, patterned inserts are depicted by p, q, r, and s, and arena wall coverings are denoted by X and Y. There was a 24-hour interval between cycle 1 and 2.

3.4.2.2 Patterned inserts

Patterned inserts were used during sample and compound phases as p, q, r and s. These were laminated paper which measured 20cm x 24cm (height x width) and were either horizontal black lines on a white background, pure grey, black spots on a white background, or pure black (Figure 12). These were used in identical pairs and were placed centrally on the longer walls of the rectangular arenas (Figure 12). These were secured to the walls with Blu Tack® and where the arena wall

coverings were used (X and Y), these were placed in front of the arena wall coverings so that both the patterned inserts and wall coverings could be seen at the same time by the mice.

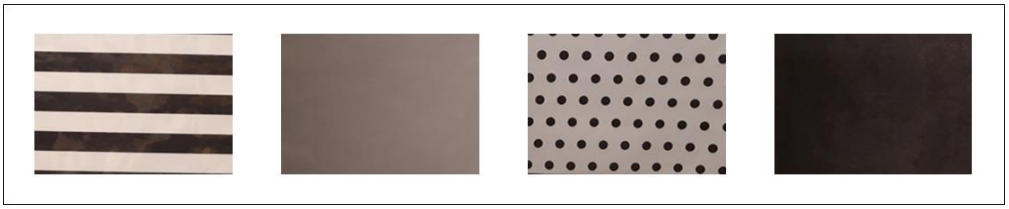


Figure 12. Patterned inserts used during Experiment 3, 4 and 5 as p, q, r and s.

3.4.2.3 Procedure

This task consisted of two cycles which each contained a sample and a compound phase, and a test. Each cycle was arranged as: 10-minute sample phase - 1-hour interval – 10-minute compound phase – 5-minute interval – 5-minute test (Figure 11). Each cycle was separated by a 24-hour interval. Half of the mice completed a blocking task during the first cycle and the other half completed a control task. During the second cycle the tasks were reversed so that all mice completed both tasks. The two tasks differed only by the patterned inserts used during sample and compound phases. For the blocking task, the same pair of patterned inserts were used for both sample and compound phases (e.g., p followed by p) and during the control task, different pairs of patterned inserts were used for each of the sample and compound phases (e.g., q followed by p). Left/right zones were used for this task. Object and patterned insert identities, and object locations during test, were counterbalanced across subjects (Table 4).

	Cycle 1				Cycle 2			
Animal	**A**	**B**	**p**	**q**	**C**	**D**	**r**	**s**
1-4	Plug	Salt	Lines	Grey	Egg	Foot	Spots	Black
5-8	Salt	Plug	Grey	Lines	Foot	Egg	Black	Spots
9-12	Plug	Salt	Grey	Lines	Egg	Foot	Black	Spots
13-16	Salt	Plug	Lines	Grey	Foot	Egg	Spots	Black

Table 4. Counterbalancing of objects and patterned inserts for Experiment 3. The same wall covering pattern was used throughout Cycle 1 as context X. A different wall covering pattern was used throughout Cycle 2 as context Y. Left and right object positions were counterbalanced during test. For example, for half of the mice A was presented on the left and for the other half A was presented on the right.

3.4.3 Results and Discussion

During the sample phase, we expected more exploration of the objects, compared with the compound phase, for the blocking task as we predicted that the objects (A) would have been primed by the patterned inserts (p→A), via retrieval-generate priming, during the compound phase, which would have reduced exploration relative to the sample phase. In contrast, we expected similar levels of object exploration between both the sample and compound phases for the control task as the objects (A) were paired with different patterned inserts during each phase (q→A and p→A respectively) thus no retrieval-generated priming would have occurred. However, exploration times were greater during the compound phase compared with the sample phase for both tasks and there were no differences between tasks (Figure 13). A mixed ANOVA with phase (sample/compound), task (blocking/control), and object location (to be novel/to be familiar) as within-subject factors revealed an effect of phase ($F(1, 15) = 63.495$, $p < .001$, $MSe = 10701.845$, $\eta_p^2 = .809$) and no other significant results (smallest $p = .071$).

The increased exploration of objects during the compound phase, was likely due to the addition of the contextual arena wall covering (X/Y) which reduced the floor

space available around the objects during this phase. A reduced floor space may have resulted in the mice being in closer proximity to the objects more frequently, than during the sample phase, when the floor space was much greater. This may have resulted in the observed increased exploration of the objects. This should not have compromised the test results as the increase appeared consistent for both tasks but, it does compromise the sample and compound phase comparison and any reduction in exploration across phases that may have otherwise occurred. However, considering only the compound phase, we would still have expected a reduction in exploration for the blocking task, via retrieval-generated priming, relative to the control condition. The absence of a difference between the two tasks suggests that a strong association may not have been formed during the sample phase, in the blocking task, as would be required for a blocking effect to occur.

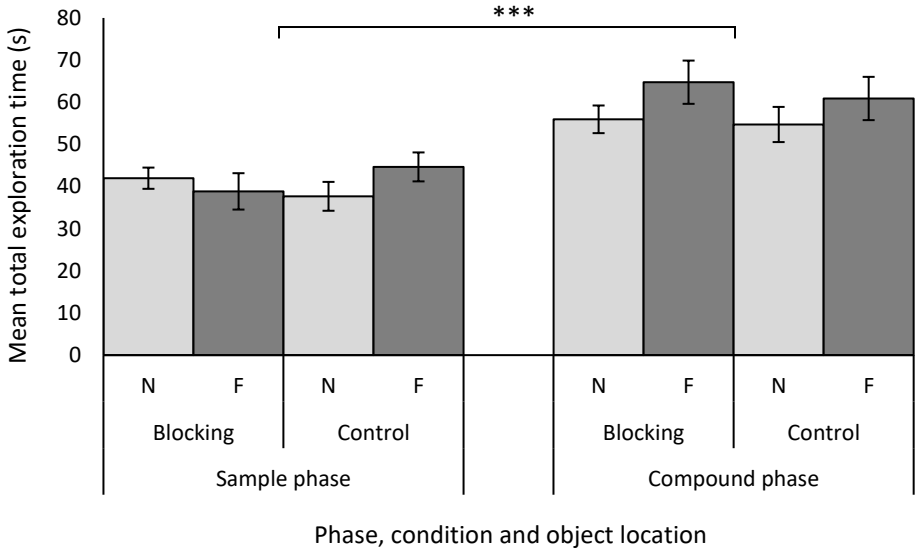


Figure 13. Mean (±SD) total exploration time (s) of objects for each phase and condition during Experiment 3. Locations were categorised in the same way as they were during the sample phases of Experiment 1 and 2. *** above a bar denotes p = <.001 between sample phases.

During test, there was a novel-familiar discrimination but no apparent blocking effect (Figure 14). Exploration of the novel and of the familiar objects were very similar between the blocking and control tasks. An ANOVA with task (blocking/control) and object (novel/familiar) as within-subject factors showed an effect of object ($F(1, 15) = 9.086$, $p = .009$, $MSe = 627.433$, $\eta_p^2 = .377$) and nothing else was significant (smallest $p = .950$). Because the effect of object novelty was so large during the initial analysis, we compared blocking ratios to test whether the novelty effect was masking any potential blocking effects. Blocking ratios were calculated for each condition using the equation: $N \div N + F$, where N and F represent the mean percentage of exploration time of the novel and of the familiar objects respectively, during the first three minutes of test. The blocking ratios were very similar for both tasks (mean ±SD: blocking task, 9.8 ± 3.8; control task 9.7 ± 5.6) and a Student's *t*-test showed no difference between them ($t(15) = .046$, $p = .964$).

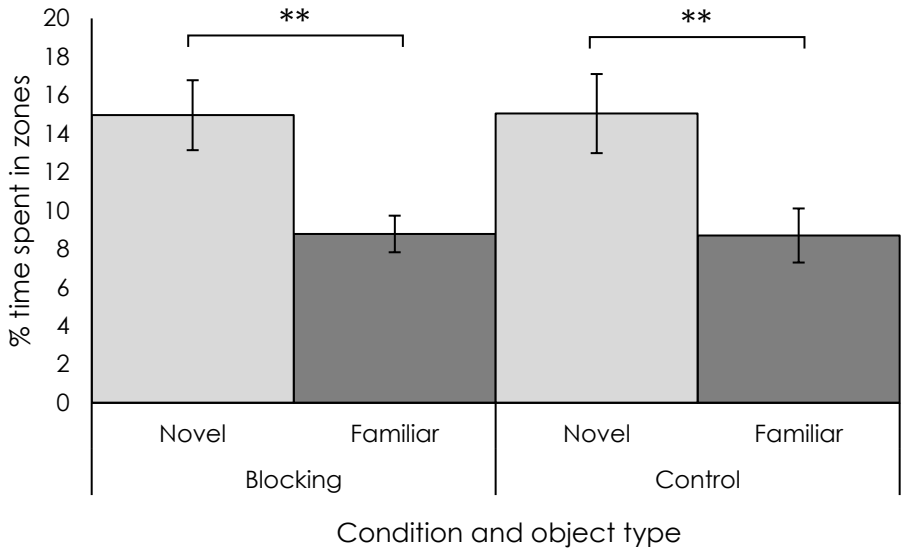


Figure 14. Mean percentage of time (±SEM) spent in each object zone in the first three minutes of test, for blocking and control tasks, during Experiment 3. Asterisks above lines denote a significant difference, $p < .010$.

During Experiment 3, no blocking effect was observed. Mice directed significantly more exploration towards the novel object than the familiar object irrespective of

condition. If a blocking effect had occurred, then we would have expected the discrimination between the novel and familiar objects, during the test phase, to have been significantly stronger during the control condition relative to the blocking condition. This would have been because during the control condition, we would have expected to see a reduction in exploration of A, via retrieval-generated priming, as A would have been successfully retrieved by X, and more exploration of B, as B would not have been retrieved by X. During the blocking condition, we would have expected to observe similar amounts of exploration of A and B, as neither A nor B would have been susceptible to retrieval-generated priming, as neither of them would have been retrieved by X. Therefore, the discrimination between A and B in the control task would have been stronger than in the blocking task. However, we observed no difference in discrimination performance between the two tasks.

This could have been because of the relatively short intervals used between phases which could have resulted in recency-based memory effects during test. SOP describes such effects as self-generated priming which, in the short-term, results in less exploration of recently experienced objects (M. A. Good et al., 2007; Mazur & Wagner, 1982; Tam et al., 2014; Wagner, 1981). Self-generated priming effects may have eclipsed effects from retrieval-generated priming. For example, during the sample phases of Cycle 1, mice would have explored both copies of object A multiple times. This could have resulted in a large proportion of elements from each copy of object A residing in A2 at the end of each phase. After the 5-minute interval between the compound phase and test, most of object A's elements would still reside in A2 thus object A would be explored less than object B, as object B would have all of its elements available for A1 activation. Reducing the possibility of self-generated priming effects interfering with effects from retrieval-generated priming was explored during Experiment 4.

3.5 Experiment 4

3.5.1 Introduction

During Experiment 3, we observed no difference in discrimination performance between the blocking and control tasks. We speculated that this may have been because of the relatively short intervals used between phases which may have allowed effects from self-generated priming to eclipse retrieval-generated priming effects. Therefore, here we replicated the previous blocking task from Experiment 3 but increased the intervals between the sample and test phases to try and remove potential self-generated priming effects (Sanderson et al., 2011).

3.5.2 Materials and method

3.5.2.1 Subjects

The subjects were 16 male C57BL/6J mice that were approximately 3 months old at the start of Experiment 4 and had previously completed Experiments 2 and 3.

3.5.2.2 Procedure

This task was identical to the Experiment 3 task (Table 3; Figure 11) except for the time intervals between the sample phases and test. Each cycle was arranged as: 10-minute sample phase - 24-hours interval – 10-minute compound phase – 24-hours interval – 5-minute test phase.

3.5.3 Results and Discussion

We expected exploration of the objects to be less in the compound phase compared with the sample phase for the blocking task and expected equal exploration between the two phases for the control task. This was because we predicted that, during the compound phase, the objects (A) would be primed by their association with the patterned inserts, in the blocking task (sample: p→A , compound: pX→A), via retrieval-generated priming, whereas the objects (A) would not be primed in the control task, as they were paired with novel patterned

inserts (sample: q→A, compound: pX→A). Similarly, to Experiment 3, there was more exploration in the compound phase compared with the sample phase for both tasks, but no difference between tasks (Figure 15). An ANOVA with phase (sample/compound), task (blocking/control), and object location (to be novel/to be familiar) as within-subjects factors revealed an effect of phase ($F(1, 15) = 23.104$, $p < .001$, $MSe = 3373.824$, $\eta_p^2 = .606$) and a phase * object location interaction ($F(1, 15) = 4.893$, $p = .043$, $MSe = 295.549$, $\eta_p^2 = .246$) and no other significant results (smallest $p = .365$). Further analysis of the interaction showed no significant effect of object location during the sample phase ($p = .337$) or the compound phase ($p = .093$).

We would have expected a reduction in exploration, for the blocking task, during the compound phase compared to the sample phase, and the general increase in exploration during the compound phase compromised this comparison. As in Experiment 3, we would still have expected a reduction in exploration, during the compound phase, for the blocking task, via retrieval-generated priming, relative to the control task where retrieval-generated priming would not occur. Again, no difference between the two tasks suggests that a strong association may not have been formed during the sample phase, in the blocking task. Therefore, it is unlikely that a blocking effect would have occurred.

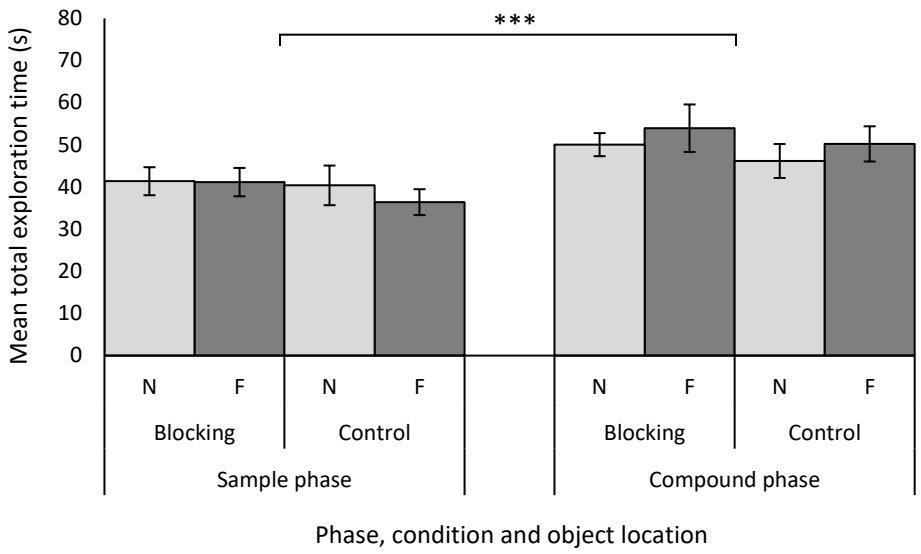


Figure 15. Mean total exploration time (±SEM) of objects during both sample phases and both conditions of Experiment 4, including object locations. Object locations were categorised as previously described. *** above a bar denotes p = <.001 between phases.

Similarly, to Experiment 3, there was an object discrimination between the novel and familiar objects, during the test, but no evidence of a blocking effect (Figure 16). Mice explored the novel and the familiar objects for similar durations during both blocking and control tasks. An ANOVA with task (blocking/control) and object (novel/familiar) as within-subject factors revealed a main effect of object ($F(1, 15) = 14.161$, $p = .002$, $MSe = 357.315$, $\eta_p^2 = .486$) but no other significant results (smallest $p = .489$). Due to the strong novel-familiar discrimination, we also calculated blocking ratios (using the same method as during Experiment 3). The blocking ratios were very similar for both tasks (mean ±SD: blocking task, 9.2 ± .9; control task, 9.1 ± .8) and a Student's t-test showed no difference between them ($t(15) = .091$, $p = .929$). Finally, we pooled the data from Experiments 3 and 4 to compare exploration of the familiar objects, during test, to explore whether there was any evidence for stimulus priming during the blocking tasks altering response levels to familiar cues relative to the control tasks. The pooled data for the exploration of familiar objects were similar between the blocking and control

tasks, which was confirmed using a Student's *t*-test which showed no difference between tasks ($t(31) = .092$, $p = .927$).

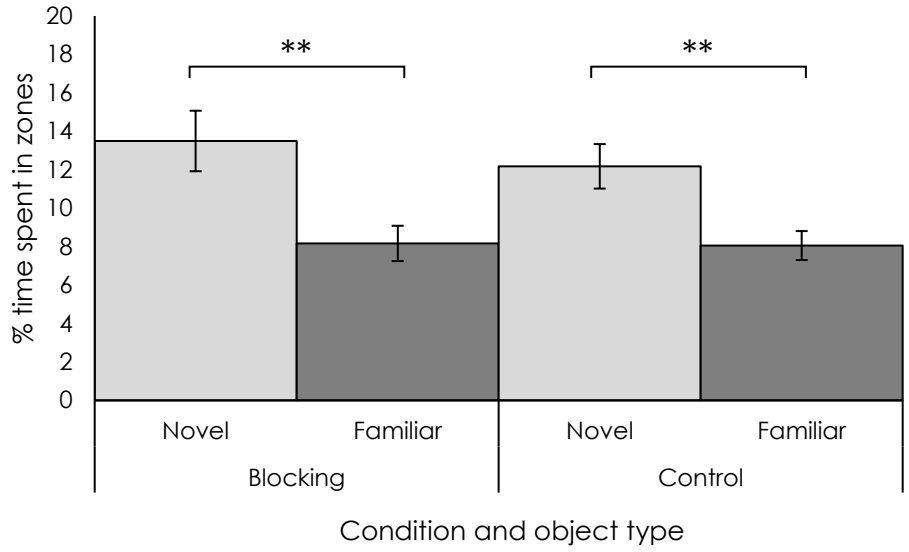


Figure 16. Mean percentage of time (±SEM) spent in each object zone for blocking and control tasks in the first three minutes of test in Experiment 4. Asterisks above lines denote a significant difference, $p < .001$.

The results from Experiment 4 were similar to those obtained during Experiment 3. As before there was no difference in discrimination performance between the two tasks. That is, the novel object was explored significantly more than the familiar object during the test phase regardless of task. During the compound phases of Experiment 3 and 4, we would have expected stimulus priming to have occurred during the blocking tasks of both experiments, via retrieval-generated priming through associations acquired during sample phases. This would have reduced exploration of the objects during the compound phases of both experiments relative to the control tasks. However, we found no evidence of stimulus priming which suggests that the blocking cues had not acquired enough associative strength, during the sample phases, and had not reached asymptote as would be required for blocking to occur (e.g., Jones & Haselgrove, 2013).

Another issue could be that the propensity for mice to explore novelty is very strong (Ennaceur, 2010; Ennaceur & Delacour, 1988) and by comparison any blocking effects that may have occurred would be relatively weaker. It has been demonstrated that differential salience of the stimuli used during blocking tasks can reduce a blocking effect or eliminate it altogether (Heckler, Kaminski, & Sloutsky, 2006; Seraganian, 2023). Thus, during Experiment 4, any potential blocking effects could have been eclipsed by the salience of the novel object. Reducing this potential issue was explored during Experiment 5.

3.6 Experiment 5

3.6.1 Introduction

Experiment 5 aimed to avoid the possibility that the propensity to explore novelty, by mice, may have eclipsed any potential blocking effects during Experiment 4. To address this issue, we used a blocking design which exposed animals to objects A and B equally, prior to test, so that neither object would be novel at test (Table 5).

Sample phase	Compound phase	Test
A + p	A + p in X	A + B in X
B + q	B + p in X	A + B in X

Table 5. Experimental design for Experiment 5. A and B represent junk objects, p and q depict rectangular patterned inserts that were placed near the objects, and X denotes a distinct pattern that covered the entirety of the arena walls.

Here, mice were exposed to object A with patterned insert p (p→A) and object B with patterned insert q (q→B), during the sample phase. They were then exposed to object A and object B both with patterned insert p, in context X (pX→A, pX→B), during the compound phase. During test, mice were exposed to object A and object B in context X (XA, XB; Table 5). The rationale for this experiment was based on SOP which proposes that during the sample phase associations

would form between the objects and patterned inserts (p→A and q→B). During the compound phase, object A would already be predicted by patterned insert p thus an X→A association would not form (sample: p→A, compound: pX→A). Object B would not be predicted by patterned insert p therefore an X→B association would form (sample: q→B, compound: pX→B). Consequently, during test we would expect more exploration of object A than object B, as object B would be susceptible to retrieval-generated priming via its prior association with X whereas object A would not.

3.6.2 Materials and method

3.6.2.1 Subjects

The subjects were 16 naïve male C57BL/6J mice that were approximately 3 months old at the start of Experiment 5.

3.6.2.2 Patterned inserts

Two of the patterned inserts from Experiment 3 and 4 were used during the sample phases of Experiment 5 (Figure 11). These were the horizontal black lines on a white background and the pure grey inserts. These were used in different combinations of identical and mismatched pairs that were placed centrally on the shorter walls of the rectangular arenas, one on the left wall and one on the right wall, both at base level (Figure 17). During the compound phase, these were placed in front of the contextual arena wall coverings so that mice could see both the patterned inserts and arena wall coverings simultaneously.

3.6.2.3 Procedure

This task consisted of a sample and compound phase and a test arranged as: 10-minute sample phase – 24-hours interval – 10-minute compound phase – 24-hours interval – 5-minute test (Figure 17).

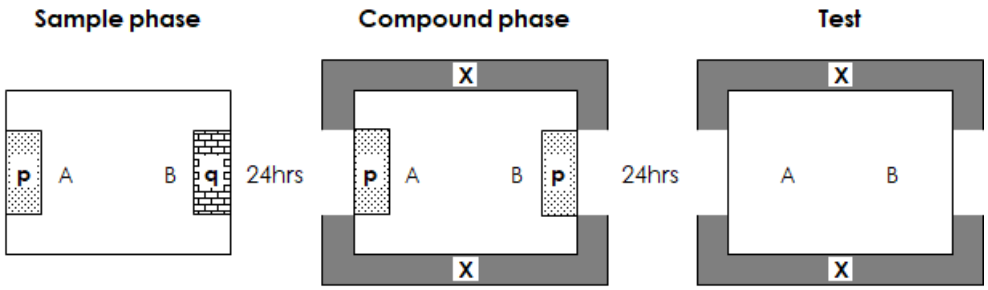


Figure 17. Schematic of the experimental procedure for Experiment 5. A and B represent target objects, p and q depict patterned inserts and X denotes contextual arena wall covering.

During the sample phase, object A was presented with patterned insert p and object B was presented with patterned insert q. During the compound phase, both objects were presented with patterned insert p within the arena context X. During test, objects A and B were presented within context X. Left/right zones were used during this task. Object and patterned insert identities and object locations were counterbalanced across subjects (Table 6). The contextual arena wall covering used (X) was identical for all subjects.

Animal	Object A	Object B	Insert p	Insert q
1-4	Plug	Salt	Lines	Grey
5-8	Salt	Plug	Grey	Lines
9-12	Plug	Salt	Grey	Lines
13-16	Salt	Plug	Lines	Grey

Table 6. Counterbalancing of objects and patterned inserts for Experiment 5. Left and right object locations were counterbalanced throughout both phases and test. For half of the mice object A was presented in the left position and for the other half of the mice it was presented in the right position.

3.6.3 Results and Discussion

Exploration time was greater during the compound phase compared with sample phase for both objects. There was no difference between exploration of object A

and B during each phase (Figure 18). A mixed ANOVA with phase (sample/compound) and object (A/B) as within-subject factors revealed an effect of phase ($F(1, 15) = 6.586$, $p = .021$, $MSe = 1191.113$, $\eta_p^2 = .305$) and no other significant results (smallest $p = .235$). Similarly, to Experiments 3 and 4, this was likely due to the addition of the arena wall covering context during this stage which reduced the floor space available around the objects. Thus, the mice again, may have been in closer proximity to the objects more often than during the sample phase which may have resulted in more exploration of the objects.

We would have expected less exploration of object A during the compound phase, both compared with exploration of object A in the sample phase and compared with exploration of object B during the compound phase. This was because SOP would predict that object A would have been susceptible to retrieval-generated priming, during the compound phase, via its prior association with p (p→A). Neither object B, during the compound phase, nor object A, during the sample phase, would have been subjected to retrieval-generated priming. An absence of both of these differences suggest that a strong association may not have formed, between object A and patterned insert p, during the sample phase, thus a blocking effect during test was unlikely to have occurred.

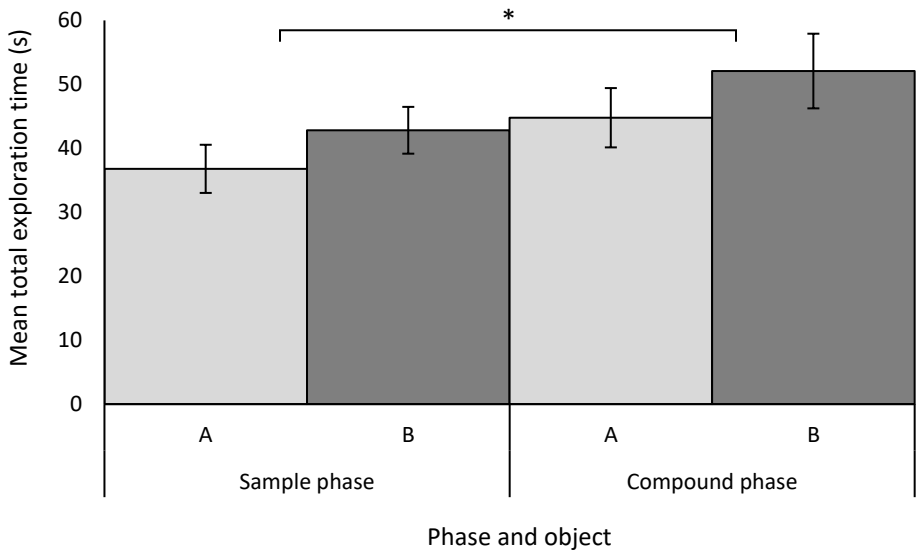


Figure 18. Mean (±SEM) total exploration time (s) of objects A and B for both the sample and compound phases during Experiment 5. * above a bar denotes p = <.050 between phases.

During test, we would have expected more exploration of object A compared with object B if blocking had have occurred. This was because object B would have been susceptible to retrieval-generated priming, that is, it would have been successfully retrieved by X (X→B). Object A would not be retrieved by X thus would not be subjected to retrieval-generated priming. However, we observed similar exploration of the two objects during test (Figure 18). A Student's *t*-test revealed no significant difference between exploration of object A and object B ($t(15) = .041$, $p = .968$).

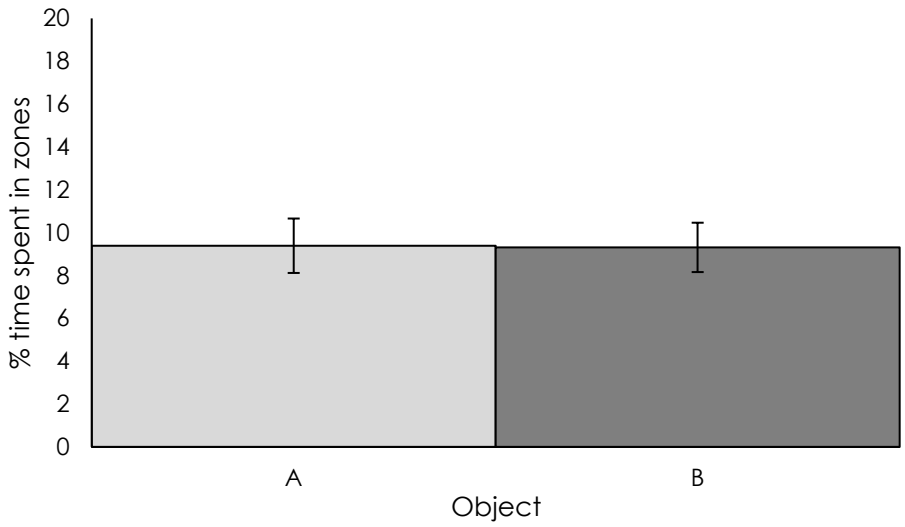


Figure 18. Mean exploration time (±SEM) of objects A and B in each of the first three minutes of test, during Experiment 5.

During Experiment 5, there were no differences in exploration between objects A and B as would have been expected if blocking had occurred. We would have expected more exploration of A than B because object A would not have been retrieved by X (sample: p→A, compound: pX→A, test: XA, XB) whereas object B would have been (sample: q→B, compound: pX→B, test: XA, XB). This could have been because the blocking cues used during the sample phase (p; patterned inserts) were not salient enough relative to the second cues that were introduced during the compound phase (X; arena wall coverings), to create a blocking effect. For example, it has been shown that in a typical blocking paradigm (A+, AB+) if the second stimulus B (in Experiment 5 this was represented by X, the arena wall coverings) was more salient than the initial stimulus A (in Experiment 5 this was represented by p, the patterned inserts) then blocking was greatly reduced or eliminated (Heckler et al., 2006). Moreover, the absence of a blocking effect has previously been suggested to be partly attributed to lower levels of attention to the stimuli, by mice, that were observed (Yamada, 2010).

It is also possible that single 10-minute sample phases may be insufficient for strong associations to form as blocking effects appear to be contingent on the

duration of stimulus exposure (Jennings & Bonardi, 2017; Sanderson et al., 2016). During the compound phase of Experiment 5, we found no evidence of an association between object A and the blocking cue p as would be required for a blocking effect to occur. Exploration of object A should have been reduced, via retrieval-generated priming, during the compound phase, relative to exploration of object A during the sample phase, and relative to exploration of object B during the compound phase, as neither object A, during the sample phase, nor object B, during the compound phase, would have been susceptible to retrieval-generated priming.

These potential issues were addressed during Experiment 6.

3.7 Experiment 6

3.7.1 Introduction

Experiment 6 aimed to address the potential issues of the salience of the patterned inserts, that were used as blocking cues, and the sample phase durations, which both may have impacted the results of Experiment 5. This is because we need conditioning to reach asymptote to establish effective blocking (Jennings & Bonardi, 2017; Jones & Haselgrove, 2013; Sanderson et al., 2016), that is, we require the blocking cues to from a strong association with the objects if they are to be effective. However, we have not found any evidence so far that the blocking cues had acquired enough associative strength to reach asymptotic levels of conditioned responding, as would be required for blocking to occur (Jennings & Bonardi, 2017; Jones & Haselgrove, 2013; Sanderson et al., 2016). We speculated that this could have been because the blocking cues were not salient enough, as would be required to get effective blocking (Heckler et al., 2006; Yamada, 2010), and because the sample phases were not sufficient for the appropriate duration of stimulus exposure, that would also be required for blocking to occur (Jennings & Bonardi, 2017; Sanderson et al., 2016).

Therefore, the number of 10-minute sample phases were doubled and the patterned inserts were replaced by textured floors as blocking cues. The textured floors

covered a large amount of floor space around the objects and the mice had to walk over them to explore the objects. This should have increased the chances of associations forming between the blocking cues (textured floors) and the objects. The textured floors were made from different materials which should have been more discriminable than the patterned inserts previously used, by both visual and tactile modalities. Rodents gather a large amount of information from their environment with their facial whiskers (vibrissae) (Brecht, 2007; Carandini & Churchland, 2013). They have been shown to use this information successfully during perceptual learning tasks (Montuori & Honey, 2016; Pacchiarini, 2019), and during a novel object recognition task (Wu, Ioffe, Iverson, Boon, & Dyck, 2013), which all used tactile stimuli. Wu et al. (2013) also provided evidence that the mice used during their study, primarily used their vibrissae to discriminate between the textured floors. When vibrissae were removed, the mice were unable to discriminate between the textured floors and when the texture cues were removed, by shielding the objects with transparent film, which only left visual cues, the mice were no longer able to discriminate between the floor stimuli. Therefore, the textured floors used during Experiment 6 should have been more salient than the previous used patterned inserts. The experimental design and rationale were the same as Experiment 5 except for the two aforementioned changes.

3.7.2 Materials and method

3.7.2.1 Subjects

The subjects were the 16 male C57BL/6J mice which had completed Experiment 5.

3.7.2.2 Textured floors

Textured floors were used as blocking cues (p), in place of the patterned inserts used previously. These measured 30cm x 20cm and were placed so that two textured floors covered the majority of the arena floor space with the arena wall

coverings (X) in place. Each individual textured floor would cover either the left or right half of the arena floor. Two types of textured floor were used. One was a 4mm thick black rubber mat with a textured square design (Figure 19A) and the other was a 2mm thick grey ethylene vinyl acetate (EVA) draw liner with a textured convex polka dot design (Figure 19B).

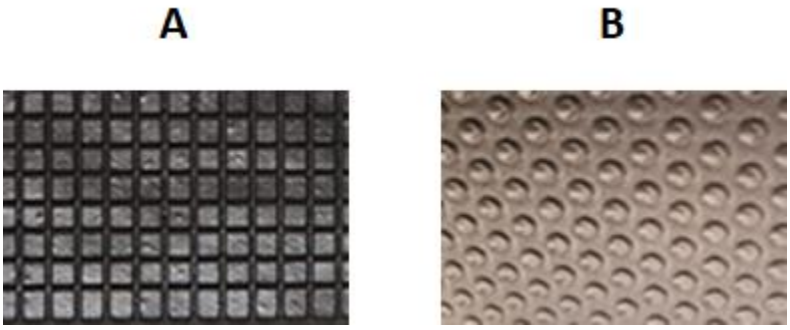


Figure 19. Textured floors used for Experiment 6. Black rubber mat (A) and grey EVA draw liner (B).

3.7.2.3 Procedure

The design for Experiment 6 was the same as Experiment 5 (Table 5), with the addition that the sample and compound phases were each run through twice, and that the blocking cue and control cue (p and q) were now textured floors (Figure 19) rather than patterned inserts. This task consisted of two replications of the sample and compound phases, and a test arranged as: 10-minute sample phase (p→A, q→B) – 1-hour interval – 10-minute sample phase (q→B, p→A) – 24-hours interval –10-minute compound phase (pX→A, pX→B) – 1-hour interval – 10-minute compound phase (pX→B, pX→A) – 24-hours interval - 5-minute test (XA, XB) (Figure 20).

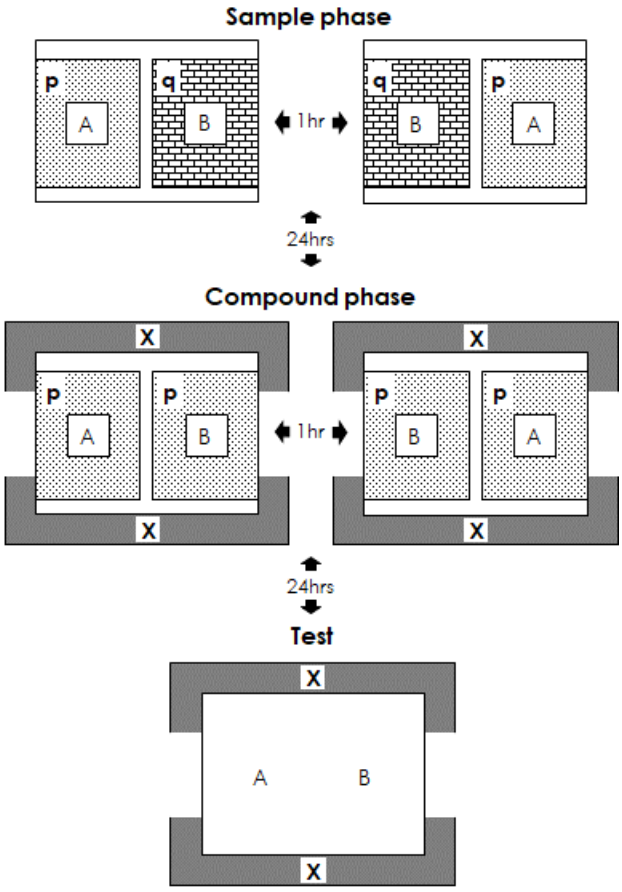


Figure 20. Schematic of experimental procedure for Experiment 6. Target objects are represented by A and B, textured floors are depicted by p and q, and contextual arena wall covering are denoted by X. During the second replication of the sample and compound phases, object and textured floor pairings were presented on the opposite side of the arena to which they had been presented during the first replication of these phases.

During the initial sample phase, object A was presented with the blocking cue p (p→A) and object B was presented with a control cue q (q→B). Each object (A and B) was placed in the centre of the textured floor that they were paired with (p or q). This arrangement was repeated during the second replication of the sample phase, but p→A and q→B were presented on the opposite sides of the arena to those initially used. During the compound phase, both objects (A and B) were presented with p and a second cue X (pX→A, pX→B). Both objects were again

placed in the centre of the textured floors that they were paired with, and a contextual arena wall covering was used as X. This arrangement was repeated during the second replication of the compound phase, except that p→A and p→B were switched to the opposite sides of the arena. During test, objects A and B were presented with X (XA, XB). Left/right zones were used during this task. Object and textured floor identities and their locations were counterbalanced across subjects (Table 7).

	Object and floor identities				**Object left and right locations**				
Animal	**A**	**B**	**p**	**q**	**Sample1**	**Sample2**	**Compound1**	**Compound2**	**Test**
1-4	Deo	Tab	EVA	Rubber	A-B	B-A	A-B	B-A	B-A
5-8	Deo	Tab	Rubber	EVA	B-A	A-B	B-A	A-B	B-A
9-12	Tab	Deo	Rubber	EVA	B-A	A-B	B-A	A-B	A-B
13-16	Tab	Deo	EVA	Rubber	A-B	B-A	A-B	B-A	A-B

Table 7. Counterbalancing of objects and textured floors and the locations that they were presented in, during Experiment 6.

3.7.3 Results and Discussion

We expected a reduction in exploration of object A during the compound phase compared to the sample phase and similar amounts of exploration for object B across both phases. However, there were no statistical differences between exploration of the two objects during the compound phase and no difference between the sample and compound phases (Figure 23). A mixed ANOVA with phase (sample/compound) and object (A/B) as within-subject factors showed no effect of phase ($F(1, 15) = 1.431$, $p = .250$, $MSe = 1525.879$, $\eta_p^2 = .087$) or object ($F(1, 15) = .511$, $p = .485$, $MSe = 210.613$, $\eta_p^2 = .033$) and no interaction between these two factors ($p = .751$). Because A appeared to suffer a larger reduction in exploration across phases, compared with B, we calculated difference scores for each object by subtracting exploration of each object during the compound phase from the exploration of each object during the sample phase. This resulted in a higher score for object A than for object B (mean ±SD: A, 11.2 ± 33.4; B, 8.4 ±

40.1). However, a Student's *t*-test showed no difference between these scores for the two objects ($t(15) = .323$, $p = .751$).

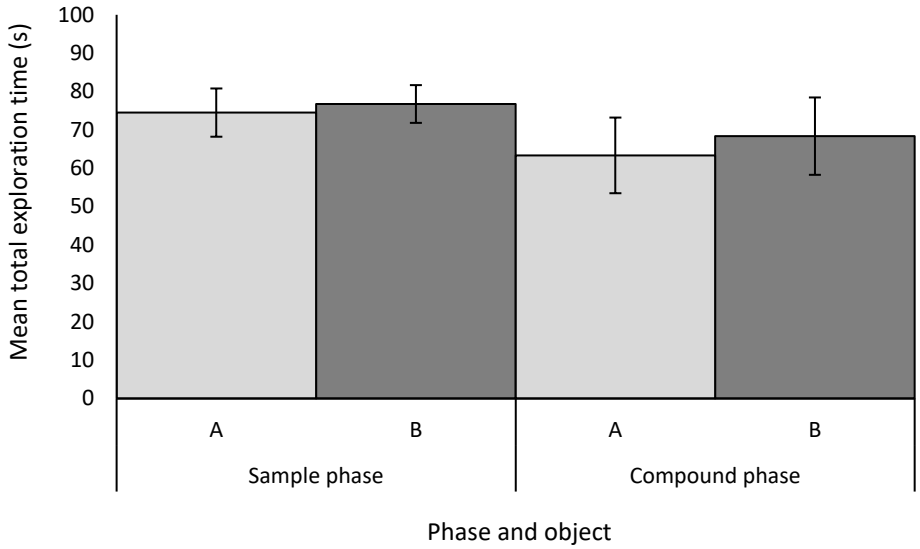


Figure 23. Mean (±SEM) total exploration time (s) of objects A and B for both the sample and compound phases during Experiment 6.

During test, we expected more exploration of object A, relative to B, if blocking had have occurred. This was because object B would have been susceptible to retrieval generated priming through its association with X whereas object A would not. In other words, B would have been successfully retrieved by X, but X would not retrieve A. However, object exploration was similar for both objects during test (Figure 24). A Student's *t*-test revealed no significant difference in exploration between the two objects ($t(15) = -.605$, $p = .554$).

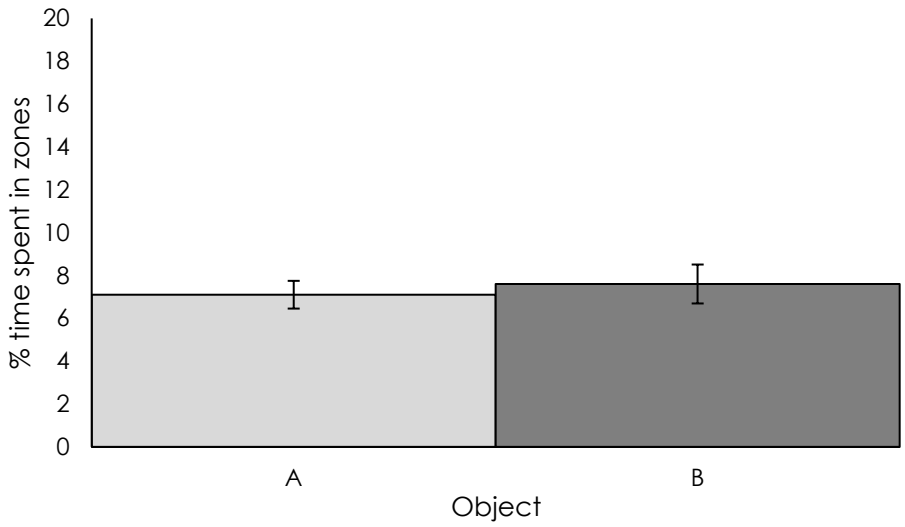


Figure 24. Mean percentage of exploration time (±SEM) of objects A and B for the first three minutes of test during Experiment 6.

During Experiment 6, there was no apparent blocking effect that would have been evident by a reduction in exploration of object B relative to A during test. This was because object B would have been retrieved in X whereas object A would not. Crucially, we would also have expected exploration of object A, during the compound phase, to have been reduced relative to the sample phase, and to have observed less exploration of A than B during the compound phase. This was because object A should have been primed by the blocking cue p, during the compound phase, via its prior association from the sample phase (p→A), but object B would not, as during the sample phase it was not associated with p (q→B). Although there were no statistical differences between exploration of the objects across phases, the mean differences were in the direction that A (11.2s) had suffered a larger reduction than B (8.4s). It is possible that with more exposure time to A and p, that exploration of A, during the compound phase, would be further reduced. If this were the case then it would suggest that the blocking cue had not acquired enough associative strength to reach asymptote and consequently would not create effective blocking (Jennings & Bonardi, 2017; Jones & Haselgrove, 2013; Sanderson et al., 2016).

3.8 General discussion

All four blocking experiments failed to demonstrate a blocking effect and provide evidence that associations underlie recognition memory. However, there are many variables and parameters that can influence the blocking effect, such as stimulus salience, stimulus duration, number of training trials, and contiguity (Heckler et al., 2006; P.-P. Liu & Luhmann, 2013; Sanderson et al., 2016; Urcelay, 2017). For example, Heckler et al. (2006) demonstrated that differential cue salience significantly affected blocking. They found that if the initially learned cue was more salient than the blocked cue, then blocking was enhanced but when the blocked cue was more salient than the initially learned cue, blocking was greatly reduced or eliminated. P.-P. Liu and Luhmann (2013) reported that blocking was modulated by cognitive load and that a secondary task during the initial stages of compound training eliminated a blocking effect.

In the current experiments, mice may not have been able to discriminate between the blocking cues because they were primarily visual in modality from a human perspective (except the textured floors), although mice may have used other sensory information to discriminate between them such as tactile or olfactory information (Carandini & Churchland, 2013). Although vision in mice is thought to be poor, it has been argued that it is far more sophisticated than previously thought, as it is extremely efficient at sampling the visual scene and is primarily used for spatial navigation (Carandini & Churchland, 2013; Huberman & Niell, 2011).

Nonetheless, mice should have been able to discriminate between the visual stimuli used as we had chosen specific 2-dimensional pattern combinations which mice can readily learn to discriminate between (Hyde & Denenberg, 1999; Makowiecki, Hammond, & Rodger, 2012; Wong & Brown, 2006). Moreover, we used larger stimuli (20cm x 24cm) than the previous study on which ours were based (12.8cm x 23.1cm, Hyde & Denenberg, 1999), which should have made our stimuli easier to discriminate between. Another difference between their study and ours was the mouse strain used, they used BXSB/MpJ (BXSB) mice, and we used

C57BL/6J (C57) mice. Although it has been well documented that visual ability can vary significantly in different strains of mice (Wong & Brown, 2006), it is unlikely to be a factor here, as C57 mice have normal vision (Wong & Brown, 2006) and learn visual discriminations well (Bussey et al., 2001; Passino & Ammassari–Teule, 1999; Wong & Brown, 2006) similarly to BXSB mice (Boehm et al., 1998; Hyde & Denenberg, 1999). However, it should be noted that BXSB mice have previously been shown to exhibit superior spatial learning and memory when directly compared directly with C57 mice (Hyde, Hoplight, & Denenberg, 1998). The final and perhaps the most crucial difference is that the mice used by Hyde and Denenberg (1999) were reinforced during 10 daily trials that were run for 3 weeks, whereas our mice received no reinforcement and were exposed to the stimuli for 10-minute durations only. Therefore, it is possible that with the absence of reinforcement and with limited exposure time to the stimuli that the mice could not discriminate between them.

Regarding the textured floors that were used as the blocking cue during Experiment 6, this should not have been the case. We would have expected the mice to have been able to discriminate between these stimuli under our experimental parameters. This was because rodents can be trained to readily discriminate between tactile stimuli, like the textured floors used (Montuori & Honey, 2016; Pacchiarini, 2019), and more importantly C57 mice have been shown to discriminate between textured floor coverings during a spontaneous novel object recognition task in which the sample phase was 5 minutes (Wu et al., 2013). However, the stimuli used as textured floors by Wu et al. (2013) were different to the ones used during Experiment 6 so we cannot confirm that the mice could discriminate between them. Therefore, the discriminability of the blocking cues may have contributed to the absence of a blocking effect.

Another possibility is that in blocking experiments with rodents, the first cue is typically conditioned over multiple trials and sessions where the cue is presented for a given duration and terminated with the presentation of an US (e.g., Bonardi et al., 2010; Sanderson et al., 2016). Thus, the degree of temporal contiguity is high, and a strong association can form. However, during the current set of

experiments this may not have been the case. For example, mice may have explored the blocking cue p (equivalent to a CS) and then generally explored the arena before exploring the object A (equivalent to an US). This would have resulted in a low degree of temporal contiguity and weakened associative strength between p and A. Furthermore, in terms of SOP, if they had spent a significant amount of time exploring p and A individually, then both stimuli would have many elements residing in A2, at any given time, and consequently both stimuli would have very few elements available to enter A1. As a strong association between the stimuli is contingent on both stimuli having elements in A1 simultaneously, this could have greatly reduced associative strength. The data from the compound phases of these experiments certainly suggest that a strong association did not form during the sample phases.

Furthermore, the exposure time that mice had during the current set of experiments may have been insufficient for the blocking cues to acquire enough associative strength to reach the asymptotic levels of conditioned responding that would be required for blocking to occur (Jennings & Bonardi, 2017; Jones & Haselgrove, 2013; Sanderson et al., 2016). For example, previous work in mice has shown that using a visual cue blocked conditioning after 200 trials but failed to after 80 trials (Sanderson et al., 2016). Each trial presented a CS for 10s followed by an US. In the SOR tasks used here it is very difficult to establish how many times each animal has experienced an equivalent of a CS→US pairing and the duration of these exposures. Therefore, it is possible that during the sample phases the blocking cues were not given the amount of attention required for a blocking effect to occur (Yamada, 2010).

3.9 Conclusion

To establish effective blocking, conditioning needs to reach asymptote (Jennings & Bonardi, 2017; Jones & Haselgrove, 2013; Sanderson et al., 2016). Therefore, in our experiments we required the blocking cues used to form strong associations with the objects in order to be effective. The current experiments provided no evidence to suggest that this had occurred. Thus, to further explore any possible

blocking effects during object recognition memory tasks, we needed to employ a task that established a strong context-object association. Once successful, a blocking experiment could be designed based on this task.

A study by Whitt et al. (2012) provided good evidence of a context-object association during an indirect object recognition memory task in rats. They exposed rats to object P in context x (xP) and then object Q in context y (yQ) with the assumption that associations would form between objects and contexts (x-P, y-Q). They then exposed rats to context x with no objects and predicted that x would associatively activate the memory of object P, and that consequently, P would be explored less relative to Q during the test that followed. After a short interval, rats were exposed to P and Q with no contexts present, during the test, and rats explored Q more than P as the authors had predicted. This task parallels the initial stage of a blocking task (e.g., A+ trials) and if this could be replicated with our mice, then further associative phases could be added (e.g., AB+ trials) which may result in a blocking effect. Therefore, during Chapter 4 we replicated Whitt et al. (2012) to further explore association-based recognition memory effects and predictions of SOP relating to retrieval-generated priming.

Chapter 4: Indirect object recognition and blocking

4.1 General introduction

A central characteristic of SOP is how it explains association-based memory and learning phenomena (Brandon et al., 2003; Sanderson & Bannerman, 2011; Uribe-Bahamonde et al., 2019; Vogel et al., 2020; Vogel et al., 2019). SOP asserts that such events occur through the process of retrieval-generated priming (Brandon et al., 2003; Mazur & Wagner, 1982; Wagner, 1981). In rodents, association-based memory is widely assessed using variants of the object-in-context task (For a review see: Sep et al., 2021) which is thought to largely depend on the hippocampal system (M. A. Good et al., 2007; Mumby et al., 2002; Spanswick & Sutherland, 2010) and associated regions (Norman & Eacott, 2005; Spanswick & Dyck, 2012; D. I. Wilson et al., 2013). Generally, the task consists of two sample phases and a test. During the sample phases, animals are usually exposed to two copies of an object in one context (e.g., Px) followed by two copies of a different object in an alternative context (e.g., Qy). During test, animals are exposed to one copy of an object from each of the sample phases presented in one of the two contexts (e.g., Px, Qx). Animals will generally explore the object that has not previously been paired with the test context (e.g., Q) more than the object that has (M. A. Good et al., 2007; Langston & Wood, 2010; Mumby et al., 2002; Norman & Eacott, 2005; Spanswick & Dyck, 2012; Spanswick & Sutherland, 2010; D. I. Wilson et al., 2013). Data from these types of tasks are consistent with SOPs retrieval-generated priming theory. SOP postulates that during test the object that was explored less was primed by its prior association with the context and the object that was explored more was not.

Two previous studies provided some evidence in support of SOPs associative account of memory in relation to these types of association-based memory tasks (Bonardi et al., 2016, 2021). Both studies used a transgenic mouse model of Alzheimer's disease ($APP_{swe}/PS1_{\Delta e9}$, hereafter APP/PS1), and their wild type littermates, as a tool to explore the processes that may underlie recognition memory performance during spontaneous object recognition tasks. The mice were

around 5 months old during the testing phases of both studies. At this age, these transgenic mice display amyloid-β protein related pathological changes in the brain, primarily in the hippocampus (Garcia-Alloza et al., 2006; Hong et al., 2016; Pedrós et al., 2014; Ruan et al., 2009; Zhu et al., 2017), but generally do not exhibit cognitive deficits in learning and behaviour (Kelly et al., 2017; Kilgore et al., 2010), although some mild cognitive deficits are already present (Holcomb et al., 1998). Crucially, at this age, they do not display cognitive deficits in the novel object recognition task either (Bonardi et al., 2011), a task widely used to assess recognition memory in rodents. This is surprising as Alzheimer's patients typically display deficits in recognition quite early in the disease continuum (Didic et al., 2010; Zola et al., 2013), therefore we would expect an object recognition deficit in these mice at this age, as they begin to show some mild cognitive deficits at around 4 months old (Holcomb et al., 1998).

Both studies used a novel object recognition task (Ennaceur & Delacour, 1988), where mice were exposed to two copies of object P and then tested with one copy of P and a novel object Q, to compare performance of the mice on that task with their performance on two other recognition memory tasks, which they argued map well onto the processes of self-generated priming (relative recency) and retrieval-generated priming (object-in-place) relatively independently. The first of these tasks was a relative recency task (Mitchell & Laiacona, 1998), where mice were sequentially presented with two copies of P and then two copies of Q, followed by a test with P and Q. For the second test, each study used a different variant of the object-in-place task (Ameen-Ali, Eacott, & Easton, 2012; Dix & Aggleton, 1999), which has been suggested as a test of associative recognition (Aggleton & Nelson, 2020), where objects were presented in an array, followed by a test with the same objects rearranged so that some of them had changed places. For example, Bonardi et al. (2016) presented mice with an array of four objects (e.g., PQRS) during a sample phase, and then switched the position of two objects during a test phase (e.g., PRQS). Bonardi et al. (2021) instead presented mice with two objects (e.g., PQ) during a sample phase, and then switched one of the objects for a copy of the other object during a test (e.g., PP).

Both studies reported that, during test, the transgenic mice exhibited no deficits during the novel object recognition tasks compared with their wild type littermates, exploring Q more than P in both cases (Bonardi et al., 2016, 2021), consistent with previous data (Bonardi et al., 2011; Jardanhazi-Kurutz et al., 2010; Kelly et al., 2017). They also reported no deficits during the relative recency tasks, mice explored P more than Q regardless of genotype. However, they both reported a selective deficit in the transgenic mice during the object-in-place tasks. Wild type littermates explored the displaced objects more than the objects that were presented in their original locations, relative to the sample phases, whereas transgenic mice directed similar amounts of exploration to all objects.

The authors interpreted these results in terms of SOP and suggested that retrieval-generated priming may have been impaired in the transgenic mice, but self-generated priming remained intact. They also suggested that both processes may contribute to the novel object recognition task, therefore, if one component was impaired then the other may have been able to compensate so that performance appeared normal. During the relative recency tasks, self-generated priming alone could have supported performance in the transgenic mice. Because the object-in-place tasks were association-based, sustained performance in these tasks would have required retrieval-generated priming.

Another similar task to the object-in-place task is the object-in-context task, in which objects are typically presented sequentially in two distinct contexts (e.g., Px, Qy), followed by a test with both objects in one of the contexts (e.g., Px, Qx), where animals generally direct more exploration towards the object that has not been previously paired with the context (M. A. Good et al., 2007; Langston & Wood, 2010; Norman & Eacott, 2005; Spanswick & Dyck, 2012; Spanswick & Sutherland, 2010; D. I. Wilson et al., 2013). Therefore, similarly to the object-in-place tasks, this task also relies on associations although because of the sequential sample phases, the relative recency of the test objects could also influence performance during these tasks (e.g., Tam et al., 2015). An alternative explanation for these results from object-in-place tasks described above (Bonardi et al., 2016,

2021) and the typical pattern of results obtained from object-in-context tasks (M. A. Good et al., 2007; Langston & Wood, 2010; Norman & Eacott, 2005; Spanswick & Dyck, 2012; Spanswick & Sutherland, 2010; D. I. Wilson et al., 2013), is based on generalisation decrement of perceptual differences of stimuli between sample and test phases (Sanderson & Bannerman, 2011; Whitt et al., 2012). In other words, animals may have discriminated the relative novelty of the object/context pairings as opposed to using associative information. That is, in the example of Px, Qy followed by a test with Px,Qx, they may have perceived object Q differently during test when it was presented in x than during the sample phase when it was presented in y, which may have resulted in Q being treated as more novel relative to P during test, as P would have been perceived equally during both phases, as it was presented in x both times (Whitt et al., 2012).

Whitt et al. (2012), explored SOPs retrieval-generated priming explanation of object-in-context data in relation to the alternative hypothesis of generalisation decrement. They presented rats with object P in context x and object Q in context y across two sample phases as would be done in standard object-in-context tasks (M. A. Good et al., 2007; Langston & Wood, 2010; Norman & Eacott, 2005; Spanswick & Dyck, 2012; Spanswick & Sutherland, 2010; D. I. Wilson et al., 2013). They included a third phase in which rats were placed in context x with no objects present. Their rationale for this phase was that according to SOP, x would associatively activate elements of P into the A2 state, via retrieval-generated priming, and consequently reduce exploratory responding to P during the test that followed. After a short interval, rats were tested with objects P and Q in a third context which neither object had prior associations with (e.g., z). Therefore, both objects should have been equally familiar during test resulting in the possibility of generalisation decrement being highly unlikely. Rats explored object Q relatively more than object P during test which was consistent with SOPs priming theory and inconsistent with the alternative hypothesis. These data provided support for SOPs retrieval-generated priming explanation for the process of association-based memory formation (Brandon et al., 2003; Mazur & Wagner, 1982; Wagner, 1981).

Chapter 4 aimed to further explore retrieval-generated priming over a series of experiments that were based on the indirect object recognition study by Whitt et al. (2012). These included replications using 4-month-old and 5-month-old C57BL/6J mice and our experimental setup described in Chapter 2. We also investigated the hypothesis that 5-month-old APP/PS1 mice would show a selective deficit in indirect object recognition memory, during this task. The prediction was based on the assumption that the task required association-based memory, which relies on retrieval-generated priming, and that this priming process would be impaired in these mice at this age (Bonardi et al., 2016, 2021). Additionally, we further explored the possibility of the occurrence of blocking effects during recognition memory tasks, as described in Chapter 3, using an experimental design based on the indirect object recognition memory task.

4.2 Experiment 7

4.2.1 Introduction

Experiment 7 aimed to replicate a previous study which demonstrated indirect recognition memory in rats (Whitt et al., 2012). In a typical object-in-context task, two target objects, P and Q, are sequentially presented over two sample phases each paired with a different context, Px and Qy, which are followed by a test with the target objects presented with one of the contexts, Px, Qx (M. A. Good et al., 2007; Langston & Wood, 2010; Norman & Eacott, 2005; Spanswick & Dyck, 2012; Spanswick & Sutherland, 2010; D. I. Wilson et al., 2013). During test, animals generally explore Q more than P. As explained above, generalisation decrement can account for these data because the target stimuli are presented simultaneously with the contextual cues during test (Whitt et al., 2012). Therefore, object-in-context tasks may be a demonstration of direct recognition memory. However, SOP can explain these data equally well using its retrieval-generated priming theory (Brandon et al., 2003; Mazur & Wagner, 1982; Sanderson & Bannerman, 2011; Wagner, 1981) which suggests that object P is associatively primed by x during test. Furthermore, the associative activation of P by x can occur in the absence of P provided that an X→P association has been previously

established (Robinson & Bonardi, 2015). In other words, object Ps representation in memory could be activated by x alone and consequently, responses to P shortly afterwards would be reduced by this indirect process of recognition memory (Robinson & Bonardi, 2015; Whitt et al., 2012).

This is exactly what Whitt et al. (2012) did with rats and what the present experiment attempted to replicate using mice. Mice were sequentially exposed to object P in context x and object Q in context y during the initial sampling stage (Table 8), which was identical to the initial stage of a typical object-in-context task. In a subsequent stage the mice were exposed to context x with no objects present. During test, objects P and Q were presented in a third context (the arena with no wall coverings present). We predicted that mice would explore object Q more than object P during test, as responding to object P would have been reduced due to its associative activation by x, during stage 2. This was based on SOPs retrieval-generated priming theory of association-based memory (Brandon et al., 2003; Mazur & Wagner, 1982; Wagner, 1981) and previous data demonstrating indirect recognition memory in rats (Whitt et al., 2012).

Stage 1		**Stage 2**	**Test**
Trial 1	Trial 2		
Px/Qy	Qy/Px	x	P + Q

Table 8. Experimental design used for Experiment 7, 11, and 12. P and Q represent junk objects and x and y depict different patterned arena wall coverings. The order of Px, Qy trials were counterbalanced across subjects so that half of the mice received Px followed by Qy and the other half received Qy followed by Px.

4.2.2 Materials and method

4.2.2.1 Subjects

The subjects were 16 naive male C57BL/6J mice that were 3 months old (N=16) at the start of the experiment.

4.2.2.2 Procedure

This task consisted of four phases that were arranged as: trial 1 – trial 2 – stage 2 – test, with a 10-minute interval between each phase (Figure 25).

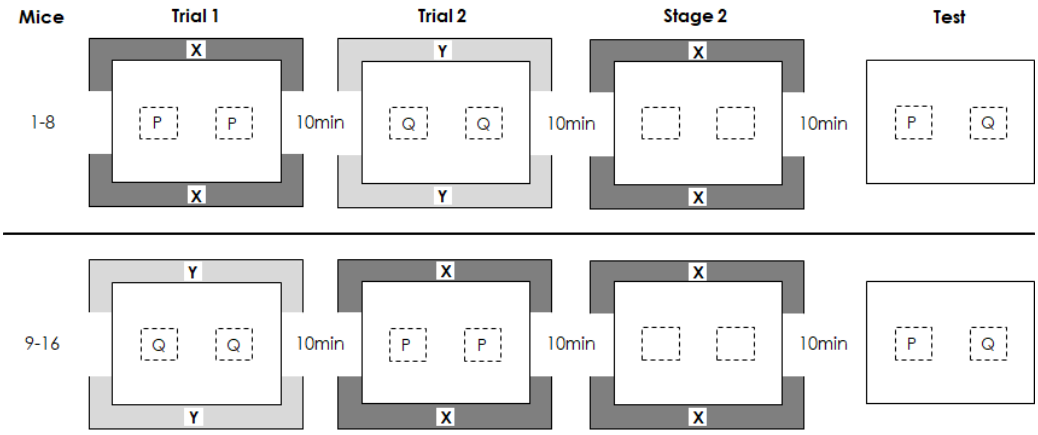


Figure 25. Schematic representation of Experiment 7. P and Q represent objects, x and y denote patterned arena wall coverings, and dashed lines depict the superimposed zones used for data collection.

During each phase, mice were allowed to explore for 5 minutes and then were returned to their home cages. Half of the mice were exposed to object P in context x, during trial 1, and object Q in context y, during trial 2; for the other half of the mice these were reversed (i.e., Qy in trial 1 and Px in trial 2). During stage 2, all mice were exposed to context x with no objects present. Mice were then tested with objects P and Q. Object and context identities were counterbalanced across subjects (Table 9). Left/right objects positions were used throughout.

Animal	**Trial 1**		**Trial 2**		**Stage 2**	**Test**	
	Object	*Pattern*	*Object*	*Pattern*	*x*	*Left*	*Right*
1-2	Deo	Blue	**Tab**	White	Blue	Deo	**Tab**
3-4	**Deo**	Blue	Tab	White	White	**Deo**	Tab
5-6	Tab	Blue	**Deo**	White	Blue	**Deo**	Tab
7-8	**Tab**	Blue	Deo	White	White	Deo	**Tab**
9-10	**Tab**	White	Deo	Blue	Blue	**Tab**	Deo
11-12	Tab	White	**Deo**	Blue	White	Tab	**Deo**
13-14	**Deo**	White	Tab	Blue	Blue	Tab	**Deo**
15-16	Deo	White	**Tab**	Blue	White	**Tab**	Deo

Table 9. Counterbalancing of objects and patterned arena wall coverings during Experiment 7. Bold font represents object used as Q.

4.2.3 Results and Discussion

Mice spent similar proportions of time in the object zones of Q and P during stage 1 sample phases (Qy, Px). This was confirmed using a Student's t-test which analysed the mean percentage of time spent in the object zones during stage 1. The Student's t-test showed no significant difference between the time spent in each of the object zones ($T(15) = -.199$, $p = .845$; Figure 26).

The data of primary interest were those from the test which demonstrated that mice directed more exploration towards object Q than object P (Figure 26), consistent with our prediction. This was confirmed using an ANOVA with object (P/Q) as a within-subjects factor and trial 1 (Px/Qy) as a between-subjects factor. Trial 1 was included in the analysis to test for the possibility of an order effect for mice that had received either Px (trial 1) then Qy (trial 2) or vice versa. This revealed a main effect of object ($F(1, 14) = 5.637$, $p = .032$, $MSe = 7.616$, $\eta_p^2 = .281$), no effect of trial 1 ($F(1, 14) = .036$, $p = .853$, $MSe = .543$, $\eta_p^2 = .003$), and no interaction between the two factors ($p = .465$).

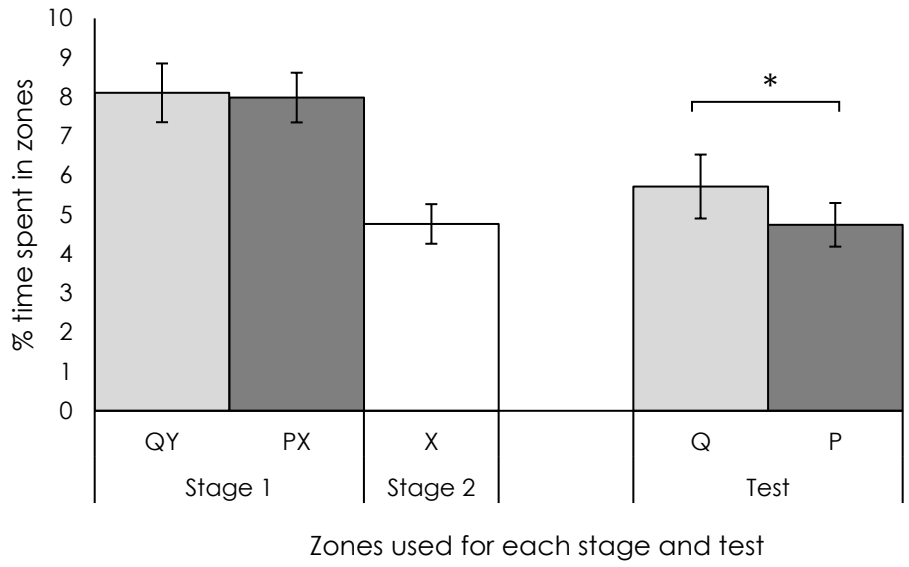


Figure 26. The left set of data denotes the mean percentage of time (±SEM) spent in the zones during stage 1 and stage 2; the right pair of data denotes the mean percentage of time (±SEM) spent in the zones during the first three minutes of the test, during Experiment 7. Asterisk above a bracket indicates $p < .05$ between time spent in object zones.

Experiment 7 successfully replicated the previous study of indirect object recognition that it was based on (Whitt et al., 2012), using mice. These data are consistent with SOP's retrieval-generated priming theory (Brandon et al., 2003; Mazur & Wagner, 1982; Sanderson & Bannerman, 2011; Wagner, 1981) and with other studies related to association-based recognition memory (M. A. Good et al., 2007; Langston & Wood, 2010; Norman & Eacott, 2005; Spanswick & Dyck, 2012; Spanswick & Sutherland, 2010; D. I. Wilson et al., 2013). Because P and Q were presented independently from x, the possibility of generalization decrement effects were unlikely, therefore the results are more suggestive of an association-based form of indirect recognition memory (Sanderson & Bannerman, 2011; Whitt et al., 2012), such as the associative retrieval mechanism proposed by SOP.

SOP suggests that these data may reflect that during Stage 1, an association was formed between the objects and the contexts (x→P and y→Q) and that during Stage 2, elements from object P were provoked into A2 through retrieval-

generated priming, due to its prior association with context x. The increased exploration of Q relative to P, during test, was therefore a consequence of Q having more elements available to enter A1 and produce a stronger response relative to P, due to P having elements already residing in A2. The degree to which responding was reduced towards P would have been dependent on the strength of the association formed between stimuli (x→P) during Stage 1 (Sanderson & Bannerman, 2011). That is, the stronger the association, the more of Ps elements that would have been activated into A2 during Stage 2. Therefore, an association that reached asymptote would have provoked the greatest reduction in responding to P during test.

These results provided evidence to support an associative account of indirect recognition memory. The task design may therefore be a useful base to build on to further explore cue competition effects that may occur during object recognition memory.

4.3 Experiment 8

4.3.1 Introduction

Retrieval-generated priming is a key aspect of SOPs theory of memory formation and is based on the rules of associative learning (Brandon et al., 2003; Mazur & Wagner, 1982; Wagner, 1981).That is, excitatory associations form between the elements of stimuli if elements from those stimuli are simultaneously provoked into the A1 state (Mazur & Wagner, 1982; Wagner, 1981). Future exposure to one of these stimuli results in the activation of the associated stimulus' elements directly into the A2 state and consequently reduces responding to that stimulus. Therefore, this priming process should be susceptible to cue competition effects, such as blocking (Kamin, 1969), because it is dependent on associative contextual information (Brandon et al., 2003; Mazur & Wagner, 1982; Vogel et al., 2020; Wagner, 1981). Thus, Experiment 8 aimed to further explore whether blocking effects could be observed during spontaneous object recognition memory tasks.

The first stage of Experiment 8 was based on the design used during Experiment 7 (Whitt et al., 2012) and consisted of two sequential sample phases designed to establish associations between two object/floor pairings (x→P, y→Q, Table 10). Textured floors were chosen for this phase because the mice had previously discriminated between them, so they should have been suitable (Appendix D; pilot study), and because we wanted to use the contextual arena wall coverings (previously used for this phase during Experiment 7) during the test phase.

Sample		**Compound**	**Test**
Trial 1	Trial 2		
Px/Qy	Qy/Px	Px + Qx in R	P + Q in R

Table 10. Experimental design for Experiment 8. P and Q represent junk objects, x and y denote textured floors, and R depicts a contextual arena wall covering.

The second stage of the experiment was a compound phase in which mice were exposed to one copy of object P and one copy of object Q, presented with floors x and y respectively. Both object/floor pairings were presented within context R. The rationale for this phase was that P would be predicted by x, thus it would have elements moved directly into A2, via retrieval-generated priming, and consequently would suffer a reduced association formation with R (Rx→P). That is that P would have less elements available to enter A1 concurrently with elements of R, thus there would be less opportunities for an excitatory association to form between the elements of R and P. Q would not be predicted by x, therefore would be surprising and able to form a strong association with R, as it would have all of its elements available to enter A1 concurrently with elements of R (Rx→Q; Rescorla & Wagner, 1972; Wagner, 1981). During the test, mice were exposed to objects P and Q in context R. We expected more exploration of P relative to Q because now Q would be predicted by R and P would not. Therefore, Q would have elements provoked directly into A2, via retrieval-generated priming, which would reduce responding to Q, whereas P would have all of its elements available to enter A1 and elicit a relatively stronger response.

4.3.2 Materials and method

4.3.2.1 Subjects

The subjects were 16 male C57BL/6J mice that had been used during a pilot study (reported in Appendix D). These were approximately 4.5 months old at the start of Experiment 8. Because these mice had completed several pilot tasks prior to this experiment, and because they were now a month older than all the mice we had previously used, we also included a second replication. This consisted of 16 naive male C57BL/6J mice that were 3 months old.

4.3.2.2 Procedure

This task consisted of four phases arranged as: trial 1 – trial 2 – compound – test. Each of the sample phases (trial 1 and trial 2) and the compound phase were 10-minutes in length and each of these phases were separated by a 1-hour interval (Figure 27). Half of the mice were exposed to object P with floor x during trial 1 and object Q with floor y during trial 2; for the remainder of mice these were reversed (e.g., Qy in trial 1 and Px in trial 2). The textured floors used were medium grade (120 grit) aluminium oxide sandpaper and galvanized steel square design wire mesh that each measured 25cm x 11.5cm, identical to the ones used during the sandpaper/mesh discrimination task of Experiment 8. During the compound phase, all mice were exposed to one copy of object P and one copy of object Q both with floor x. These were both presented in context R, which was an arena wall covering which covered all four of the arena walls with a distinctive pattern. Mice were then tested for their responses to P and Q presented in R. Left/right object positions were used throughout and object and floor identities and their locations were counterbalanced across subjects (Table 11).

Animal	Trial 1		Trial 2		Compound		Test	
	Object	*Floor*	*Object*	*Floor*	*Left*	*Right*	*Left*	*Right*
1-2	Plug	Mesh	**Salt**	**Sand**	**Salt**	Plug	**Salt**	Plug
3-4	**Plug**	**Mesh**	Salt	Sand	Salt	**Plug**	Salt	**Plug**
5-6	Salt	Mesh	**Plug**	**Sand**	Salt	**Plug**	Salt	**Plug**
7-8	**Salt**	**Mesh**	Plug	Sand	**Salt**	Plug	**Salt**	Plug
9-10	**Salt**	**Sand**	Plug	Mesh	Plug	**Salt**	Plug	**Salt**
11-12	Salt	Sand	**Plug**	**Mesh**	**Plug**	Salt	**Plug**	Salt
13-14	**Plug**	**Sand**	Salt	Mesh	**Plug**	Salt	**Plug**	Salt
15-16	Plug	Sand	**Salt**	**Mesh**	Plug	**Salt**	Plug	**Salt**

Table 11. Counterbalancing of objects, textured floors, and their locations during Experiment 8. Bold text denotes the object that was used as P and the floor that was used as x. During the compound phase all objects were presented with floor x. During the compound phase and test, the contextual arena wall covering R was in place.

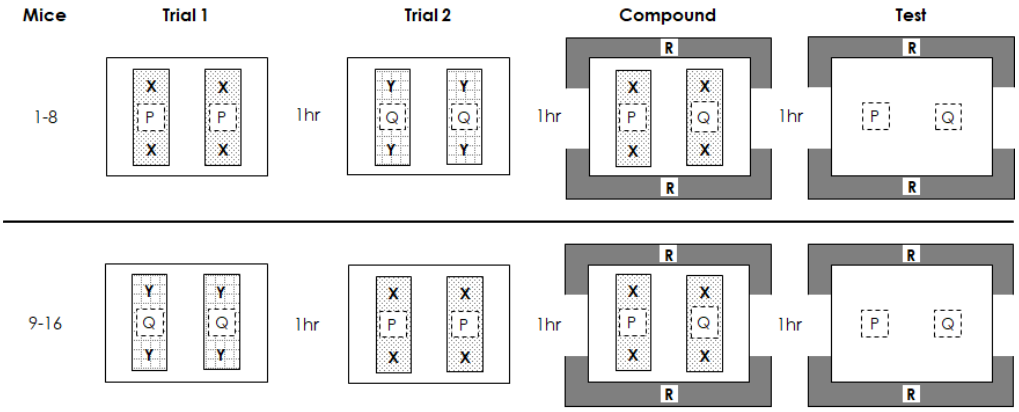


Figure 27. Schematic representation of Experiment 8. P and Q represent junk objects, x and y depict textured floors, R denotes a contextual arena wall covering, and dashed lines represent superimposed zones used for data collection.

4.3.3 Results and Discussion

During the sample phase, mice from both replications spent similar amounts of time exploring objects P and Q in context x and y respectively (Figure 28). This was explored using an ANOVA with object arrangement (Px/Qy) as a within-

subjects factor and replication (1/2) as a between-subjects factor, which analysed the percentage of time that mice spent within the object zones during the sample phase. This revealed no effect of object arrangement ($F(1, 30) = .021$, $p = .886$, $MSe = .264$, $\eta_p^2 < .001$) or replication ($F(1, 30) = .968$, $p = .333$, $MSe = 3.790$, $\eta_p^2 = .031$) and no interaction between them ($F(1, 30) < .001$, $p = .984$, $MSe = .005$, $\eta_p^2 < .001$).

During the compound phase, mice form both replications again spent similar amounts of time exploring objects P and Q in context x (Figure 28). We predicted reduced responding to P relative to Q during this phase because P should have been successfully retrieved by x and Q should not. For the same reason, we also expected to see a reduction in responding to P compared to responding to P during the sample phase. However, responding to both P and Q was greater during the compound phase relative to the sample phase. This was investigated using an ANOVA with phase (sample/compound) and object (P/Q) as within-subject factors and replication (1/2) and trial 1 (Px/Qy) as between-subjects factors. This showed a main effect of phase ($F(1, 28) = 10.387$, $p = .003$, $MSe = 89.376$, $\eta_p^2 = .271$) and returned no other significant results (smallest $p = .197$). There could have been a difference between the exploration of P and Q in x, during the compound phase, but any difference may have been transient. Therefore, we analysed the raw exploration data collected during the compound phase. These data showed similar amounts of exploration for P and Q for this phase. This was explored uisng an ANOVA with object (P/Q) and minute (1-10) as within-subjects factors and replication as a between-subjects factor (1/2). This revealed a main effect of minute ($F(6.2, 185.3) = 3.708$, $p = .001$, $MSe = 37.830$, $\eta_p^2 = .110$) and a minute*replication interaction ($F(6.2, 185.3) = 2.947$, $p = .008$, $MSe = 30.067$, $\eta_p^2 = .089$) and nothing else was significant (smallest $p = .153$). There was a simple main effect of minute during both replications (replication 1: $p = .001$; replication 2: $p < .001$). These results confirmed that there were no significant differences in exploration of the two objects during this phase.

During test, we observed no apparent blocking effect. That is, the percentage of time mice spent exploring P was not greater than the percentage of time that they spent exploring Q (Figure 28), as SOP would have predicted. This was confirmed by an ANOVA, with object (P/Q) as a within-subjects factor and replication (1/2) and trial 1 (Px/Qy) as between-subjects factors, which revealed a main effect of replication ($F(1, 28) = 10.431$, $p = .003$, $MSe = 191.938$, $\eta_p^2 = .271$), no effect of object ($F(1, 28) = .031$, $p = .861$, $MSe = 1.189$, $\eta_p^2 = .001$) and no other significant results (smallest $p = .540$). The mice in replication one appeared to have explored the objects less, during test, than the mice used in replication two (mean ±SEM, replication one, P = 8.4 ± .8, Q = 8.0 ± 1; replication 2, P = 11.7 ± 6.2, Q = 11.3 ± 6.6). Mice also explored the objects more during test than in the compound phase. This was possibly because the floors had been removed and the mice appeared to direct a good proportion of their time to the floors when they were in place. This was confirmed with an ANOVA with phase (compound/test) and object (P/Q) as within-subject factors and replication (1/2) and trial 1 (Px/Qy) as between-subjects factors. This revealed a main effect of phase ($F(1, 28) = 44.437$, $p < .001$, $MSe = 571.384$, $\eta_p^2 = .613$) and a phase*replication interaction ($F(1, 28) = 14.400$, $p < .001$, $MSe = 185.161$, $\eta_p^2 = .340$) and nothing else was significant (smallest $p = .250$). During both replications the objects were explored significantly more during test than the compound phase (replication 1, $p = .048$; replication 2, $p < .001$).

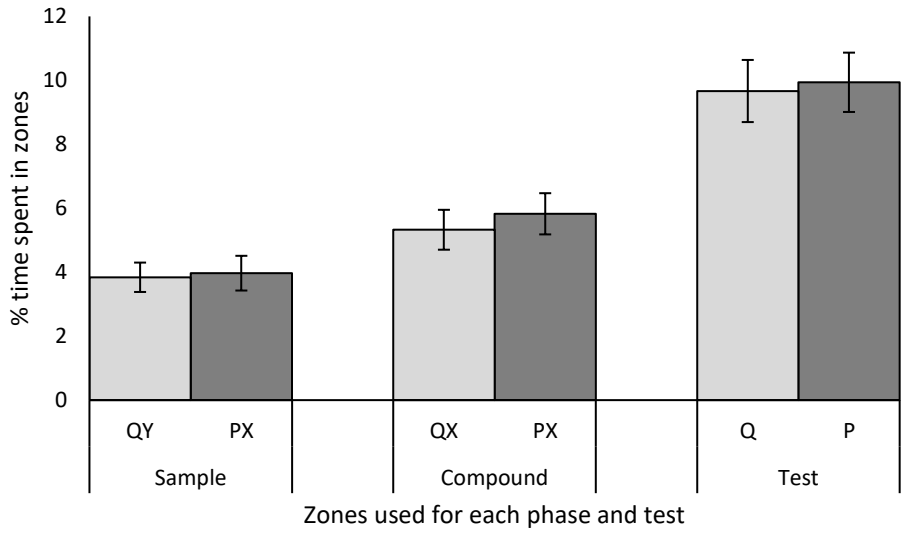


Figure 28. Mean (±SEM) percentage of time spent in the zones during both phases and test. The left set of data denotes the sample phase, the central data represents the compound phase, and the right pair of data depicts the first three minutes of the test, during Experiment 8.

Experiment 8 did not provide any evidence for a blocking effect during the spontaneous object memory task used. If blocking had occurred, we would have expected mice to have directed more attention towards object P than Q, during the test, because Q would have been retrieved by R (sample: y→Q, compound: xR→Q, Test: R→Q), whereas P would not (sample: x→P, compound: xR→P, Test: R→P; e.g., Brandon et al., 2003; Vogel et al., 2019). That is, during the compound phase, P would not have been surprising or unexpected, because of its prior association with x, therefore it would have suffered a reduction in associative strength with R (Rescorla & Wagner, 1972). Consequently, during the test, it would not have been retrieved by R and would have all of its elements available to enter A1 and produce a relatively stronger response. Instead, mice directed similar amounts of exploration towards both objects.

There was also no indication, during the compound phase, that an association had been formed, during the initial sample phase, between P and x. If a strong x→P association had formed, then we would have expected exploration of P to have

suffered a reduction in exploration relative to Q during the compound phase. This was because during the compound phase, P would have been retrieved by x, via retrieval-generated priming where many of its elements would have been provoked into A2, reducing exploratory responses to P, whereas Q would not be predicted by x, thus retrieval-generated priming would not have occurred, therefore all of Qs elements would be available to enter A1 and produce a relatively stronger response. This was surprising as the sample phase arrangement paralleled that of stage 1 of Experiment 7, which provided some evidence of an x→P association, and the time for each trial during Experiment 8 had been increased to 10 minutes, which should have improved the chances of strong associations forming. Furthermore, the blocking cues used were discriminable by mice during Experiment 8 which were also the same mice that completed the first replication of Experiment 8. Taken together, we would have expected strong associations to have formed (x→P, y→Q) during the sample phase of Experiment 8.

One possible reason for the absence of formed associations was that the mice appeared to direct a lot more of their attention towards the textured floors than to the objects. This could have been because the textured floors were likely to have been highly salient to the mice due to their tactile properties (Brecht, 2007; Carandini & Churchland, 2013; Wu et al., 2013). Unfortunately, we did not track mice's exploration of the floors so we cannot confirm this. However, when compared to the mice from Experiment 7, exploration of P during the initial Px sample phase was much lower (Mean ± SEM, Experiment 8: 3.97 ± .54; Experiment 7: 8.1 ± .75), which suggests that this may have been the case. If exploration of x (equivalent to a CS) far exceeded that of P (equivalent to an US) then a strong association between them would be unlikely to form because of inconsistent CS-US pairings. That is, if the CS is repeatedly explored but the US is only occasionally explored following this, then both excitatory and inhibitory associations can occur and consequently may cancel each other out.

Learning theory would predict extinction and SOP explains this by postulating that, when the CS and US are explored temporally contiguously, excitatory

associative strength between them is incrementally increased because of concurrent A1 activation of both the CS and US (e.g., Brandon et al., 2003; Vogel et al., 2020). When the CS is explored without the US, elements of the US are provoked into A2, via retrieval-generated priming, through its association with the CS. Consequently, inhibitory associations occur between the elements of the CS and US, resulting in decrements in associative strength, because of simultaneous A1 and A2 activity of the CS and US respectively. Since association formations between the CS and US are presumed to result from excitatory minus inhibitory associations (Vogel et al., 2020), associative strength could remain unchanged as these associations may have effectively cancelled each other out (Brandon et al., 2003). One way to reduce this possibility in future experiments might be to significantly decrease the size of the textured floors to prevent mice from interacting with them when not in close proximity to the objects.

4.4 Experiment 9

4.4.1 Introduction

Experiment 9 was a replication of the indirect object recognition task (Whitt et al., 2012) used during Experiment 7 but used transgenic APP/PS1 mice and their wild type littermates, as opposed to C57BL/6J mice. Our aim was to add to previous reports of apparent association-based memory being impaired in these mice, during modified object-in-place tasks (Ameen-Ali et al., 2012; Dix & Aggleton, 1999), at 5 months of age (Bonardi et al., 2016, 2021). Although at this age these mice have suffered amyloid-β protein related alterations in the brain (Garcia-Alloza et al., 2006; Hong et al., 2016; Pedrós et al., 2014; Ruan et al., 2009; Zhu et al., 2017), they are generally not impaired in learning and memory tasks (Bonardi et al., 2011; Kelly et al., 2017; Kilgore et al., 2010), though some mild cognitive deficits start to be observed (Holcomb et al., 1998). The authors that reported the deficits during the modified object-in-place tasks (Bonardi et al., 2016, 2021), interpreted their results in terms of SOP (Mazur & Wagner, 1982; Wagner, 1981) and suggested that the impairment may have been in retrieval-generated priming

but not in self-generated priming. They speculated that the general absence of deficits in these mice in typical SOR tasks, at this age, may be because in many tasks both priming processes contribute to performance and that self-generated priming may be able to compensate enough to yield levels of performance that seem normal.

Thus, we predicted that 5-month-old APP/PS1 mice would be impaired relative to their wild type littermates on the indirect object recognition memory task. This was based on the results of Experiment 7, which added to the suggestion that the task may be dependent on retrieval-generated priming (Whitt et al., 2012), and on previous data which demonstrated selective deficits in association-based tasks, in these mice, which may reflect impaired retrieval-generated priming (Bonardi et al., 2016, 2021).

4.4.2 Materials and method

4.4.2.1 Subjects

The subjects were 29 APPswe/PS1dE9 transgenic mice, 12 positive (3 males and 9 females) and 17 wild type animals (8 males and 9 females). Our initial aim was to use 32 animals with genotype and sex equally balanced. However, our breeding facility was unable to produce this number thus we had to continue the experiment with an unbalanced, suboptimal sample size.

4.4.2.2 Procedure

This task was identical to the task used during Experiment 7 (Figure 25/Table 8). Object and context identities, and object positions were counterbalanced across subjects and genotypes (Table 11).

Animal	**Trial 1**		**Trial 2**		**Stage 2**	**Test**	
	Object	*Pattern*	*Object*	*Pattern*	*x*	*Left*	*Right*
Wild type mice							
1-2	Deo	Blue	**Tab**	White	Blue	Deo-**Tab**	**Tab**-Deo
3-4	**Deo**	Blue	Tab	White	White	**Deo**-Tab	Tab-**Deo**
5	Deo	Blue	**Tab**	White	Blue	Deo	**Tab**
6	**Deo**	Blue	Tab	White	White	Tab	**Deo**
7-8	**Tab**	Blue	Deo	White	White	Deo-**Tab**	**Tab**-Deo
9-10	Tab	Blue	**Deo**	White	Blue	**Deo**-Tab	Tab-**Deo**
11	Tab	Blue	**Deo**	White	Blue	**Deo**	Tab
12	**Tab**	White	Deo	White	White	**Tab**	Deo
13	**Tab**	Blue	Deo	White	White	**Tab**	Deo
14	**Deo**	Blue	Tab	White	White	**Deo**	Tab
15	Deo	Blue	**Tab**	White	Blue	Deo	**Tab**
16	**Deo**	Blue	Tab	White	White	**Deo**	Tab
17	Tab	Blue	**Deo**	White	Blue	Tab	**Deo**
APPswe mice							
1-2	**Deo**	Blue	Tab	White	White	**Deo**-Tab	Tab-**Deo**
3	Deo	Blue	**Tab**	White	Blue	Deo	**Tab**
4-5	Tab	Blue	**Deo**	White	Blue	**Deo**-Tab	Tab-**Deo**
6-7	**Tab**	Blue	Deo	White	White	Deo-**Tab**	**Tab**-Deo
8	Deo	Blue	**Tab**	White	Blue	**Tab**	**Deo**
9	Deo	Blue	**Tab**	White	Blue	Deo	**Tab**
10	**Deo**	Blue	Tab	White	White	**Deo**	Tab
11	Tab	Blue	**Deo**	White	Blue	Tab	**Deo**
12	**Tab**	Blue	Deo	White	White	**Tab**	Deo

Table 11. Counterbalancing of objects, their locations during test, and the patterned arena wall coverings used during Experiment 7. Bold font represents object used as Q.

4.4.3 Results and Discussion

Three female mice were excluded from the analyses due to low levels of exploration (the exclusion criterion for this was reported in Chapter 2). One of them was wild type and the other two were APPswe.

During stage 1 of Experiment 9, the mice directed similar amounts of exploration towards objects P and Q regardless of genotype (Figure 29). However, males explored the objects more than females (mean percentage of time ± SEM: males, 6.1 ± .7; females, 4.9 ± .6). The mean percentage of time spent in the zones of objects Q and P by the mice, during stage 1, was explored using an ANOVA with object (Q/P) as a within-subjects factor and genotype (APPswe/wild type) and sex (male/female) as between-subjects factors. This showed a main effect of sex ($F(1, 22) = 4.375$, $p = .048$, $MSe = 32.938$, $\eta_p^2 = .166$) and that genotype approached but did not reach significance ($F(1, 22) = 3.309$, $p = .083$, $MSe = 24.914$, $\eta_p^2 = .131$) and returned no other significant results (smallest $p = .178$).

During the test, mice explored object P more than object Q independent of genotype, sex, and which object they explored first during trial 1 (Figure 29). The mean percentage of time spent in the zones of objects Q and P, during the first three minutes of test was analysed using a mixed ANOVA with object (P/Q) as a within-subjects factor and genotype (APPswe/wild type), sex (male/female) and trial 1 (Px/Qy) as between-subjects factors. There was an effect of object ($F(1, 18) = 7.177$, $p = .015$, $MSe = 31.394$, $\eta_p^2 = .285$) and a 3-way interaction between object, sex, and genotype that approached but did not reach significance ($F(1, 18) = 3.635$, $p = .073$, $MSe = 15.900$, $\eta_p^2 = .168$) and nothing else was significant (smallest $p = .155$).

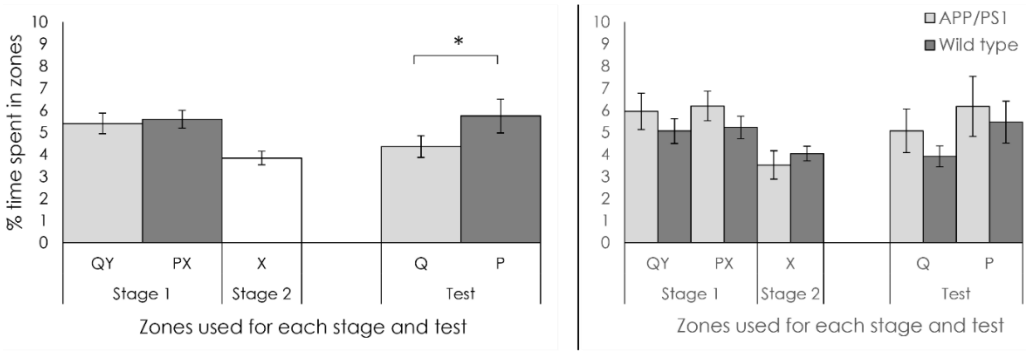


Figure 29. The left panel shows the overall data independent of genotype, and the right panel shows each genotype separately, for Experiment 9. For each panel, the mean percentage of time (±SEM) spent in the zones are reported. In both panels, the left set of data represents stage 1 and stage 2, and the right pair of data corresponds to the first three minutes of the test.

During the test, mice explored object P significantly more than object Q independent of genotype. This was the reverse effect to that observed during Experiment 7 and was inconsistent with our prediction. Similar results have been reported in rats with excitotoxic hippocampal lesions (Honey & Good, 2000a, 2000b). During this study, the lesioned rats directed more orientation towards a primed stimulus than an unprimed stimulus whereas control rats exhibited the opposite orienting response. The results of this study were discussed in relation to SOP (Honey & Good, 2000a, 2000b) and it was suggested that hippocampal damage may have resulted in reductions in either, the weighting of elements in the A1 state via attention (greater weighting produces a stronger response) or in the perceived intensity of the target (e.g., the proportion of elements provoked into the A1 state is smaller in hippocampal rats versus controls). They suggested that either of these consequences could dramatically influence the direction of the priming effect.

They described the potential variation in the SOP priming effect further. In the context of Experiment 9, this would be described as follows. Context x places a proportion of object P's elements into A2 (P_{A2}, e.g., $P_{A2} = 0.5$) which reduces the number of elements that P has available to move into A1 (1 - P_{A2}). Of these

available elements, only a proportion will move into A1 (P_1). The strength of the response elicited by P is a combined effect of the proportion of P's elements primed by x into A2 (P_{A2}) and the proportion of P's elements ($P_1(1 - P_{A2})$) provoked into A1 by P. The effect of elements residing in A1 and A2 have separate weighting factors (W_1 and W_2 respectively). If elements in A1 generate a stronger response than elements in A2 (i.e., $W_1 > W_2$) and an impromptu presentation of P moves all of its elements to A1 (i.e., $P_1 = 1$), then the presentation of x prior to P should reduce the response elicited by P. However, if P is less intense and consequently can only provoke a smaller number of elements into A1 (e.g., $P_1 = 0.25$) then presentation of x prior to P could enhance responding. Specifically, provided that P_1 is less than 1, the proportion of elements that are primed by x ($P_1(1 - P_{A2}) + P_{A2}$) will be greater than the proportion activated when P is unannounced (P_1). Therefore, whether x enhances or reduces responding to P depends on the values of W_1 and W_2. Simply put, if the elements in A1 are no more likely to generate a response than elements in A2 (i.e., $W_1 = W_2$) and $P_1 < 1$, then P will generate a stronger response when it is preceded by x than when it is not. However, if A1 is more effective at producing a stronger response than A2 (i.e., $W_1 > W_2$) and an unannounced presentation of P moves all of its elements to A1 (i.e., $P_1 = 1$), then presenting x prior to P will reduce responding to P.

This may indicate that lower levels of object exploration may have resulted in a reverse-priming effect (Donegan, 1981). For the APP/PS1 mice used during Experiment 9, this may have been due to the hippocampal damage present at this age (Garcia-Alloza et al., 2006; Hong et al., 2016; Pedrós et al., 2014; Ruan et al., 2009; Zhu et al., 2017). However, it is puzzling that the same results were obtained from the wild type controls as these mice should have no hippocampal burden at this age. If the effect was a result of an attentional deficit, then although unlikely, it is possible that the wild type mice could also have shown a similar reduction in attention. In the APP/PS1 mice the attentional deficit would likely be due to pathological changes in the brain whereas in the wild type mice it would be due to a behavioural change which resulted in less object exploration. For example, wild

type littermates have been shown to display modified behaviour which more closely mimics that of their knockout mice littermates (Kalbassi, Bachmann, Cross, Roberton, & Baudouin, 2017). In this study, mice with a deletion in Neuroligin-3 (Nlgn3) exhibited deficits in social behaviour as did their wild type littermates. However, when Nlgn3 was re-expressed in the knockout mice, both the wild type and knockout mice displayed normalised social behaviour.

Apart from the strain of mice, the main difference between the C57BL/6J mice used during Experiment 7 and the wild type mice used here were their ages during the tasks (3 months-old and 5 months-old respectively). It is possible that older mice directed less attention towards objects than younger mice. For example, when C57BL/6J mice at 2 and 6 months old were compared during a modified novel object recognition memory task, the 2-month-old mice displayed significantly higher levels of exploration during the sample phase than the 6-month-old mice (Wu et al., 2013). The data from Experiment 7 and 10 are consistent with this notion. During Experiment 7, mice displayed more exploratory behaviour across trial 1 and 2 than did the older wild type mice of Experiment 9 (Mean % time ± SEM, Experiment 9: 5.1 ± .37; Experiment 7: 8.0 ± .48). We explored the possibility that age may have been the confounding variable during this task by running another replication, using 5-month-old C57BL/6J mice, in Experiment 10.

4.5 Experiment 10

4.5.1 Introduction

Experiments 7 and 9 produced contrasting results. Apart from the different strains used between experiments, the main difference between them was the ages of the mice (3 and 5 months-old during Experiment 7 and 9 respectively). We speculated that reduced attention towards objects by older mice, an occurrence that has been reported in mice undertaking these types of memory task (Wu et al., 2013), may impact upon the test results obtained from the indirect recognition memory task. Thus, to explore whether the indirect recognition memory task was age sensitive

we replicated the task again, during Experiment 10, using both 3- and 5-month-old mice. We then combined these data with the data from Experiment 7 so that we had two replications for each age group.

4.5.2 Materials and method

4.5.2.1 Subjects

The subjects were 48 male C57BL/6J mice. At the start of the experiment, the first two replications (N=16 per replication) of mice were 5 months old and the third replication (N=16) were 3 months old. During the first replication of 5-month-old mice, three had to be removed from the experiment due to very low levels of exploration (the criterion for exclusion was described in Chapter 2). Therefore, a second replication of 5-month-old mice was included.

4.5.2.2 Procedure

This task was identical to the task used in Experiment 7 (Figure 25/Table 8). A between-subjects design was used so that the task was completed by the first replication of mice (5 months old), and then by the second replication of mice (5 months old), and finally the third replication of mice (3 months old). These data were then combined with the data from Experiment 7, which resulted in 4 separate replications, two for each age group.

4.5.3 Results and Discussion

Two outliers were excluded from these analyses, they were 3-month-old mice (the criterion for exclusion was described in Chapter 2). Mice of both ages spent similar amounts of time exploring objects P and Q during stage one, but there was some overall variance in this exploration between age and replication (Figure 30). An ANOVA with trial type (Px/Qy) as a within-subjects factor, age (3/5) as a between-subjects factor and replication (2, 3, 4) as a covariate showed effects of age ($F(1, 40) = 14.993$, $p < .001$, $MSe = 113.087$, $\eta_p^2 = .273$) and replication ($F(1, 40) = 29.271$, $p < .001$, $MSe = 220.779$, $\eta_p^2 = .423$) but no effect of trial type ($F(1,$

40) = .001, p = .975, MSe = .005, η_p^2 < .001) and no interactions between these factors (smallest p = .895).

During the test, mice directed similar amounts of exploration towards objects P and Q, and this appeared not to differ between ages or replications (Figure 30). This was explored using an ANOVA with object (P/Q) as a within-subjects factor, age (3/5) and trial 1(Px/Qy) as between-subjects factors, and replication (2, 3, 4) as a covariate. This revealed no effects of age (F(1, 38) = .605, p = .441, MSe = 10.449, η_p^2 = .016), replication (F(1, 38) = 2.907, p = .096, MSe = 50.177, η_p^2 = .071), object (F(1, 38) = .164, p = .688, MSe = 1.814, η_p^2 = .004), trial 1 (F(1, 38) = .172, p = .681, MSe = 2.971, η_p^2 = .005) and no interactions between these factors (smallest p = .091, object*trial 1 interaction).

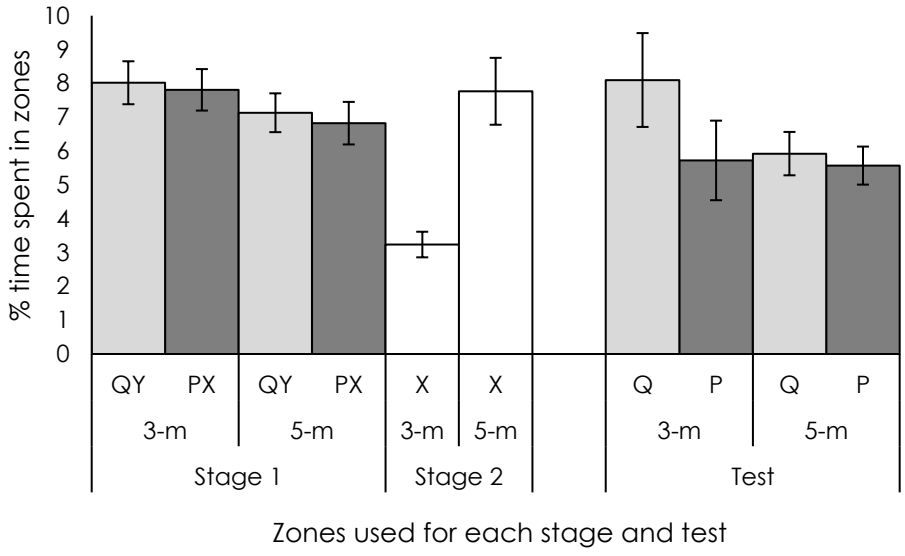


Figure 30. The left set of data denotes the mean percentage of time (±SEM) spent in the zones during stage 1 and stage 2; the right pair of data represents the mean percentage of time (±SEM) spent in the zones during the first three minutes of the

test, during Experiment 10. The ages of the mice are shown as 3-m and 5-m which depict 3-months-old and 5-months-old respectively.

Next, we combined the data from Experiment 7 with Experiment 10 to further analyse potential differences between different ages and replications of mice. We first explored stage 1 where we found no differences in exploration levels of Q and P, between 3- and 5-month-old mice or between each replication (Figure 31). An ANOVA with trial type (Px/Qy) as a within-subjects factor, age (3/5) as a between-subjects factor, and replication (1/2/3/4) as a covariate was used to explore these data. This indicated no effect of age ($F(1, 56) = 2.650$, $p = .109$, $MSe = 32.763$, $\eta_p^2 = .045$), replication ($F(1, 56) = 1.385$, $p = .244$, $MSe = 17.125$, $\eta_p^2 = .024$), or trial type ($F(1, 56) = .012$, $p = .913$, $MSe = .054$, $\eta_p^2 < .001$) and no interactions between these factors (smallest $p = .751$).

Next, we examined the test data which showed no differences in exploration of Q and P, but did show some variance between replications. This was analysed using an ANOVA with object (P/Q) as a within-subjects factor, trial 1 (Px/Qy) and age (3/5) as between-subjects factors, and replication (1/2/3/4) as a covariate. This revealed an effect of replication ($F(1, 54) = 4.450$, $p = .040$, $MSe = 73.951$, $\eta_p^2 = .076$), no effects of age ($F(1, 54) = .267$, $p = .607$, $MSe = 4.442$, $\eta_p^2 = .005$), object ($F(1, 54) = .004$, $p = .950$, $MSe = .033$, $\eta_p^2 < .001$), or trial 1 ($F(1, 54) = .002$, $p = .968$, $MSe = .027$, $\eta_p^2 < .001$), and no other significant results (smallest $p = .105$; Figure 31).

To explore whether exploration levels during the stage 1 sample phases affected discrimination during the test, we analysed the test data and included the raw total object exploration times for stage 1 as a covariate. These data revealed that there was some variance in exploration levels, but this did not appear to affect discrimination of the objects during the test. To explore this we used an ANOVA with object (P/Q) as a within-subjects factor, trial 1 (Px/Qy) as a between-subjects factor, and total object exploration during stage 1 as a covariate. This revealed an

effect of exploration ($F(1, 51) = 28.734$, $p < .001$, $MSe = 336.681$, $\eta_p^2 = .339$) and no other significant results (smallest $p = .087$; object*trial 1 interaction).

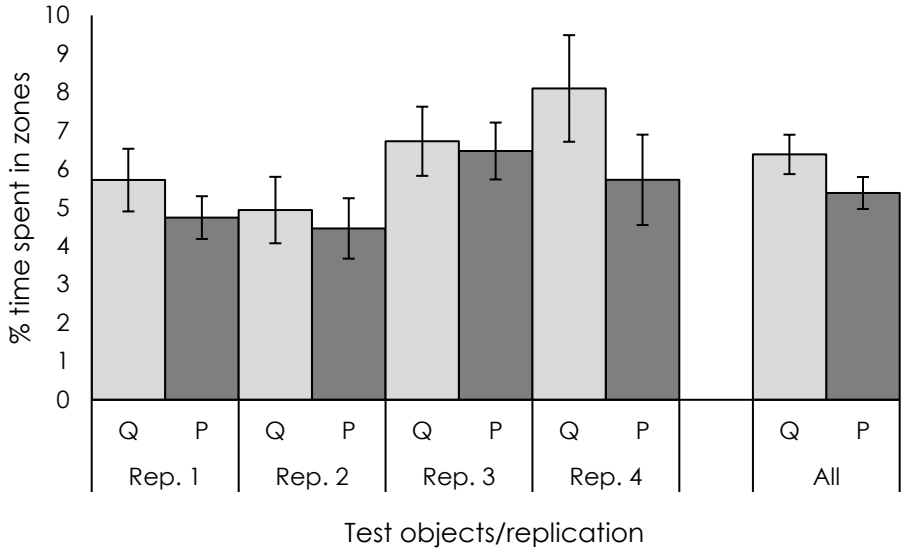


Figure 31. Mean percentage of time (±SEM) spent in the zones during the test for each replication of Experiment 7 and 10. Replication 1 denotes the 3-month-old mice used during Experiment 7; replications 2 and 3 depict the 5-month-old mice used during the first and second replications of Experiment 10, respectively; replication 4 represents the 3-month-old mice used during the third replication of Experiment 10.

Experiment 10 explored the possibility that indirect object recognition memory effects may be sensitive to the age of the mice used. Our transgenic mice displayed a reverse-priming effect which we hypothesised may have been because of low levels of object exploration by these mice. Thus, we wanted to investigate whether older mice would explore objects less than younger mice, which has previously been shown (Wu et al., 2013), and whether this would produce a reverse-priming effect. Moreover, for indirect recognition to occur a certain level of exploration would be required for a strong association to form between the objects and contexts. According to SOP, this is because for excitatory associations to form, the stimuli (e.g., x and P) must both have elements simultaneously provoked into the

A1 state. With more exploration, x and P will consequently both have more elements activated into A1, and the more elements they have concurrently in A1, the stronger the association will be (Mazur & Wagner, 1982; Wagner, 1981). Therefore, a stronger association would result in more elements of P being associatively activated into A2 during exposure to x, thus during the test, exploration of P would be greatly reduced relative to Q. However, we found no evidence to support any differences between older (5-month-old) and younger (3-month-old) mice, during the indirect object recognition memory recognition task. We also failed to replicate the effect we observed during Experiment 7.

When we combined the data from Experiments 7 and 10, we also saw no apparent effect. However, the mean percentage of exploration for objects P and Q during the test, for each replication, were all in the direction of Q being explored more than P. This suggests that there may have been a very weak effect. Associative effects such as this require conditioning to reach asymptote if they are to be effective (e.g., Jennings & Bonardi, 2017; Jones & Haselgrove, 2013; Sanderson et al., 2016). Therefore, if there was a weak effect across the four replications, then the priming cue x had not acquired enough associative strength with P to reach the asymptotic levels of conditioning required for a strong priming effect to have occurred. This could have been because of the relatively low levels of exploration during the stage 1 sample phases. For example, our mice explored the objects during these sample phases for around 7-8 percent of the total exposure time. In contrast, the rats used during the study by Whitt et al. (2012) explored the objects during the same phases for approximately 20-25 percent of the total exposure time. This could explain why we failed to replicate their experiment which included two successful replications using rats. When directly compared, during this type of object recognition memory task, it has been reported that rats and mice exhibit similar levels of object exploration to one another (Stranahan, 2011). Thus, it is unlikely that the variation in the total object exploration observed, between our study and the study by Whitt et al. (2012), resulted from species differences and instead is more likely to have occurred due to other experimental parameters. In fact, their arenas were identical in size to the ones we used. Because rats are

substantially larger than mice, the relative size of the arenas used by them and us were very different. The objects that they used were also much larger than the ones we used. This would have reduced the floor space available for their rats to explore, in between exploring the objects, relative to the floor space available for our mice. This may account for the relatively low levels of exploration by our mice as they would have had far more space to occupy and explore, in between object exploration, relative to the rats used during the study by Whitt et al. (2012).

In summary, we failed to replicate the results of Experiment 7 and found no evidence of age differences during the task used. Failure to demonstrate indirect object recognition memory effects may have been due to the low levels of object exploration that the mice exhibited.

4.6 General discussion

Chapter 4 examined indirect object recognition memory effects and further explored the possibility of blocking effects during object recognition memory tasks. SOP suggests that animal's performance during association-based memory tasks, such as the tasks used during this chapter, are modulated by an associative priming process termed retrieval-generated priming (Mazur & Wagner, 1982; Wagner, 1981). When an object is presented within a specific context (e.g., object P in context x), SOP postulates that both P and x will have elements activated into A1 simultaneously and consequently, that an excitatory association will form between the elements of P and x (x→P). A later encounter with x will provoke elements of P directly into A2 which will indirectly reduce responses to P in the short term. That is, if P is encountered with x, P would suffer reduced responding. If P is encountered shortly after x, as it was during our Whitt et al. (2012) replication experiments, P would also suffer reduced responding. The reduction in responding to P is proportional to the number of elements associatively activated into A2 by x. The greater the number of elements activated, the greater the reduction. The number of elements activated is dependent on the strength of the x→P association (Brandon et al., 2003; Mazur & Wagner, 1982; Wagner, 1981).

Experiment 7 successfully replicated the indirect object recognition memory results reported by Whitt et al. (2012). Mice were sequentially presented with object P in context x and object Q in context y, with task order counterbalanced. They were then presented with x with no objects present. This was followed by a test with P and Q where mice directed more exploration towards Q relative to P. This was consistent with SOP's theoretical retrieval-generated priming process which suggests that x→P and y→Q associations would have formed during the initial sample phases. When presented with x, some of P's elements would have been activated into A2. Therefore, during the test that followed, responding to P would have been reduced relative to Q because P would be less surprising, as it would have had relatively more elements residing in A2, thus Q would have more elements available to enter A1 and elicit stronger responding compared with P.

Experiment 9 replicated Experiment 7 using APP/PS1 transgenic mice and their wild type littermates. A reverse-priming effect was observed in that mice directed more exploration towards P than Q. The results from the APP/PS1 mice were consistent with SOP and with previous work that suggested that there was a selective deficit in retrieval-generated priming in these mice, at this age (Bonardi et al., 2016, 2021). The hippocampal damage present in APP/PS1 mice at this age (Garcia-Alloza et al., 2006; Hong et al., 2016; Pedrós et al., 2014; Ruan et al., 2009; Zhu et al., 2017) may have resulted in a reduction in the weighting of elements in the A1 state, via reduced attention (Honey & Good, 2000a, 2000b). However, the same results were observed for the wild type littermates which was a little puzzling. We speculated that this could have been due to a reduction in attention in these mice that arose because of the mice modifying their behaviour to closer mimic that of their littermates, an effect that has previously been reported in transgenic mice and their wild type littermates (Kalbassi et al., 2017).

Apart from strain differences, we also suggested that the results of Experiment 9 may have been due to the mice being older than the mice used during Experiment 7. We directly tested whether age influenced indirect object recognition memory effects during Experiment 10. We found no evidence to support this and failed to

replicate the results of Experiment 7. When we combined the data from the two experiments (7 and 10) we saw no significant difference between exploration of P and Q. However, all the means for all four replications were in the direction of Q being explored more than P. We suggested that this may have indicated that a weak effect was present and that this may have been due to the low levels of object exploration exhibited by the mice. According to SOP, a strong effect would have required a strong x→P association to have formed (Brandon et al., 2003; Mazur & Wagner, 1982; Wagner, 1981). The strength of such an association would be determined by the number of elements that x and P have activated in A1 simultaneously during the sample phase. The more elements x and P have concurrently in A1, the stronger the excitatory association between them would be. Therefore, low levels of object exploration would have resulted in fewer elements of P being activated into A1 and fewer opportunities for this to have occurred concurrently with elements of x. With a weak x→P association, x would only have been able to prime a small number of P's elements into A2. During the test that followed, the relatively small number of P's elements residing in A2 may not have been sufficient to significantly reduce responding to P relative to Q.

These low levels of exploration, and the impact that this may have had on excitatory association formation, may also account for the results of Experiment 8 where we failed to provide evidence of a blocking effect, during an object recognition memory task. During the compound phase of the blocking task used, we would have expected a reduction in exploration of P, relative to Q, if a strong x→P association had formed earlier, during the sample phase. Instead, we observed no differences in exploration between P and Q, during the compound phase, which suggested that the blocking cue x had not acquired the levels of associative strength with P that would have been required for blocking to have occurred (Jennings & Bonardi, 2017; Jones & Haselgrove, 2013; Sanderson et al., 2016).

For future work, low levels of object exploration could be improved by reducing the floor space so that mice come into close proximity with the objects more often

and by moving the objects nearer to the corners of the arena. Both of these measures have previously been shown to increase object exploration in mice (Pacchiarini, 2019). This could also strengthen the x→P association because x and P would be in closer proximity to one another, therefore, the temporal contiguity between them would be more favourable for association formation. In other words, excitatory associative strength between them would incrementally increase at a faster rate because of more concurrent A1 activation of both X and P (Brandon et al., 2003; Vogel et al., 2020).

Another possible explanation for the failure to detect a reliable priming effect is that self-generated priming could have impacted the test performance of the mice. For example, during the sample phases, P and Q would be explored and both would have elements provoked into A1. These elements would decay into A2 and then slowly begin to decay back to inactive. Because the time between the sample phases and the test were short (10 minutes between each phase, trial 1, trial 2, stage 2, and test), it is likely that both P and Q would have had many elements residing in A2, during the test, because of object exploration during the sample phases. Elements of Q and P residing in A2 would have reduced responding towards these objects. Because P would have had many elements residing in A2 during stage 2, when mice were presented with x alone, P would have had very few elements available to be moved directly into A2 via retrieval-generated priming, through its prior association with x. Therefore, the relative difference between the number of elements that P and Q would have had in A2, during the test, may have been very small. This possibility could be avoided by moving stage 2 (x alone) and the test to the following day. This would allow the A2 activity produced during the sample phases to have dissipated by the test. For example, in a relative recency task (Mitchell & Laiacona, 1998) where two pairs of identical objects are sequentially presented over two sample phases (e.g., A and A – interval – B and B), followed by a test with one object from each pair (e.g., A and B), animals generally explore the less recent object more during test (e.g., A). SOP explains this typical test result by suggesting that self-generated priming reduced responding to B relative to A. When the retention interval (the time between the

second sample and test) is 5-15 minutes this effect seems robust, whereas when the retention interval is extended beyond this the effect becomes unreliable (Hatakeyama et al., 2018; Mitchell & Laiacona, 1998; Tam et al., 2014). SOP accounts for this by suggesting that an increased retention interval may allow sufficient time for the elements of both objects to decay from A2 back to inactive. Therefore, using an interval of 24 hours between the sample phase and stage 2 (x alone) should allow enough time for A2 activity to dissipate and not interfere with test performance.

Furthermore, moving these phases should not negatively impact indirect object recognition memory effects if these effects are a product of retrieval-generated priming. This is because retrieval-generated priming is dependent on formed associations that should persist over time. For example, Ramsaran, Sanders, and Stanton (2016) exposed rats to two pairs of objects sequentially presented over two sample phases (e.g., A and A – interval – B and B), each pair in a different context (e.g., xA and yB), and then tested them with a one copy of each object presented in one of the contexts (e.g., xA and xB). During the test, rats explored the object not associated with the test context (e.g., B) more than the object that had a prior association with it (e.g., A). These results are consistent with SOPs retrieval-generated priming theory which suggests that A would have been retrieved by x during the test, whereas B would not. Ramsaran et al. (2016) reported that this effect was equally robust when the retention interval was either 5 minutes or 24 hours. Therefore, any effects of indirect object recognition memory should be preserved if the interval between the sample phases and stage 2 (x alone) were to be extended to 24 hours.

4.7 Conclusion

Chapter 4 provided some evidence that was consistent with SOP during Experiment 7 but failed to replicate these data during Experiment 10. A potential issue throughout this series of experiments was low levels of object exploration exhibited by the mice. Generally, rats and mice display similar amounts of object exploration during these types of tasks (e.g., Stranahan, 2011). However, our mice

exhibited significantly lower object exploration compared with the rats used by Whitt et al. (2012), which may explain the inconsistencies in our data compared with theirs. Future work should try to address this issue through modifications in the experimental setup such as by significantly reducing the floor space around the objects and by moving the objects closer to the corners of the arena. These improvements should result in much greater levels of object exploration and remove this as a potential confounding variable.

Chapter 5: Recency-based object discrimination

5.1 General introduction

Chapter's 3 and 4 were focused on retrieval-generated priming and thus used tasks that likely required association-based memory processes. These included object-in-context, indirect object recognition and blocking tasks which have all been suggested to reflect such associative processes (e.g., Rescorla & Wagner, 1972; Sep et al., 2021; Whitt et al., 2012). Chapter 5 instead focused on SOPs other nonassociative priming process; self-generated priming (Mazur & Wagner, 1982; Wagner, 1981). Self-generated priming occurs when a stimulus is encountered multiple times within a short timeframe. Consequently, this repeated exposure to the stimulus reduces responding towards the stimulus over time (i.e., short term habituation, Sanderson & Bannerman, 2011).

The relative recency spontaneous object recognition task (Mitchell & Laiacona, 1998) consists of two pairs of identical objects sequentially presented over two sample phases (e.g., A and A – interval – B and B), followed by a test with one object from each pair (e.g., A and B) where subjects generally explore the less recent object more during test (e.g., A). This task has been widely used with rats and mice to explore various memory processes and their underlying neural mechanisms (G. R. Barker et al., 2007; Bonardi et al., 2016, 2021; M. A. Good et al., 2007; Hannesson et al., 2004; Mitchell & Laiacona, 1998; Nelson et al., 2011; Sanderson et al., 2011; Tam et al., 2015; Tam et al., 2014). Evidence has been provided that specific brain sites are necessary for recency discriminations (For a review see: Aggleton & Nelson, 2020) with the most support for the involvement of the prefrontal cortex (G. R. Barker et al., 2007; Hannesson et al., 2004; Mitchell & Laiacona, 1998; Nelson et al., 2011), perirhinal cortex (G. R. Barker et al., 2007; Hannesson et al., 2004; Warburton & Brown, 2010, 2015), and hippocampus (Albasser, Amin, Lin, Iordanova, & Aggleton, 2012; G. Barker & Warburton, 2011; M. A. Good et al., 2007; Kesner, Hunsaker, & Ziegler, 2010). This task maps well onto self-generated priming and it is unlikely that retrieval-generated priming contributes to test performance. Because the context used during the sample and

test phases for both objects is the same throughout, associations formed with the context should be matched for both objects thus it is doubtful that retrieval-generated priming supports performance during this task (Robinson & Bonardi, 2015; Sanderson et al., 2011). However, context→object associations formed during sample phase 1 would have the opportunity to extinguish during sample phase 2.

The influence of manipulating the sample-sample and retention intervals have previously been explored (Hatakeyama et al., 2018; Mitchell & Laiacona, 1998; Tam et al., 2014). For example, Mitchell and Laiacona (1998) investigated the impact of increasing the retention interval from 1 hour to 168 hours (with increments of 6, 24 and 72 hours) using rats and found that recency discrimination was robust from 1 to 24 hours but had dissipated from 72 hours. Inconsistent with these results, Hatakeyama et al. (2018) performed a similar experiment with rats and reported a strong recency discrimination with a retention interval of 15 minutes but not at 3 or 75 minutes. The main difference between the two tasks was that different durations were used for the sample-sample intervals, 1 hour (Mitchell & Laiacona, 1998) and 125 minutes (Hatakeyama et al., 2018). Interestingly, Hatakeyama et al. (2018) also tested the effect of using different durations for the sample-sample interval and reported that recency performance was good using 65 minutes, significantly improved using 125 minutes, and disappeared at a short 11-minute interval. Similarly, Tam et al. (2014) also compared the effect of using a sample-sample interval of 5 minutes and 2 hours both with retention intervals of 5 minutes. They reported that recency performance was significantly better when the interval was 2 hours than when it was 5 minutes.

Taken together, these results indicate that increasing the sample-sample interval increases recency performance and that shorter retention intervals are more likely to retain this performance. This is exactly what most theories including SOP would predict. SOP would explain this by suggesting that a longer sample-sample interval would allow more time for the first sampled objects elements to decay from A2 to inactive, therefore, it would have relatively more elements available to

enter A1, during test, compared with the second sampled object. A short retention interval would mean that the second sampled object would have many elements still residing in A2 during test, thus, responding to this object would be less likely. Both manipulations increase recency performance by increasing the difference between levels of A2 activity, between the two objects, during the test.

A further prediction of SOP is that an enhancement effect of recency discrimination performance should be observed if an intervening distractor stimulus is placed between the two sampled objects during the sample-sample interval (Wagner, 1981). SOP postulates that the additional stimulus elements, from the distractor stimulus, will compete for space in the activation states thus would accelerate the decay of existing elements, of the first sampled object, residing in A1 and A2 (Mazur & Wagner, 1982; Wagner, 1981). Therefore, during the later test, the first sample object will have relatively more elements that have returned to inactive, compared with a no distractor task, and consequently have more available to enter A1 and elicit strong responding.

Evidence for distractor stimuli resulting in enhanced recency discrimination has been provided indirectly, through temporal separation effect studies (But also see: G. Barker et al., 2019; Kesner, Gilbert, & Barua, 2002; Templer & Hampton, 2013) which explored the ability of animals to remember the order in which stimuli had been experienced. For example, Templer and Hampton (2013) trained rhesus monkeys (*Macaca mulatta*) to identify which of two images from trial-unique sequences of five images had been presented first. Following training, they explored the effects that temporal spacing and intervening images would have on order discrimination (Experiment 4). To do this they presented monkeys with lists of 5 images where either image 3 (temporal spacing) or image 1 (intervening image) was omitted and then tested them for order discrimination between images 2 and 4. For example, for temporal spacing they were presented with 1, 2, _, 4, 5, where "_" depicts omitted image, and for intervening image they were presented with _, 2, 3, 4, 5. Therefore, the test images, 2 and 4, had previously been separated by either a temporal interval or by an intervening image. The authors

reported that the monkey's discrimination performance was significantly better during the intervening image task than the temporal spacing task, which suggests that the intervening distractor image enhanced recency discrimination performance.

Distractor effects have also been investigated during taste aversion learning studies (e.g., Batsell Jr, Barclay, Vespi, Cain-Kellman, & Harding, 2023; Kwok & Boakes, 2012; Kwok, Harris, & Boakes, 2017; Kwok, Livesey, & Boakes, 2012; Revusky, 1971) and it has been shown that memory decay over time can be accelerated with the addition of interfering stimuli (e.g., Kwok et al., 2012). For example, during seminal work by Revusky (1971), rats were given access to a saccharin solution, which would serve as the target taste, followed 15 minutes later by a vinegar solution that would serve as the overshadowing stimulus, or water as a control. After 1 hour, rats were given a lithium chloride (LiCl) injection to induce illness. Two days later rats were tested for their aversion to saccharin, and it was reported that vinegar overshadowed the taste aversion to saccharin. In other words, the aversion to saccharin was reduced when an interfering stimulus (vinegar) was experienced during the interval between presentation of the target taste (saccharin) and the (LiCl) injection, an effect that has been replicated in subsequent studies (e.g., Kaye, Gambini, & Mackintosh, 1988; Kwok et al., 2012). Moreover, distractor effects can be influenced by factors such as their salience (Kaye, Gambini, et al., 1988; Revusky, 1971; Robertson & Garrud, 1983) and their placement relative to target stimuli, such as proximal compared with distal presentation (Kwok et al., 2017) as well as proactive and retroactive presentations (Kwok & Boakes, 2012; Kwok et al., 2012). To our knowledge, distractor effects have not been explored in a relative recency object recognition task.

Chapter 5 explored possible distractor effects which SOP predicts should occur during spontaneous object recognition memory tasks (Mazur & Wagner, 1982; Wagner, 1981). We used modified versions of the standard relative recency task (e.g., Mitchell & Laiacona, 1998) which included additional objects placed within the sample-sample interval or prior to the first sampled object. We also attempted

to manipulate the salience of the distractors used and investigated the potential effect that their placement within the sample-sample interval may have. We used a 2-hour sample-sample interval and a 5-minute retention interval throughout to try and maximise recency discrimination (Hatakeyama et al., 2018; Mitchell & Laiacona, 1998; Tam et al., 2014). During experiment 12, we used 5-month-old APP/PS1 mice to test a further prediction of SOP related to the recency-based distractor task. Previous work in these mice, at this, has identified a selective deficit in association-based tasks but not recency-based ones (Bonardi et al., 2016, 2021), which SOP would suggest may reflect that these mice have a deficit in retrieval-generated priming, but self-generated priming remains intact.

5.2 Experiment 11

5.2.1 Introduction

Experiment 11 tested a prediction of SOP in relation to distractor stimuli. The prediction was that a distractor object presented during the sample-sample interval of a relative recency memory task would increase recency discrimination performance during the test that followed (Mazur & Wagner, 1982; Wagner, 1981). SOP suggests that this would be because when a distractor stimulus is experienced after a target stimulus, there is competition for space in A1 and A2 which results in increased decay of the targets elements from these activation states. To test this prediction, we used a standard relative recency task (Mitchell & Laiacona, 1998) where animals are exposed to objects A and B sequentially over two sample phases and then tested with A and B together. We added a third object which was presented either during the sample-sample interval as a distractor stimulus (ACB) or prior to the first sample as a control condition (CAB).

Task	Sample phases and intervals								Test
Standard	C	55-min	A	2-hours			B	5-mins	A and B
Distractor			A	55-min	C	55-min	B	5-mins	A and B

Table 12. Experimental design for Experiment 11. A and B represent the target objects and C depicts the distractor objects. Sample phases were 10 minutes in duration and the test was 5 minutes.

We hypothesised that recency discrimination would be enhanced when the third object was presented between the target objects compared to when it was presented prior to the target objects. This would be consistent with SOP as the rate of decay of object A's elements, from A1 and A2, would only be accelerated when the distractor was experienced after A and not when it was experienced prior to this object (Kaye, Swietalski, et al., 1988a; Mazur & Wagner, 1982). An alternative hypothesis is that both distractors could proactively interfere with the target objects (Bartko, Cowell, Winters, Bussey, & Saksida, 2010; Engelmann, 2009). That is, when the distractor was presented prior to the target objects, it would proactively interfere with the memory of object A and enhance recency discrimination. When it was presented between them, it would proactively interfere with the memory of object B, but would not interfere with the memory of A, and would reduce recency discrimination. In this instance then we may expect recency discrimination to be better when the distractor is presented prior to the target objects and worse when presented between them. However, data from temporal separation studies suggest that the inclusion of distractors between target stimuli increases discrimination as opposed to decreasing it (Kesner et al., 2002; Templer & Hampton, 2013). For example, Templer and Hampton (2013) reported better discrimination when an intervening distractor occurred between two target items compared to when no distractors occurred, and the intervals between the two target items were of equivalent duration.

5.2.2 Materials and method

5.2.2.1 Subjects

The subjects were 16 male C57BL/6J mice that were approximately 3 months old at the start of the experiment. These mice had previously completed Experiments 5 and 6.

5.2.2.2 Procedure

This experiment consisted of two individual tasks which each had four phases, three sample phases and a test phase (Table 12). All sample phases were 10 minutes in duration and test phases were 5 minutes. Half of the mice completed a standard task first and the other half completed a distractor task. This was then reversed so that all mice completed both tasks. Left/right zones were used for this experiment and object identities, their locations during test, and task order were counterbalanced across subjects (Table 13).

Standard task

Mice were first exposed to two copies of object C followed by a 55-minute interval and then they were presented with two copies of object A. After a 2-hour interval, mice were exposed to two copies of object B followed by a 5-minute interval and then tested with one copy of A and one copy of B.

Distractor task

Mice were first presented with two copies of object A followed by a 55-minute interval and then they were exposed to two copies of object C. After another 55-minute interval, mice were presented with two copies of object B, followed by a 5-minute interval, and then tested with one copy of each of the object's A and B.

Animal	Task order	Objects			Test	
		C	A	B	Left	Right
1-4	Standard	Egg	Star	Ping	Star	Ping
	Distractor	Lego	Foot	Silver	Silver	Foot
5-8	Standard	Lego	Ping	Star	Star	Ping
	Distractor	Egg	Silver	Foot	Silver	Foot
9-12	Distractor	Egg	Star	Ping	Star	Ping
	Standard	Lego	Foot	Silver	Silver	Foot
13-16	Distractor	Lego	Ping	Star	Star	Ping
	Standard	Egg	Silver	Foot	Silver	Foot

Table 13. Counterbalancing of objects, their test locations, and task order during Experiment 11.

5.2.3 Results and Discussion

Mice directed similar amounts of exploration towards the objects during all the sample phases of both tasks. This was explored using an ANOVA with task (standard/distractor) and object (A/B/C) as within-subject factors. This revealed no effect of task ($F(1, 15) = .148$, $p = .705$ $MSe = 169.070$, $\eta_p^2 = .010$) or object ($F(2, 30) = .230$, $p = .796$ $MSe = 139.996$, $\eta_p^2 = .015$) and no interaction between them ($F(1.5, 22.2) = 1.073$, $p = .340$ $MSe = 510.683$, $\eta_p^2 = .067$).

During the test phases, mice performed well on the recency discrimination during the distractor task but not on the standard task (Figure 32). During the distractor task they explored the less recent object A far more than object B (mean exploration (± SEM): A, 17.7s ± 1.4s; B, 8.5s ± 1.3s). During the standard task they appeared to have a slight preference for object A over B, but this was not significant (mean exploration (± SEM): A, 13.3s ± 1.5s; B, 10.9s ± 1.6s). This was investigated using a Student's t-test which compared the mean discrimination index of each task and revealed a difference between the two tasks ($T(15) = -3.319$, $p = .005$). The mean discrimination index for each individual task was then compared against chance (zero) using one sample t-tests. These showed a strong discrimination in the distractor task ($T(15) = 6.433$, $p < .001$) but no

discrimination in the standard task despite the mean being in the right direction (T(15) = .531, p = .147).

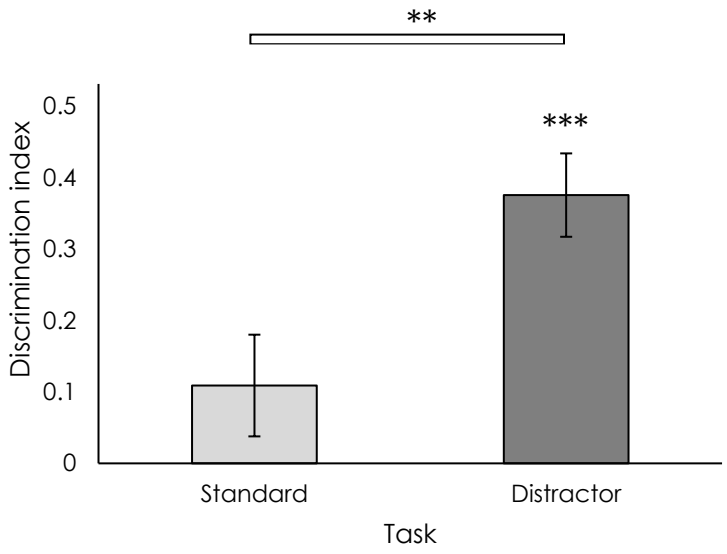


Figure 32. Mean discrimination index (±SEM) during the test phase of Experiment 11. Asterisks above bracket denotes p <.01 between tasks and asterisks above bars indicates p < .001 versus chance (0).

Experiment 11 demonstrated that relative recency discrimination performance was enhanced by the inclusion of a distractor object presented between the target objects compared with presentation prior to them. This finding is consistent with our hypothesis and the SOP prediction (Mazur & Wagner, 1982) as well as with previous reports from temporal separation studies in rats and monkeys (Kesner et al., 2002; Templer & Hampton, 2013). These data are inconsistent with the hypothesis that the distractors cause proactive interference of the target objects (Bartko et al., 2010; Engelmann, 2009). In this case we would have expected recency discrimination to be enhanced when the distractor object was presented prior to the target objects compared with presentation between them, the reverse effect to that observed during Experiment 11.

According to the SOP interpretation, during the test of the standard task, objects A and B would both have many elements residing in the A2 state. However, object A would have relatively fewer elements in A2 than object B because object A's

elements would have had relatively more time to decay back to inactive. Therefore, object A would have more elements available to enter A1 and elicit a stronger response than object B. Although we did not observe a recency discrimination during this task, as we may have expected, the means were in the direction of more exploration of A than B consistent with the SOP suggestion. During the distractor task, the decay of elements from A2 to inactive for object A, would have been accelerated by the addition of object C, during the sample-sample interval. Thus, during the test, object A would have far more elements available to enter A1 and elicit a very strong response, compared with the standard task, whereas object B would not, as most of its elements would still reside in A2, similarly to the standard task. This offers one explanation of why we observed an enhanced relative recency discrimination with the inclusion of a distractor object presented between two target objects.

These data provide some evidence that using the SOP model may be a suitable theoretical framework for studying recency-based object recognition memory effects similarly to previous work (Bonardi et al., 2016, 2021; Tam et al., 2014). However, these data can also be explained by other hypotheses such as the discrimination between relative strengths of memory traces (Ennaceur, 2010; Fortin, Agster, & Eichenbaum, 2002). For example, memory traces for recently experienced stimuli may be stronger than those for stimuli experienced earlier and these differences in trace strength could be used by the animals to judge the order in which stimuli were presented (Fortin et al., 2002). Moreover, these memory traces may be susceptible to depletion over time and be subject to interference (Kwok et al., 2012). That is, memory traces of a stimulus decay because of time passing and may decay at a faster rate if the stimulus is followed by the presentation of another stimulus, and stimuli from different modalities may decay at different rates (Kwok et al., 2012). Therefore, it is possible that during the distractor task, the memory trace of object A decayed faster than during the standard condition as it was followed by two objects (C and B) as opposed to one object (B). In both tasks object B was not followed by any other objects thus its memory trace would be equally strong in both tasks. Hence, a discrimination

between the memory traces of objects A and B would have been stronger during the distractor than the standard task.

To attempt to minimise alternative explanations for these data, we explored further predictions that were potentially more unique to SOP during Experiments 13, 14, and 15.

5.3 Experiment 12

5.3.1 Introduction

Experiment 12 was a replication of the distractor task used during Experiment 11 but using 5-month-old APP/PS1 mice and their wild type littermates instead of C57BL/6J mice. This Alzheimer's mouse model exhibits amyloid-β protein related brain alterations by the age of 5 months old, primarily in the cortex and hippocampus (Garcia-Alloza et al., 2006; Hong et al., 2016; Pedrós et al., 2014; Ruan et al., 2009; Zhu et al., 2017). However, they do not generally display deficits in learning and memory tasks before the age of 6 months (Kelly et al., 2017; Kilgore et al., 2010) and crucially, their performance during the novel object recognition memory and relative recency tasks also appear unaffected (Bonardi et al., 2011; Bonardi et al., 2016, 2021; Jardanhazi-Kurutz et al., 2010; Kelly et al., 2017).

Human subjects with Alzheimer's disease, and subjects who have experienced a hypoxic episode known to cause damage to the hippocampus, exhibit deficits during temporal separation tasks compared to healthy controls (R. O. Hopkins, Kesner, & Goldstein, 1995; Madsen & Kesner, 1995). Temporal separation tasks can be very similar to our distractor task. For example, Madsen and Kesner (1995) used a temporal distance of 2 with recency held constant (e.g., exposed to item A, followed by items C, then D, then B, followed by a test with A and B), which is the equivalent of our distractor task but using two distractors (e.g., C and D). They found that healthy controls discriminated between the test items better with two distractors (~90% correct) compared with no distractors (~70% correct), whereas the subjects with Alzheimer's disease performed badly in both tasks (~44% correct

during both tasks). Similarly, R. O. Hopkins et al. (1995) found that hypoxic subjects performed significantly worse than healthy controls also using a temporal distance of 2. They reported that healthy control subject's recency discrimination performance was better with the distractors (~90% correct) than without them (~76%). The hypoxic subject's performance was worse than the controls for both tasks and there appeared to be no distractor effect in these subjects (~66% correct during both tasks). Therefore, because our mice likely had already suffered a pathological burden in the hippocampus (Garcia-Alloza et al., 2006; Hong et al., 2016; Pedrós et al., 2014; Ruan et al., 2009; Zhu et al., 2017) it is possible that a distractor effect may not be present in these animals. However, the subjects used during the study by Madsen and Kesner (1995) displayed mild to moderate memory deterioration, prior to the study, whereas APP/PS1 mice at the age we were using them generally do not (Bonardi et al., 2011; Kilgore et al., 2010).

Two previous studies used alternative spontaneous object recognition task variants, to further explore recognition memory in these mice at 5 months old (Bonardi et al., 2016, 2021). They compared the performance of the transgenic mice and their wild type littermates during novel object, relative recency, and object-in-place tasks. They reported a deficit in the transgenic mice, in the object-in-place tasks only. They interpreted their findings using SOP and suggested that the mice may have had intact self-generated priming and a selective deficit in retrieval-generated priming. This would allow the mice to perform well in the recency discrimination tasks but not the object-in-place tasks as these tasks may primarily rely on self-generated and retrieval-generated priming respectively. In terms of the novel object task, they proposed that it is possible that both priming processes are used for the task and that one may have compensated for the other, in the transgenic mice, so that performance appeared unaffected.

Based on these previous studies which showed no deficit in typical relative recency tasks (Bonardi et al., 2016, 2021), we predicted that the transgenic mice used during Experiment 12 would not show a deficit during our standard task because this task was very similar to a typical relative recency task. However,

based on the data for how humans with hippocampal damage perform during these types of tasks (R. O. Hopkins et al., 1995; Madsen & Kesner, 1995), how the transgenic mice would perform during our distractor task was more difficult to predict. Based on the suggestion that relative recency primarily depends on self-generated priming (Robinson & Bonardi, 2015; Sanderson et al., 2011), which should not be impaired in these mice at 5 months old (Bonardi et al., 2016, 2021), we predicted that transgenic mice would not be impaired on the distractor task.

5.3.2 Materials and method

5.3.2.1 Subjects

The subjects were APPswe/PS1dE9 transgenic mice and their wild type littermates. Our aim was to use 16 positive and 16 negative animals with an equal balance of sex. However, our transgenic breeding unit was only able to breed 15 positive (7 females and 8 males) and 15 wild type animals (8 females and 7 males), so this was our initial sample. Later, we received a second batch of mice from the breeding unit which consisted of 12 positive (3 males and 9 females) and 17 wild type animals (8 males and 9 females), so these were ran as a second replication. These mice had previously completed Experiment 9.

5.3.2.2 Procedure

This task was identical to the task used during Experiment 11 (Table 12). Object identities, their locations during test, and task order were counterbalanced across subjects similarly to Experiment 11 (Table 13).

5.3.3 Results and Discussion

Two mice were excluded from the analyses, one due to low levels of exploration (wild type female; the exclusion criterion was described in Chapter 2) and another because it died during the experiment (APP/PS1 male).

During the sample phases of the experiment, mice directed similar amounts of exploration towards objects A, B and C, irrespective of genotype and replication.

However, there was some variability in the total amount of object exploration during each task. During the standard task, males mean (±SD) total exploration of the objects was more (269.2 ± 91.9) than females (221.6 ± 81.7) but during the distractor task, exploration was similar between males (256.5 ± 62.4) and females (240.4 ± 87.0). This was confirmed using an ANOVA with task (standard/distractor) and object (A/B/C) as within-subject factors and genotype (positive/negative), sex (male/female), and replication (1/2) as between-subject factors. The ANOVA revealed a task*sex interaction ($F(1, 49) = 4.568$, $p = .038$ $MSe = 2887.477$, $\eta_p^2 = .085$) and no other significant results (smallest $p = .101$). Simple main effects showed that males explored the objects more than the females, during the sample phases, in the standard ($p = .045$) but not the distractor task distractor task ($p = .542$). Overall, the exploration levels during the sample phases seemed lower than that of Experiment 11. We compared the total exploration across all sample phases between the two experiments using a Student's t-test which showed greater exploration during Experiment 11 than 14 ($T(71) = 2.720$, $p = .008$). This reduction in exploration may have reduced recency discrimination and the distractor effect during the test. For this reason we included the total object exploration during the sample phases as a covariate during the test analysis.

During the test phase, there appeared to be no distractor effect for the group overall or within each of the genotypes (Figure 33). In all cases the means were in the right direction for an effect, but any potential differences between tasks were nonsignificant. The wild type mice exhibited a stronger recency discrimination than the APP/PS1 mice, but the apparent differences between genotypes were also nonsignificant (mean exploration (± SEM): APP/PS1, standard task, A, 10.1s ± 1.2, B, 8.2s ± 1.4s, distractor task, A, 11.5s ± .9s, B, 8.8s ± 1.2s; wild type, standard task, A, 10.2s ± .8s, B, 8.3s ± 1.4s; distractor task, A, 13.3s ± 1.0s, B, 8.1s ± 1.2s). There was some variability between replications, but this variability only appeared to be in the wild type males. Discrimination index test data were explored using an ANOVA with task (standard/distractor) as a within-subject factor, genotype (positive/negative), sex (male/female), and replication (1/2) as between-subject factors, and total object exploration during the sample phases as a

covariate. This revealed no main effect of task ($F(1, 48) = 1.769$, $p = .190$ $MSe = .197$, $\eta_p^2 = .036$), no task*genotype interaction ($F(1, 48) = .028$, $p = .868$ $MSe = .003$, $\eta_p^2 < .001$), no effect of sample phase exploration ($F(1, 48) = .977$, $p = .328$ $MSe = .111$, $\eta_p^2 = .020$), that there was a replication*sex*genotype three way interaction ($F(1, 48) = 9.069$, $p = .004$ $MSe = 1.027$, $\eta_p^2 = .159$) and nothing else was significant (smallest $p = .112$). The interaction was explored which showed that there was an effect of replication in male wild type male, but not females (males: $p = .007$; females: $p = .079$), and no effect of replication in APP/PS1 mice of either sex (males: $p = .947$; females: $p = .076$).

To further explore recency discrimination, during the test phase, one sample t-tests were performed which compared discrimination indexes against chance (zero). These showed that for all of the mice combined, recency discrimination was very good during the standard ($T(56) = 3.848$, $p < .001$) and distractor tasks ($T(56) = 5.436$, $p < .001$; Figure 33). For APP/PS1 mice, recency discrimination was in the right direction in the standard task, but failed to reach significance ($T(25) = 2.018$, $p = .054$), and was good in the distractor task ($T(25) = 3.256$, $p = .003$), and for wild type mice, recency discrimination was good in both tasks but appeared better in the distractor task ($T(30) = 4.374$, $p < .001$) than the standard task ($T(30) = 3.346$, $p = .002$; Figure 33).

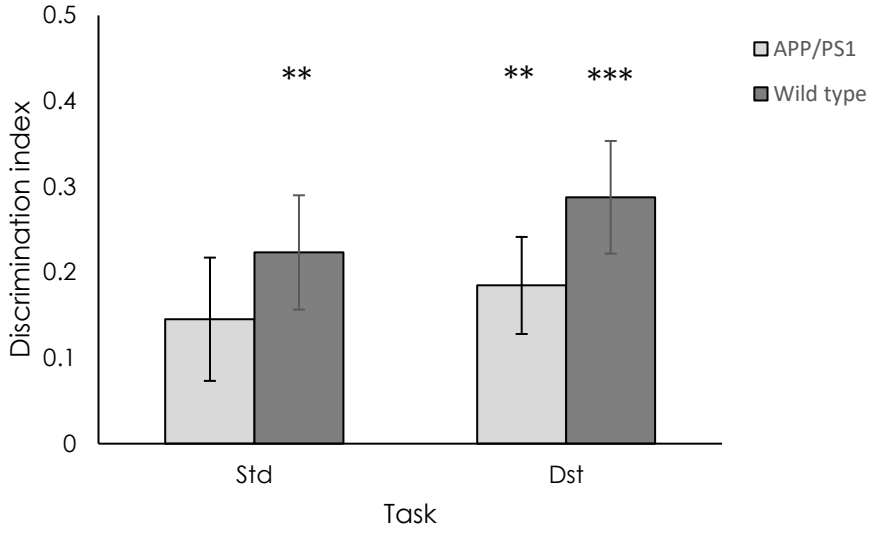


Figure 33. Mean discrimination index (±SEM) for the standard and distractor tasks during Experiment 12 broken down by genotype. Asterisks above a bar denotes a significant difference from chance (0), *** $p < .001$, ** $p < .010$.

Experiment 12 failed to replicate the distractor stimulus effect observed during Experiment 11. This was inconsistent with our prediction that mice of both genotypes would display a distractor effect. However, recency discrimination was very good during both tasks, independent of genotype, which is consistent with previous work showing intact relative recency in these mice at this age (Bonardi et al., 2016, 2021). Interestingly, when analysing the test data individually to test recency discrimination versus chance, it appeared that, for both genotypes, recency discrimination performance improved from the standard to the distractor task. It also appeared that the transgenic mice may have had a slight deficit in the standard task. This might suggest that the impact of the distractor is subtle and that a larger sample size may be needed in these mice to obtain a significant distractor effect. One observed difference between these mice and the mice used during Experiment 11, was that the transgenic mice explored the objects less across the sample phases. This reduction in exploration could have reduced any possible distractor effects during the test for recency discrimination.

SOP asserts that a distractor effect may arise through competition between stimuli for space in memory (Mazur & Wagner, 1982; Wagner, 1981). This competition for elemental space within the A1 and A2 activation states can accelerate the decay of the first presented object A's elements from these states back to inactive. During the test for recency discrimination, if object A's elements have suffered accelerated decay, then the discrimination is enhanced because the second target, object B, will have many elements residing in A2, via self-generated priming, which consequently will reduce responding to B whereas A will have relatively more elements available to enter A1 and generate higher responding. Therefore, if mice explore the objects less during the sample phases, then there may be less competition for space in memory between stimuli because there will be fewer elements in each of the activation states and more space available for existing elements to occupy.

A reduction in exploratory behaviour in the APP/PS1 mice could have been due to the hippocampal burden present at this age, in these mice, which includes synapse loss, mitochondrial dysfunction, tau hyperphosphororilation, and detectable soluble and insoluble levels of Aβ40 and Aβ42 in hippocampus (Garcia-Alloza et al., 2006; Hong et al., 2016; Pedrós et al., 2014; Ruan et al., 2009; Zhu et al., 2017). For example, a study which used rats with excitotoxic hippocampal lesions found that these animals directed more exploration towards a primed stimulus than an unprimed stimulus, which was the opposite of that observed with control rats (Honey & Good, 2000b). The authors suggested that this may have been due to an attentional deficit in the lesioned animals. Whether this was the case in our APP/PS1 mice was unclear. Another reason for their reduced object exploration could have been because the increased locomotor activity present in this strain of transgenic mice (Cheng, Logge, Low, Garner, & Karl, 2013; Hulshof et al., 2022). Irrespective of the mechanism, the behavioural phenotype of reduced attention in the APP/PS1 mice could have influenced their wild type littermate's behaviour (Kalbassi et al., 2017) and resulted in the wild type mice directing reduced attention towards the objects. There are also other explanations for the reduced exploratory behaviour of the transgenic mice which include strain and age

differences. Although the transgenic mice used were based on C57BL/6J mice, they are not the same strain and can behave differently due to various factors such as environmental variability or genetic background. For example, differences in their handling, home cage environment, noise, and other factors can significantly alter behaviour (Bailey, Rustay, & Crawley, 2006; Ferrari, Palanza, Parmigiani, & Rodgers, 1998). Because our C57BL/6J mice were bred and maintained at Charles River UK and our transgenic mice were bred and maintained at the University of Nottingham, it is likely that the two strains experienced differences in environmental factors prior to the experiments. Also, different genetic backgrounds for the same model (e.g., APP/PS1 mice can be based on different strains of C57BL/6J mice) can directly influence their behavioural phenotype (Bailey et al., 2006) which may account for disparities in behavioural responses across experiments (Cheng et al., 2013). Moreover, exploratory behaviour has been reported to be significantly higher in younger mice (2 months old) than older mice (6 months old; Wu et al., 2013) which is consistent with what we observed, although our age differences were closer together (3 and 5 months old during Experiments 11 and 12 respectively).

In summary, levels of object exploration during the sample phases may impact distractor effects. For a distractor to impact significantly upon self-generated priming effects, it may be necessary for higher levels of object exploration to create the level of competition for memory space required to accelerate elemental decay enough to enhance recency discrimination.

5.4 Experiment 13

5.4.1 Introduction

The distractor effect observed during Experiment 11 was consistent with that predicted by SOP (Mazur & Wagner, 1982; Wagner, 1981). However, this effect could also be explained by other theories such as a discrimination between the relative strengths of memory traces for the target objects, which may have been susceptible to interference thus enhancing discriminability between them

(Ennaceur, 2010; Fortin et al., 2002; Kwok et al., 2012). For example, object A would have had a weaker memory trace than object B during the test in the standard task, because A's memory trace would have had more time to weaken than B's. During the distractor task, object C would have further weakened the strength of object A's memory trace. Therefore, during the test, the animals would have discriminated between the relative strengths of the two memory traces and explored A more in both tasks as it would have had a weaker memory trace. Objects A's memory trace would also have been weaker in the distractor task than in the standard task which would have enhanced discriminability between A and B. Therefore, for Experiment 13, we wanted to test a further prediction more unique to SOP that was related to factors that may influence distractor effects. Factors that have been found to modulate distractor effects include relative similarity between the distractor and target stimuli (e.g., Kaye, Swietalski, & Mackintosh, 1988b; Robertson & Garrud, 1983), and the distractor placement relative to the target (e.g., Kaye, Swietalski, et al., 1988a; Kwok et al., 2017; Robertson & Garrud, 1983).

In terms of relative similarity, Robertson and Garrud (1983) assessed rats for habituation of their neophobic responses towards novel taste stimuli. Rats naturally display neophobia towards new taste stimuli, that is they only consume small amounts initially, and increased consumption with repeated exposure is taken to reflect habituation of neophobia (Shanks, Preston, & Stanhope, 1986). Post distractor effects on this habituation differed depending on the similarity between the target and distractor stimuli. When the target taste stimulus was followed by a dissimilar distractor stimulus (sucrose followed by coffee) habituation was disrupted, but when the target was followed by a similar distractor (lemon followed by coffee), it was not. Similarly, in a study by Kaye, Swietalski, et al. (1988a), when rats were given a target taste stimulus followed by a dissimilar distractor (lemon followed by saline solution), habituation to the target was disrupted, but when the target was followed by a similar distractor (lemon followed by coffee), habituation remained largely unaffected. Because we have demonstrated a post distractor effect on habituation to the target, in our

experiments that is evident as a disruption in habituation of the target object A, by the distractor object C, it may be that the distractor object is perceived as dissimilar to the target objects. The junk objects we used were all different sizes, shapes, and colours and the mice always discriminated between them during pretraining which suggests that they were sufficiently different and may have been perceived as dissimilar.

In terms of distractor placement relative to the target, Kwok et al. (2017) reported an effect of placing a distractor either proximal or distal to a target during a taste aversion learning experiment. They tested when a distractor would be most effective in overshadowing the acquisition of taste aversion learning, with sucrose as the target taste (CS), hydrochloric acid (HCl) as the distractor, and lithium chloride (LiCl) as the unconditioned stimulus (US). They reported that when a distractor was presented later in the CS-US interval it produced greater overshadowing compared to when it was presented earlier in the interval. They also ran a simulation of SOP (based on Wagner, 1981) which predicted the opposite effect, that the impact of a distractor stimulus would be greater when it was presented earlier, as opposed to later, within the interval. SOP explains this by suggesting that the target objects elements would be subjected to accelerated decay over a longer duration when placed earlier, relative to later, during the interval (Figure 34; Kwok et al., 2017).

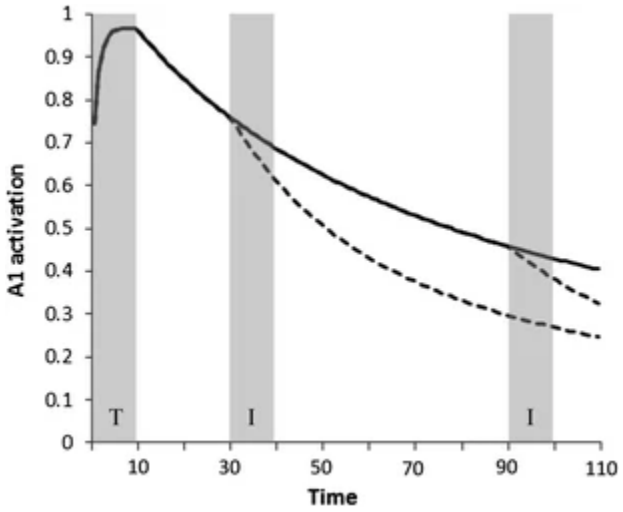


Figure 34. Simulation ran by Kwok et al. (2017) and the figure they provided. The figure depicts the A1 activation strength of a stimulus trace based on SOP (Wagner, 1981). T represents the target stimulus and I denote either an early or late distractor stimulus. The solid line shows the proportion of the target's elements active in A1 and how these decay over time. The dashed lines show how decay rates are accelerated when a distractor is placed early or late in the trace interval.

Kwok et al. (2017) suggested that their results may have been restricted to taste aversion learning which is primarily focused on how distractor stimuli can disrupt association formation. In terms of SOP, this would correspond to elements of the CS being displaced from A1 by the distractor which would restrict learning as there would be less CS elements in A1 concurrently with elements of the US (Wagner, 1981; Mazur & Wagner, 1982), thus would be focused primarily on A1 decay (e.g., Kwok et al., 2017). However, because our distractor task uses the same context for sample and test phases, it is unlikely that associations support performance during the task because object context associations would be almost equal for both target objects (Robinson & Bonardi, 2015; Sanderson et al., 2011). Therefore, instead it is more likely that task performance is a product of self-generated priming, which can be thought of as short-term habituation (i.e., Sanderson & Bannerman, 2011), and that the distractor disrupts this habituation to object A, which results in more exploration of A during the test. For this reason,

we may see a different effect in our distractor task compared with taste aversion learning. In fact, an earlier study compared the effect of distractor placement (earlier versus later) during both habituation and overshadowing tasks and reported a clear dissociation between the two (Kaye, Gambini, et al., 1988). They found that habituation of neophobia to a target (vinegar solution) was disrupted more by an early compared with a late distractor, but conditioning to the same target was overshadowed more effectively by the late compared with the early distractor. Because we are investigating recency discrimination performance rather than learning overshadowing is not really an issue.

Therefore, we predicted that, during our relative recency distractor task, a distractor placed early in the sample-sample interval would enhance recency performance more than a distractor placed later in the sample-sample interval. This would be consistent with the prediction from the SOP simulation (Figure 34) and with previous work on the effect of distractor stimuli on habituation (Kaye, Gambini, et al., 1988). We explored this prediction using two versions of the distractor task previously used during Experiment 11. During one task, the distractor was presented early during the 2-hr interval, shortly after object A, and during the other task, it was presented late during the interval, shortly before object B (Table 14).

Task	**Sample phases and intervals**							**Test**
Proximal	A	15-min	C	95-mins		B	5-mins	A and B
Distal	A	95-mins		C	15-min	B	5-mins	A and B

Table 14. Experimental design for Experiment 13. A and B represent the target objects and C depicts the distractor objects. Sample phases were 10 minutes in duration and the test was 5 minutes.

5.4.2 Materials and method

5.4.2.1 Subjects

The subjects were 16 male C57BL/6J mice that were approximately 3 months old at the start of the experiment. They had previously completed Experiment 8.

5.4.2.2 Procedure

This experiment consisted of two individual tasks which each had four phases, three sample phases and a test (Table 14). Mice were exposed to two copies of object A during an initial sample phase. This was followed by either a 15-minute (proximal task) or a 95-minute (distal task) interval after which time the mice were presented with two copies of object C. Exposure to object C was then followed by either a 95-minute (proximal task) or a 15-minute (distal) interval after which time mice were presented with two copies of object B. Exposure to object B was followed by a 5-minute interval and then mice were tested with one copy of object A and one copy of object B. Half of the mice completed the proximal task first and the other half completed the distal task. This was then reversed so that all mice completed both tasks. For the first task, the top left/bottom right zones were used and for the second task, the top right/bottom left zones were used. Object identities, their test locations, and task order were counterbalanced across subjects using the same method as used during Experiment 11 (Table 13).

5.4.3 Results and Discussion

Mice directed similar amounts of exploratory behaviour towards the objects during all the sample phases for both tasks (Table 15). This was investigated using an ANOVA which compared the mean total exploration times (s) for all objects across both tasks, using task (proximal/distal) and object (A/B/C) as within-subject factors. The analysis showed no effect of task ($F(1, 15) = .042$, $p = .840$ $MSe = 22.330$, $\eta_p^2 = .003$) or object ($F(1.4, 20.6) = .490$, $p = .550$ $MSe = 482.796$, $\eta_p^2 = .032$) and no interaction between them ($F(2, 30) = 1.871$, $p = .171$ $MSe = 1082.625$, $\eta_p^2 = .111$).

Proximal			Distal		
A	C	B	A	C	B
77.6 ± 38.7	86.5 ± 30.7	79.9 ± 30.2	81.1 ± 37.2	74.8 ± 29.2	91.0 ± 30.1

Table 15. Mean (±SD) exploration times (s) during all sample phases during Experiment 13.

During the test, recency discrimination was very strong for both the proximal and distal distractor tasks (mean exploration (± SEM): proximal, A, 13.9s ± 1.6s, B, 7.0s ± 1.4s; distal, A, 17.5s ± 2.3s, B, 6.3s ± .8s; Figure 35). However, there appeared to be no difference in the distractors effect when its temporal position was moved earlier or later in the sample-sample interval. Recency discrimination was compared using two One sample t-tests which compared the mean discrimination indexes of each task to chance (zero). These tests revealed a strong recency discrimination in both the proximal ($T(15) = 5.625$, $p < .001$) and distal ($T(15) = 4.937$, $p < .001$; Figure 35) tasks. A Student's t-test was used to compare the mean discrimination indexes of both tasks to one another. This showed no difference between the two tasks ($T(15) = -.223$, $p = .827$). Because we did not include a control condition (standard task) to test for a distractor effect during Experiment 13, we compared these data with the test data from Experiment 11. The pooled data showed that recency discrimination was greater during the three tasks which contained a distractor in the sample-sample interval (distractor, proximal, and distal tasks) compared to the task which did not (standard task). An ANOVA was used to compare the mean discrimination indexes for the four tasks used during the two experiments (standard, distractor, proximal, and distal). This revealed an effect of task ($F(3, 60) = 3.926$, $p = .013$ $MSe = .310$, $\eta_p^2 = .164$) and post hoc tests (using the Holm correction to adjust p) indicated that recency discrimination was significantly weaker in the standard task than with the distractor ($p = .040$), the proximal ($p = .040$), and the distal ($p = .027$) tasks. These results suggest that there was a distractor effect during both the proximal and distal tasks of Experiment 13.

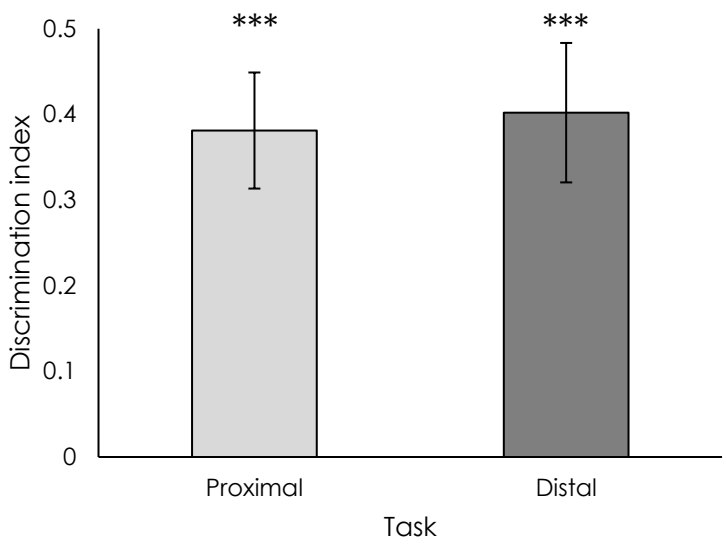


Figure 35. Mean discrimination index (±SEM) during the test for Experiment 13. Asterisks above bars indicates $p < .001$ versus chance (0).

During Experiment 13 there was a strong recency discrimination during the tests for both tasks. Recency discrimination levels were comparable to the distractor task of Experiment 11 and were significantly stronger than the standard task of that experiment. Taken together this suggests that there was a distractor effect present during both tasks of Experiment 13. However, there were no differences between the proximal and distal placements of the distractor during the tasks which suggests that there was no effect of moving the distractor object earlier or later within the sample-sample interval. These data are inconsistent with the prediction from the SOP simulation (Figure 34) and with previous data reported for distractor placement effects on habituation and taste aversion learning (Kaye, Gambini, et al., 1988; Kwok et al., 2017), where a distractor has been shown to be more effective in disrupting habituation when presented earlier (Kaye, Gambini, et al., 1988), but more effective in disrupting conditioning when presented later (Kaye, Gambini, et al., 1988; Kwok et al., 2017). In contrast to this empirical data, our experiment failed to detect any differences in the distractors effect relative to its temporal placement within the interval.

This may have been because the difference between the distractor placement, between the two tasks, was insufficient to detect an effect. Earlier we suggested that during the relative recency object recognition task, and the modified version used for our distractor task, it is unlikely that associations between the objects and their contextual surroundings aid with recency performance, as both objects will have had almost equal opportunity to form associations with the contextual surroundings because the context remained constant throughout the sample and test phases (Robinson & Bonardi, 2015; Sanderson et al., 2011). In terms of SOP then, recency discrimination performance is likely largely dependent on self-generated priming and not strongly influenced by retrieval-generated priming (Mazur & Wagner, 1982; Wagner, 1981). Therefore, the strength of a recency discrimination is determined by the relative difference in the quantity of elements, of the target objects, residing in A2 during the test. As the test always occurs 5 minutes after the presentation of object B, the quantity of B's elements in A2 during the test is likely to remain constant throughout all tasks, whereas the quantity of object A's elements in A2, will likely differ across different tasks because the presentation of a distractor object after A accelerates the decay of A's elements from A2. Moving the distractor nearer or further from object A appeared to have no effect on this as the simulation predicted (Figure 34). However, this may have been because the effect of accelerated decay on object A's elements, over a longer versus a shorter duration, may have been a very small difference that was not detectable over the duration used. In other words, object A may have had fewer elements in A2 during the proximal task than in the distal task, but this difference relative to the quantity of object B's elements in A2, was not enough to significantly alter recency discrimination. During the study by Kaye, Gambini, et al. (1988), the distractor placement differed by 210 minutes and an effect was observed, but during our experiment the distractors' placement only differed by 70 minutes, which may have been insufficient to generate a detectable effect. Therefore, over a longer time interval a difference may have been detected.

In summary, moving the distractor objects position within the sample-sample interval appears to have no influence on its recency discrimination enhancement

effect. It is possible that the interval used here was of an insufficient duration to identify any possible effects of moving the distractor or that there simply may be no effect.

5.5 Experiment 14

5.5.1 Introduction

Experiment 13 failed to provide evidence that the distractor objects' temporal position, relative to object A, impacts its recency discrimination enhancement effect, as may be predicted by SOP and from previous evidence reported for distractor placement effects on habituation and taste aversion learning (Kaye, Gambini, et al., 1988; Kwok et al., 2017). Therefore, Experiment 14 aimed to test an alternative prediction of SOP related to distractor stimuli effects. The salience of the distractor used has previously been suggested to have an impact on its effectiveness (Kaye, Gambini, et al., 1988; Revusky, 1971; Robertson & Garrud, 1983). For instance, Kwok et al. (2017) suggested that distractor effects may depend on their salience and intensity. For example, taste stimuli may produce more interference than a tone or light and a highly aversive taste may interfere more with an association than a mildly aversive taste. This is consistent with SOP, as a less salient distractor would compete less with the target object for space in A1 and A2 (Mazur & Wagner, 1982; Wagner, 1981).

Shanks et al. (1986) provided some evidence (Experiment 3) that was consistent with the SOP prediction, during a taste aversion learning study. They demonstrated that when a distractor (salt solution or sucrose solution) was novel, it disrupted habituation of neophobia to a target (vinegar solution), but when it was familiar, it did not. Similar results have also been reported during a study where it was found that a novel distractor was more effective in disrupting conditioning of a flavour aversion when compared with a familiar distractor (Best, Gemberling, & Johnson, 1979). To test this theory of SOP, we used two modified versions of the relative recency distractor task used during Experiment 11. One condition included a distractor which should have been more salient (a novel object) to the mice

whereas the other condition included one which should have been less salient (a familiar object) (Table 16). We predicted that the more salient distractor would compete more for memory space than the less salient distractor, as SOP would predict (Mazur & Wagner, 1982; Wagner, 1981). Thus, the task which used the more salient distractor would produce a greater recency discrimination enhancement than the task that used the less salient distractor.

Task	Sample phases and intervals								Test
CACB	C	55-min	A	55-min	C	55-min	B	5-mins	A and B
DACB	D		A		C		B		A and B

Table 16. Experimental design for Experiment 14. A, B and C represent different junk objects.

5.5.2 Materials and method

5.5.2.1 Subjects

The subjects were 16 naïve male C57BL/6J mice that were approximately 3 months old at the start of the experiment.

5.5.2.2 Procedure

This experiment consisted of two individual tasks which each had five phases. Four sample phases, each separated by a 55-minute interval, and then a test phase 5 minutes after the final sample phase (Table 16). During each of the sample phases, mice were exposed to two copies of an object which were either object C (CACB) or object D (DACB) for sample phase 1, object A for sample phase 2, object C for sample 3, and object B for sample 4. They were then tested with one copy of object A and one copy of object B. Half of the mice completed a familiar distractor task first (CACB), which should have reduced the salience of C, and the other half completed a novel distractor task (DACB), which should have increased the salience of C. This was then reversed so that all mice completed both tasks.

The experiment was run over 4 days and 8 mice completed one task each day. For example, mice 1-8 completed the CACB task on day 1 and then the DACB on day 3, and mice 9-16 completed the DACB task on day 2 and the CACB task on day 4. Left/right objects zones were used throughout and object identities, their test locations, and task order were counterbalanced across subjects (Table 17).

Animal	**Task order**	**Objects**				**Test**	
		C/D	**A**	**C**	**B**	**Left**	**Right**
1-4	CACB	Salt	Star	Salt	Ping	Star	Ping
	DACB	Bulb	Foot	Lego	Silver	Silver	Foot
5-8	CACB	Lego	Ping	Lego	Star	Star	Ping
	DACB	Bulb	Silver	Salt	Foot	Silver	Foot
9-12	DACB	Bulb	Star	Salt	Ping	Star	Ping
	CACB	Lego	Foot	Lego	Silver	Silver	Foot
13-16	DACB	Bulb	Ping	Lego	Star	Star	Ping
	CACB	Salt	Silver	Salt	Foot	Silver	Foot

Table 17. Counterbalancing of objects, their test locations, and task order for Experiment 14.

5.5.3 Results and Discussion

During the sample phases of Experiment 14, mice explored the objects for similar amounts of time except for object B which mice directed more exploration towards, and this was the same during both tasks (Table 18). This was investigated using a mixed ANOVA which anaylsed the mean total exploration of objects during all sample phases. Task (CACB/DACB) and sample phase (1, 2, 3, 4) were included as within-subject factors. There was a main effect of sample phase ($F(3, 45) = 4.233$, $p = .010$, $MSe = 1713.233$, $\eta_p^2 = .220$) and no other significant results (smallest $p = .640$). Post hoc comparisons (using the holm correction to adjust p) revealed that the mice explored the objects for longer during the fourth sample phase than the first ($p = .021$), second ($p = .036$), or third ($p = .036$) sample phases, and no other differences were significant (all other comparisons $p = 1$). This was

an unexpected result that may have affected the recency discrimination during the test. According to SOP, object B would have had relatively more elements in A2, during the test, than it would otherwise have had if exploration during the sample phases had been the same as the other objects. Therefore, the recency discrimination may have been enhanced as relatively less exploration would have been directed towards B during the test. However, because this was the same during both tasks it should not have affected any apparent differences between the two tasks.

Familiar distractor				Novel distractor			
C	A	C	B	D	A	C	B
63.2±30.6	60.8±27.8	59.3±28.8	73.6±23.0	57.8±23.5	62.9±34.3	64.3± 6.8	78.3±35.4

Table 18. Mean (±SD) exploration times for all sample phases during both tasks of Experiment 14.

Crucially, if the salience of object C was different between conditions, as we had hoped to achieve, then we would have expected more exploration of the novel (DACB) versus the familiar (CACB) distractor during the third sample phase. However, the data showed no apparent differences in exploration, of the distractors during the third sample phase, between the two tasks. This was explored using a Student's t-test which showed no difference in object exploration between tasks during the third sample phases ($T(15) = -0.529$, $p = .605$). During the third sample phase, we would also have expected exploration of object C to have been lower than the exploration of object C during the first sample phase, for the CACB task, because it should have been familiar to the mice which should have reduced exploration towards it. During the third sample phase of the DACB task, exploration of object C should have been the same as that of object D, during the first sample phase, as both objects should have been equally novel to the mice during these phases. However, the data revealed no differences in exploration of these objects between sample phases. This was investigated using a mixed ANOVA with task (CACB/DACB) and sample phase (1/3) as within subject factors. The ANOVA revealed no significant differences and importantly, there

was no interaction between task and sample phase ($F(1, 15) = 1.134$, $p = .304$, $MSe = 426.939$, $\eta_p^2 = .070$) as we would have expected if there had have been a reduction in responding to C during the CACB task.

Neither of our expectations were observed during the data analyses which suggested that there was no difference in distractor salience between the two tasks, and therefore, it was unlikely that there would be any difference between the two tasks during the test for recency discrimination (mean exploration (± SEM): CACB, A, 11.6s ± 2.5s, B, 4.7s ± 1.4s; DACB, A, 10.3s ± 2.2s, B, 6.1s ± 1.6s). This was exactly what we found when we compared the test data from both tasks. The mean discrimination indexes for the two tasks were compared using a Student's t-test which showed no difference between them ($T(15) = .667$, $p = .515$; Figure 36). We then compared each task's discrimination index to chance (zero) using One-sample t-tests. This indicated that there was a good recency discrimination in the CACB task ($T(15) = 3.247$, $p = .005$) but not in the DACB task even though the means were in the right direction ($T(15) = 1.647$, $p = .120$; Figure 36). This was in the opposite direction to our prediction and was surprising as we would have expected good discrimination during both of these tasks.

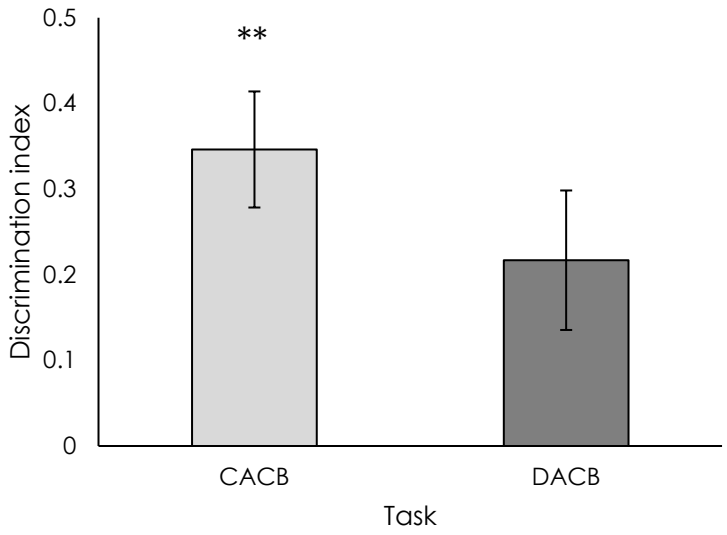


Figure 36. Mean discrimination index (±SEM) for the familiar (CACB) and novel (DACB) distractor tasks during Experiment 14. Asterisks above bars indicates $p <$ .010 versus chance (0).

During Experiment 14, we observed no difference between the more salient (DACB, novel) and less salient (CACB, familiar) distractor tasks. We expected exploration of the distractor object C to have been reduced during the third sample phase of the CACB task, when compared with the exploration of object C during the first sample phase of the CACB task, and when compared with the exploration of object C during the third sample phase of the DACB task. However, there was no evidence to support such a reduction as would be expected if the distractor object in the CACB task had been less salient and perceived as a familiar object by the mice. If the distractor objects during both tasks were perceived as novel by the mice, as the data suggested, then logically we would have expected the same results during both tasks. An earlier study by Shanks et al. (1986) reported no difference in disruption, between a novel and familiar distractor, to the habituation of neophobia to a target flavour (vinegar solution), similar to the data from Experiment 14. To establish a familiar distractor, they exposed rats to a distractor flavour (salt solution or sucrose solution) for 5 minutes on two consecutive days (10 minutes total exposure time). This familiar distractor stimulus interfered equally, with recognition of the target stimulus, compared with the novel distractor

stimulus. However, in a follow up experiment they increased the exposure time during the familiarisation phase of the familiar distractor. Rats were now given 5 minutes of exposure to the familiar distractor on 8 consecutive days (40 minutes total exposure time). As a result of increasing the familiarity of the distractor, they found that habituation was not disrupted by the familiar distractor relative to the novel distractor, which is consistent with SOP (Mazur & Wagner, 1982; Wagner, 1981) and our prediction. Therefore, it is likely that our mice required far more preexposure to the object, that would be used as the less salient, familiar distractor, for a difference between the two tasks (CACB and DACB) to have been present.

A more puzzling result was that recency discrimination was only good in the CACB and not in the DACB. We would have expected a good recency discrimination during both tasks, based on the distractor task of Experiment 11, because both tasks included a distractor stimulus. The main difference between these two tasks and the distractor task of Experiment 11 is that these tasks both included an object presented prior to the target object A. Up to this point we have assumed that the junk objects used are dissimilar from one another, as they appear to create interference when placed post target (after object A), and based on previous work this may suggest that they do not cause interference when placed prior to the target (before object A). For example, Robertson and Garrud (1983) reported a post distractor disruption to habituation when the distractor was dissimilar to the target but not when it was similar, possibly because when it was similar the animals perceived both stimuli as the same stimulus thus the distractor could not compete for A1 space as effectively because of overlapping elements. The same effect was subsequently replicated by Kaye, Swietalski, et al. (1988a; Experiment 1). Robertson and Garrud (1983) also reported that a pre distractor disruption to habituation only occurred when the distractor was similar to the target but not when it was dissimilar, consistent with our assumption. However, in a second experiment, Kaye, Swietalski, et al. (1988a) reported that a dissimilar distractor (saline solution) disrupted habituation whether it was presented pre or post target (lemon solution). Therefore, it is likely that the pre target object used during some of our distractor tasks (standard task, Experiment 11; CACB and

DACB, Experiment 14) influenced the recency discrimination during the tests. So far, we have not compared our data with a standard relative recency task which should answer the question of how a pre and post distractor influence recency discrimination performance during spontaneous object recognition tasks. This was explored during Experiment 15.

5.6 Experiment 15

5.6.1 Introduction

It is possible that the pre target objects used during some of our experimental tasks influenced the recency discrimination during those tasks. For example, when we presented object C prior to object A during the standard task of Experiment 11 this could have influenced discrimination performance during the test for this task. Pre target distractors have been shown to disrupt habituation of rats' neophobic responses towards novel flavours under certain circumstances (Kaye, Swietalski, et al., 1988a; Robertson & Garrud, 1983), and the level of disruption to habituation appears to be dependent on the distractor's relative similarity to the target stimulus (Kaye, Swietalski, et al., 1988a). For example, Kaye, Swietalski, et al. (1988a) reported that a distractor (saline or coffee) presented before a target solution (lemon) disrupted habituation to the target solution and that saline disrupted habituation more than coffee. They suggested that this could have been because saline was perceived as less similar to lemon than was coffee. The authors interpreted their results in terms of generalisation of habituation between distractor and target solutions – that is, the more similar the distractor and target solution were, the greater the generalisation would be between them, so that the presentation of the distractor, prior to the target solution, would more likely have resulted in generalised habituation to the target solution.

Exp.	Task	Objects and intervals								Task	One-sample	Student's
11	Standard	**C**	55-mins	**A**	2-hours			**B**	5-mins	**AB**	$p = .147$	$p = .005$
	Distractor			**A**	55-mins	**C**	55-mins	**B**		**AB**	$p < .001$	
13	Proximal	**A**	15-mins	**C**	95-mins			**B**	5-mins	**AB**	$p < .001$	$p = .827$
	Distal	**A**	95-mins			**C**	15-mins	**B**		**AB**	$p < .001$	
14	CACB	**C**	55-mins	**A**	55-mins	**C**	55-mins	**B**	5-mins	**AB**	$p = .005$	$p = .515$
	DACB	**D**		**A**		**C**		**B**		**AB**	$p = .120$	

Table 19. Experimental designs used for Experiments 11, 13, and 14. One sample t-test results are reported for each task, which compared mean discrimination indexes to chance (0), and Student's t-test results are reported which compared the mean discrimination indexes between the tasks of each experiment.

During Experiment 11, we presented a distractor object before object A during the standard task. (Table 19). We found that test discrimination was worse in the standard task – more specifically, recency discrimination between A and B was better if distractor C was presented after A. In Experiment 13 the distractor was always presented after A; but in the proximal condition it was shortly after object A in one task, and shortly before B in the distal condition (Table 19). Discrimination between A and B was very good in both conditions, and comparable to that of the distractor task in Experiment 11. Taken together these two experiments suggest that a distractor placed after A increases discrimination between A and B, and this improvement in performance doesn't seem to depend on *how long* after A the distractor is presented. During Experiment 14, distractor objects were presented both before and after object A (Table 19), and we observed inconsistent discrimination between A and B: the discrimination was better when the post-trial distractor was relatively familiar (CACB) than when it was not (DACB). Up to this point we had assumed that pre-trial distractors did not influence discrimination performance. However, the results of this experiment did

not seem to give a clear indication of this and so this assumption may have been incorrect.

To explore this possibility, Experiment 15 compared recency discrimination performance between three tasks. Two of the tasks were identical to the standard and distractor tasks used during Experiment 11, but we changed the names of these tasks to pre-distractor and post-distractor respectively to better reflect the research question, and the third task was a relative recency task that included no distractor stimuli (Table 20). We predicted that recency discrimination performance would be good in the no-distractor task and that this performance would either be reduced (pre-distractor) or enhanced (post-distractor) depending on the placement of the distractor relative to target object A.

Task	**Sample phases and intervals**								**Test**
Pre	C	55-min	A	2-hours			B	5-mins	A and B
No			A	2-hours			B	5-mins	A and B
Post			A	55-min	C	55-min	B	5-mins	A and B

Table 20. Experimental design for Experiment 15 and the three tasks used: pre-distractor, no-distractor, and post-distractor. A and B represent the target objects and C depicts the distractor objects. Sample phases were 10 minutes in duration and the test was 5 minutes.

5.6.2 Materials and method

5.6.2.1 Subjects

The subjects were 16 male C57BL/6J mice that were approximately 3 months old at the start of the experiment. These mice had previously completed Experiment 11.

5.6.2.2 Procedure

This experiment consisted of three individual tasks that each included two (no-distractor) or three (pre-distractor and post-distractor) sample phases and a test (Table 20). The sample phases were 10 minutes, and the tests were 5 minutes in duration. This was a within-subjects design, so all mice completed all three tasks. Left/right zones were used throughout and task order, object identities and their locations were counterbalanced across subjects (Table 20).

Pre-distractor task

Mice were first exposed to two copies of object C followed by a 55-minute interval and then they were presented with two copies of object A. After a 2-hour interval, mice were exposed to two copies of object B, followed by a 5-minute interval and then tested with one copy of A and one copy of B.

No-distractor task

Mice were first exposed to two copies of object A followed by a 2-hour interval and then they were presented with two copies of object B. After a 5-minute interval, mice were exposed to one copy of object A and one copy of object B.

Post distractor task

Mice were first presented with two copies of object A followed by a 55-minute interval and then they were exposed to two copies of object C. After another 55-minute interval, mice were presented with two copies of object B, followed by a 5-minute interval, and then tested with one copy of each of the object's A and B.

Animal	1-2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Task order	Pst	Pst	Pst	Pst	No	No	No	No	No	Pre	Pre	Pre	Pre	Pre	Pre
	No	No	Pre	Pre	Pst	Pst	Pst	Pre	Pre	Pst	Pst	Pst	No	No	No
	Pre	Pre	No	No	Pre	Pre	Pre	Pst	Pst	No	No	No	Pst	Pst	Pst
C	Sal	Sal	Sal	Sal	-	-	-	-	-	Sal	Sal	Sal	Sal	Sal	Sal
	-	-	Leg	Leg	Sal	Sal	Sal	Sal	Sal	Leg	Leg	Leg	-	-	-
	Leg	Leg	-	-	Leg	Leg	Leg	Leg	Leg	-	-	-	Leg	Leg	Leg
A	Sta	Pin	Pin	Sta	Sta	Sta	Pin	Pin	Sta	Sta	Sta	Pin	Pin	Sta	Pin
	Foo	Sil	Foo	Sil	Foo	Foo	Sil	Foo	Sil	Sil	Sil	Foo	Sil	Sil	Foo
	Tab	De	Tab	De	Tab	De	De	Tab	De	Tab	De	Tab	Tab	De	Tab
B	Pin	Sta	Sta	Pin	Pin	Pin	Sta	Sta	Pin	Pin	Pin	Sta	Sta	Pin	Sta
	Sil	Foo	Sil	Foo	Sil	Sil	Foo	Sil	Foo	Foo	Foo	Sil	Foo	Foo	Sil
	De	Tab	De	Tab	De	Tab	Tab	De	Tab	De	Tab	De	De	Tab	De
Left	Sta	Sta	Sta	Pin	Sta	Sta	Sta	Sta	Pin	Sta	Sta	Sta	Sta	Sta	Sta
	Foo	Foo	Foo	Foo	Foo	Foo	Foo	Foo	Foo	Foo	Foo	Foo	Foo	Foo	Foo
	Tab	Tab	Tab	Tab	Tab	Tab	Tab	Tab	Tab	Tab	Tab	Tab	Tab	Tab	Tab
Right	Pin	Pin	Pin	Sta	Pin	Pin	Pin	Pin	Sta	Pin	Pin	Pin	Pin	Pin	Pin
	Sil	Sil	Sil	Sil	Sil	Sil	Sil	Sil	Sil	Sil	Sil	Sil	Sil	Sil	Sil
	De	De	De	De	De	De	De	De	De	De	De	De	De	De	De

Table 20. Counterbalancing of objects, their test locations, and task order during Experiment 15. A and B depict target objects, C denotes distractor objects, and left and right refer to the object's positions during test.

5.6.3 Results and Discussion

During the sample phases of Experiment 15, mice spent similar amounts of time exploring all objects independent of the task (Figure 37). Exploration of objects A and B across tasks was investigated using an ANOVA, which analysed the mean total exploration time for each object, with task (pre/no/post) and object (A/B) as within-subject factors. This showed no main effect of task ($F(2, 30) = .295$, $p = .747$, $MSe = 232.403$, $\eta_p^2 = .019$), or object ($F(1, 15) = .259$, $p = 618$, $MSe = 176.313$, $\eta_p^2 = .017$), and no interaction between them ($F(2, 30) = 2.771$, $p = .079$, $MSe = 1633.709$, $\eta_p^2 = .156$). We then explored exploration of object C, relative to A and B, during the pre and post tasks which showed similar amounts of exploration of C between the two tasks. This was analysed using an ANOVA with task (pre/post) and object (A/B/C) as within-subject factors which revealed that

there was no main effect of task ($F(1, 15) = .028$, $p = .870$, $MSe = 20.720$, $\eta_p^2 = .002$), that object approached but did not reach significance ($F(2, 30) = 3.148$, $p = .057$, $MSe = 1262.878$, $\eta_p^2 = .173$), and that there was no interaction between the two factors ($F(2, 30) = .652$, $p = .528$, $MSe = 315.098$, $\eta_p^2 = .042$).

During Experiment 12, we hypothesised that the absence of a significant recency effect may have been due to the lower levels of exploration, relative to those of Experiment 11, during the sample phases. A reduction in exploration, during the sample phases, may have resulted in less competition for space in memory because there would have been less elements in each of the activation states, at any given time, thus more space available for new elements to occupy without the need to outcompete existing elements for space. To test this hypothesis, we compared the total exploration across the sample phases of Experiment 15 to those of Experiments 11 and 12. We predicted that if exploration levels were similar to Experiment 11, then there would be a strong recency discrimination enhancement effect during the test (pre versus post), but if exploration levels were more similar to Experiment 12, then there would be a much weaker effect. We compared these using an ANOVA which revealed an effect of total exploration ($F(2, 86) = 4.443$, $p = .015$ $MSe = 90438.659$, $\eta_p^2 = .094$). The total exploration (mean ± SD) for each experiment was: 602 ± 113.6 for Experiment 11, 490.0 ± 154.1 for Experiment 12, and 473.5 ± 123.1 for Experiment 15, and post hoc tests (using the Holm correction to adjust p) indicated that the exploration was greater during Experiment 11 than both 14 ($p = .019$) and 17 ($p = .024$), but did not differ between 14 and 17 ($p = .685$). Therefore, we expected any recency discrimination enhancement effect to be relatively weak between the pre and post tasks.

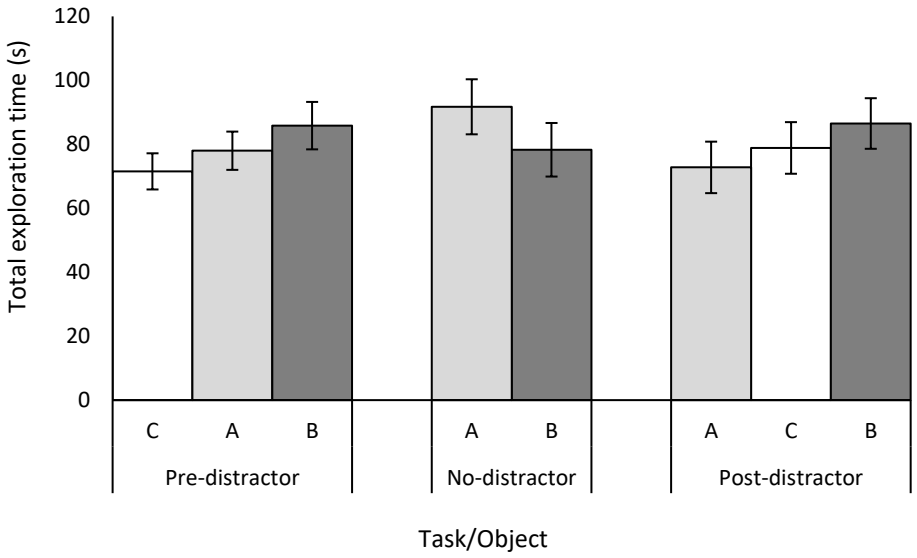


Figure 37. Mean (±SEM) total exploration time (s) for the objects during all sample phases, and tasks, during Experiment 15.

During the test phase of Experiment 15, recency discrimination performance appeared to be increased with the inclusion of a post distractor object and decreased with the inclusion of a pre distractor object, relative to a task that included no distractor objects (Figure 38). Recency discrimination was significant in both the no-distractor and post-distractor tasks but not in the pre-distractor task (mean exploration (± SEM): pre, A, 14.5s ± 1.9s, B, 10.3s ± 1.47; no, A, 11.6s ± 1.4s, B, 6.6s ± 1.3s; post, A, 15.8s ± 1.4s, B, 5.5s ± .8s). The observed test data (Figure 38) suggested that exploration of object A increased, and exploration of object B decreased across tasks but these observed differences were not statistically significant. To explore this, we compared the mean discrimination indexes between the three tasks using a repeated measures ANOVA with discrimination index as a within-subjects factor. The observed mean discrimination indexes increased across tasks (pre: .17, no: .29, post: .45), but this difference was not statistically significant ($F(2, 30) = 2.627$, $p = .089$ $MSe = .326$, $\eta_p^2 = .149$; Figure 38). We explored post hoc comparisons (using the Holm correction to adjust *p* values) because, based on the results of Experiment 11, we expected a significant difference between the pre and post distractor tasks.

Although the observed mean discrimination indexes appeared to differ (pre: .17, post: .45), in the post hoc comparisons (using the Holm correction to adjust *p*) any difference between them was not significant (pre vs post, $p = .088$; pre vs no, $p = .425$; no vs post, $p = .425$). We then compared each task's discrimination index to chance (zero) using one-sample t-tests. These indicated that recency discrimination, although in the right direction, was not significant in the pre-distractor task ($T(15) = 2.024$, $p = .061$), but was significant in the no-distractor task ($T(15) = 3.145$, $p = .007$), and in the post-distractor task ($T(15) = 6.258$, $p < .001$; Figure 38).

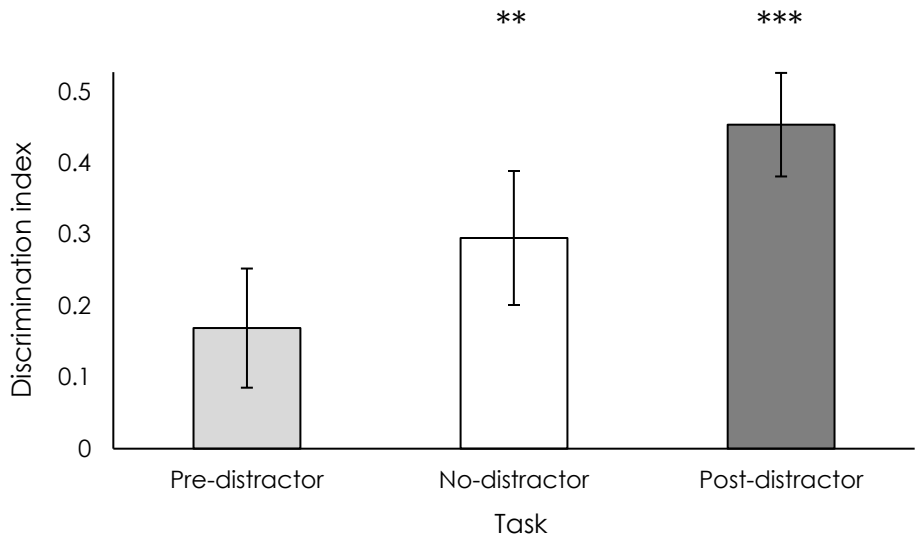


Figure 38. Mean (±SEM) discrimination indexes during the test phase of Experiment 15. Asterisks above a bar denote a significant difference from chance (0), ** $p < .010$, *** $p < .001$.

These data suggest that there may have been weak distractor effects that were not statistically detectable in these mice using this sample size, thus a larger sample size may have been required to achieve the appropriate level of statistical power needed to explore these types of effects. This was consistent with our prediction that lower levels of exploration during the sample phases would result in a weaker effect. With this in mind, we combined the pre-distractor and post-distractor data

from Experiments 11 and 15 and analysed these data. These analyses revealed that recency discrimination was significant in both tasks and that there was a significant difference between the two types of tasks, in the direction of greater responding to A during the post-distractor tasks (Figure 39). To explore this, we used an ANOVA with task (pre/post) as a within-subjects factor and experiment as a between-subjects factor (13/17). The ANOVA showed that there was a main effect of task ($F(1, 30) = 23.075$, $p < .001$ $MSe = 1.217$, $\eta_p^2 = .435$; Figure 39) and no other significant results (lowest $p = .419$). One-sample t-tests, which compared the discrimination indexes of Experiments 11 and 15 combined for the two tasks to chance (zero), showed a significant discrimination in both the pre ($T(31) = 2.562$, $p = .015$) and post ($T(31) = 8.953$, $p < .001$; Figure 39) distractor tasks. Overall, the results presented here suggest that a larger sample size may have been required to explore distractor effects in mice, and that this may be due to mice often exhibiting low levels of object exploration during the sample phases.

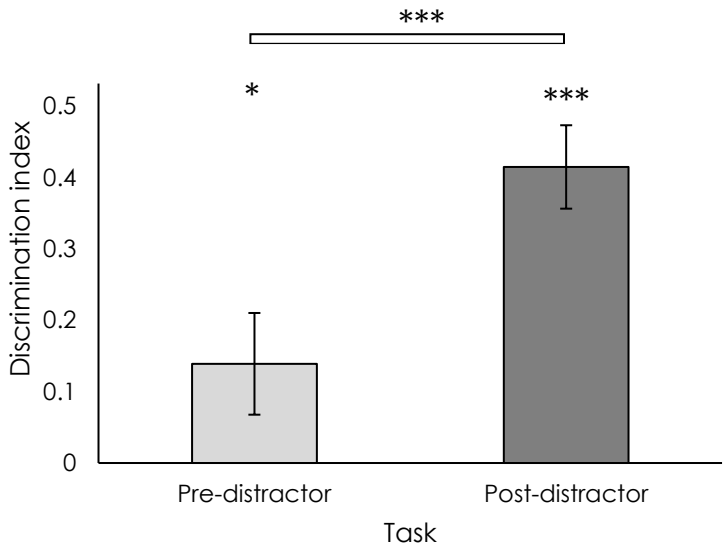


Figure 39. Combined mean (±SEM) discrimination indexes, for the pre-distractor and post-distractor tasks, during the test phases of Experiments 11 and 15. Asterisks above a bar denote a significant difference from chance (0), * $p < .050$, *** $p < .001$, and above a bracket depict a significant difference between tasks, *** $p < .001$.

The data obtained during Experiment 15 suggested that a pre-distractor object may have reduced recency discrimination performance, and that a post-distractor object may have had the opposite effect and enhanced it, compared with a recency discrimination with no distractor objects. This is consistent with our prediction. However, although the observed mean discrimination indexes and the individual recency discrimination performances for each task were suggestive of this, there were no statistically significant differences between the three tasks. This may have been due to the low levels of exploration during the sample phases which, based on the sample and test data of Experiments 11, 12 and 15, appeared to weaken distractor effects. Therefore, a larger sample may be required to gain adequate statistical power to detect such effects. Certainly, when we combined the pre and post distractor tasks of Experiment 11 and 15, we observed robust statistical differences between the tasks as well as significant recency discrimination performance during both individual tasks. This suggests that a sample size of 32 may be needed when using C57BL/6J mice to explore distractor effects during relative recency object recognition tasks.

SOP explains the potential distractor effects during Experiment 15, by suggesting that they may have arisen through competition for memory space in A1 and A2, between the elements of the distractors and target stimuli (objects A and B; Mazur & Wagner, 1982; Wagner, 1981). During a relative recency discrimination test, SOP suggests that the object explored more (e.g., A) has relatively fewer elements residing in A2 than the other object (e.g., B), thus has more elements available for A1 activation and stronger responding. It also suggests that the number of A's elements residing in A2 at test, could be further reduced when a distractor is presented after A, such as during the post-distractor task. SOP explains the enhancement effect, that may have occurred during the post-distractor task, by suggesting that elements of object C competed with and accelerated the decay of A's elements that already resided in A1 and A2. Thus, during the test A would have had more elements available, that had decayed back to inactive, which could have been provoked into A1 and generated stronger responding, compared with a task where a distractor did not precede A (e.g., as in the no-distractor task). If a

post-distractor task results in enhanced exploration of A because A has relatively fewer elements in A2 when it is followed by a distractor, then in a task where A is preceded by a distractor (e.g., pre-distractor task), and exploration of A is reduced, then this may suggest that A had relatively more elements in A2 when it was preceded by a distractor. Therefore, it is possible that when A is followed by C, the decay of A's elements is accelerated, and when A is preceded by C, the decay of A's elements is slowed down. Thus, habituation to A is greater when A is preceded by a distractor than when it is followed by one. Using this logic, during the post-distractor task, the decay of object B's elements would have been slowed down by the presentation of object C prior it. This would have resulted in more habituation to object B than in the other two tasks although the effect would be very small because of the short interval between object B and the test. The observed discrimination indexes from the test data, and the analyses of recency discrimination performance during each task, suggested that this may have been the case, but a larger sample size would be required to confirm or reject these potential effects.

In summary, Experiment 15 demonstrated that recency discrimination performance, during the test, was significantly above chance during the no-distractor and post-distractor tasks but not significant during the pre-distractor task, which suggests that the pre-distractor object reduced recency discrimination. The observed mean discrimination indexes also indicated a reduction in the pre-distractor task as well as an enhancement in the post-distractor task. However, there were no significant difference between the three tasks which suggests no effect of distractor objects. A second replication of this experiment may be required to increase statistical power and confirm or reject pre and post target distractor effects during relative recency object recognition.

5.7 General discussion

Chapter 5 explored the effects that distractor objects placed prior to, or after, target objects may have on relative discrimination performance. To explore this, we used relative recency object recognition tasks (Mitchell & Laiacona, 1998) which consisted of two pairs of identical objects sequentially presented over two sample phases (e.g., A and A – interval – B and B), followed by a test with one object from each pair (e.g., A and B). During this task animals typically explore the less recent object A more during test. We placed an additional object either within the sample-sample interval, prior to the first presented object, or both of these to explore distractor effects. In terms of SOP (Wagner, 1981), relative recency object recognition tasks map well on to self-generated priming, and it is unlikely that retrieval-generated priming supports performance during these tasks because the context used is constant throughout (Robinson & Bonardi, 2015; Sanderson et al., 2011). Therefore, recency discrimination likely arises because of the relative number of elements residing in A2 for the test objects. SOP also asserts that distractor objects should have an impact upon the decay of elements from A2 during these tasks (Mazur & Wagner, 1982; Wagner, 1981). SOP predicts that, a distractor presented after object A will accelerate the decay of A's elements from A2 and increase responding to A during the test, that this effect should be increased if the distractor is presented proximally within the sample-sample interval, compared with distally, and that a salient distractor should have a greater effect on the decay of A's elements compared with a less salient distractor.

These predictions were explored across a series of experiments during Chapter 5. The first of these experiments, Experiment 11, demonstrated that a distractor object placed between the target objects enhanced relative recency discrimination performance, when compared to a distractor object placed prior to them. This was consistent with SOP's prediction (Mazur & Wagner, 1982) and with previous reports from temporal separation studies (Kesner et al., 2002; Templer & Hampton, 2013), and was in contrast to theories of proactive interference, which

predict the opposite effect on recency discrimination (Bartko et al., 2010; Engelmann, 2009).

Experiment 12 was a replication of Experiment 11 but used APP/PS1 transgenic mice, an Alzheimer's mouse model, and their wild type littermates. We were unable to replicate the distractor effect in these mice, although we did confirm that relative recency discrimination was intact, at this age, consistent with previous reports (Bonardi et al., 2016, 2021). The observed mean discrimination indices suggested that there may have been a distractor effect, but a statistical analysis comparing these did not support the observation. However, exploration of the objects during the sample phases was significantly lower than in Experiment 11 which suggests that a certain level of exploration may be required for a significant distractor effect. Lower levels of exploration during the sample phases were also observed during Experiment 15, compared with Experiment 11, and again there was no significant difference between tasks where the distractor was placed either prior to or after the first target object A. However, the observed mean discrimination indexes again suggested that an effect may have been present. The differences in exploration between experiments, and their potential effect on the test data, are consistent with SOP (Mazur & Wagner, 1982; Wagner, 1981). SOP suggests that, with less exploration of the objects, there would be relatively fewer elements in A1 and A2 at all stages, therefore less competition for space within the activation states and consequently more space for elements to occupy. This would result in fewer elements being subjected to accelerated decay thus a reduction in the distractor effect.

Because the distractor effect appeared to be subtle, it may have been difficult to detect using our sample size of 16 mice per experiment (for Experiment 12 we used as close to this number as possible: 16 female and 11 male APP/PS1 mice, and 17 female and 15 male wild type mice). That is, a sample size of 16 mice may have been insufficient to achieve the level of statistical power needed to detect distractor effects consistently. This suggestion was supported when we combined the results of Experiments 11 and 15, which resulted in significantly robust

differences between the pre and post distractor tasks. This suggests that a sample size of 32 may be more appropriate for investigating distractor effects in these types of tasks when using C57BL/6J mice.

Experiment 13 investigated the apparent effect reported in Experiment 11 further by varying the placement of the distractor object, placing it either shortly after A or shortly before B. SOP would predict that an earlier placement would result in a greater effect than later placement, which has been reported for habituation during taste aversion learning (Kaye, Gambini, et al., 1988). The opposite effect has been shown during taste aversion conditioning (Kaye, Gambini, et al., 1988; Kwok et al., 2017). However, our results showed that distractor placement, either proximally or distally, within the sample-sample interval was irrelevant to the distractor's impact on recency discrimination performance although the absence of an effect could have been because the temporal difference between the two arrangements was not sufficient to detect such an effect. For example, Kaye, Gambini, et al. (1988) used a temporal spacing of 210 minutes difference, between the distractor's placements, and reported an effect whereas we used 70 minutes difference and saw no effect.

Because a 2-hour sample-sample interval may be optimal for these types of tasks (Hatakeyama et al., 2018; Tam et al., 2014), we were reluctant to increase the duration of the interval that we used during our tasks. Instead, during Experiment 14, we explored an alternative prediction of SOP using the same temporal spacing, between the target and distractor objects, that we had used during our previous distractor experiments. The prediction was that the salience of the distractor should increase or decrease its effectiveness (Mazur & Wagner, 1982; Wagner, 1981). Unfortunately, we were unable to establish distractors that were more or less salient than one another (a novel and a familiar object respectively) and, unsurprisingly, saw no significant differences between the two tasks. It has been shown that 10 minutes of exposure time is not sufficient to establish a stimulus as familiar enough for its interference to differ from that of a novel stimulus, and that an exposure time of 40 minutes can be sufficient to observe such a difference

(Shanks et al., 1986). Therefore, we could run the experiment again but increase the exposure time, during the pre-target familiarisation phase, from 10 minutes to 40 minutes. Shanks et al. (1986) also ran their familiarisation sample phases over multiple consecutive days, thus if we replicated Experiment 14, it may be more effective to divide the 40-minute pre-target familiarisation phase into four 10-minute sessions spread over four consecutive days.

Because we were unable to establish novel and familiar distractors during Experiment 14, we expected to see good recency discrimination during both tasks due to the apparent distractor effect, based on the results of Experiment 11. However, we only observed this in one task but not the other. We wondered if this may have been because a pre-target distractor had a reduction effect which may have interfered with any post-target enhancement effects. Experiment 15 explored this possibility by comparing three relative recency tasks, one with a pre-target distractor, one with no distractor, and one with a post-target distractor. Although we could not detect any significant differences between the three tasks, the observed mean discrimination indexes, and their individual statistical analyses, suggested that a pre-distractor may have reduced recency discrimination and that a post-distractor may have enhanced it, relative to no distractors.

These data were inconsistent with some of the previous data reported from habituation of neophobia to taste stimuli experiments, which have demonstrated that distractors disrupted habituation to targets similarly, whether they were presented before or after the target (Kaye, Swietalski, et al., 1988a). Other previously reported data are consistent with ours. For example, Artigas, Sansa, and Prados (2012) reported more disruption to habituation when the target was followed by a distractor compared to when it was preceded by one. However, this was only when using distractor and target stimuli that were similar to one another. When using dissimilar stimuli, distractor placement appeared irrelevant. Similarly, an earlier study reported a disruption to habituation to the target when the distractor followed the target and not when it preceded it (Robertson & Garrud, 1983). However, this was only when the stimuli were dissimilar. When similar

stimuli were used, a reversed effect was reported; disruption to habituation to the target was observed when the distractor preceded the target and not when it followed it. The differences between the distractor effects produced when target and distractor stimuli are similar or dissimilar from one another has previously been directly investigated (Kaye, Swietalski, et al., 1988a). Kaye, Swietalski, et al. (1988a) reported that the magnitude of the distractor effect was a function of relative similarity, and that saline (dissimilar) caused more disruption to habituation to a target (lemon) than coffee (similar), and that this was not a consequence of coffee being less salient than saline. This was confirmed in one of their other experiments where they established that coffee was at least as effective as saline in disrupting habituation of a different target (saline sucrose mixture). Thus, they suggested that distractor effects may be modulated by the relative similarity between the distractors and the target stimuli used. Therefore, it is possible that distractor effects are dependent on the specific stimuli used during each study, and that these differences may help to explain the different results reported. So, because our experiments used junk objects and not taste stimuli, this may explain why our results are inconsistent with some of the previously reported data from taste stimuli studies.

5.8 Conclusion

We provided some evidence of distractor effects although we were unable to replicate them reliably. This could have been due to the lower levels of exploration observed during the sample phases of the replications. Future work could implement measures to try and increase object exploration, such as increased duration of sample phases or reduced available floor space around the objects which may encourage more object interaction. During Experiment 14, we were unable to establish distractors that were more or less salient than one another, possibly because our familiarisation sample phases were too short. Therefore, Experiment 14 should be replicated using a much longer duration for the familiarisation sample phases, preferably divided over multiple consecutive days. Furthermore, Experiment 15 suggested that a pre-target distractor may have had a

reduction effect on recency discrimination. Therefore, to prevent the possibility of reduction effects that pre-target distractors may have on recency discrimination performance during the test, the familiarisation sample phases should be moved away from the other experimental phases. For example, the familiarisation sample phases could be run over the four days prior to the day that the rest of the experimental phases take place. Experiment 15 also needs to be replicated to confirm or reject the possibility that distractor objects can produce reduction and enhancement effects, depending on their placement relative to target objects, on later recency discrimination performance.

Chapter 6: General discussion

6.1 Summary

6.1.1 Background

During this thesis, I have explored the suitability of using an alternative approach to study recognition memory that sidesteps many of the complexities associated with other methods used. For example, recognition memory has been traditionally assessed in non-human animals using tasks such as serial recognition, delayed matching to sample, and delayed non-matching to sample tasks (e.g., M. Eacott et al., 1994; Fahy et al., 1993; Mumby et al., 1990; Rothblat & Hayes, 1987; Suzuki et al., 1997; Turchi et al., 2005; F. A. W. Wilson & Rolls, 1993; Zola et al., 2000), which offer poor translation to humans because humans are generally not tested under regimes of elevated fear or hunger levels (Dere et al., 2007; Ennaceur & Delacour, 1988). Furthermore, they also require levels of pretraining that 'normal' recognition does not require. In contrast, research in humans has often been based on subjective psychological experience (e.g. Berry et al., 2006; Espinosa-García et al., 2017), which can require verbal judgements that are generally not appropriate for animal models or for dementia patients. Although Fortin, Wright, and Eichenbaum (2004) successfully trained rats to differentially respond to new and old items across a series of response biases, which paralleled human confidence judgements, but this required intensive training. Furthermore, recognition memory processes have often been defined by their underlying neural mechanisms (Aggleton & Nelson, 2020; Warburton & Brown, 2010, 2015) but the evidence supporting these definitions has often been criticised (e.g., Squire et al., 2007). Our alternative approach uses the spontaneous object recognition task (Ennaceur & Delacour, 1988), a task that exploits rodents' propensity to explore novel objects more than familiar ones and that requires no training or schedules of reinforcement. This task is translatable to humans using a conceptually identical task where objects are instead displayed visually and eye-tracking software used to measure gaze duration – as humans generally gaze longer at novel vs familiar

items (e.g., Fagan, 1970; Nitka et al., 2020). We combine using variants of the spontaneous object recognition task with the Sometimes Opponent Process (SOP) theory of associative learning and memory (Brandon et al., 2003; Wagner, 1981), which defines recognition simply, as a reduction in behavioural response to a previously encountered stimulus (compared to a novel one). SOP asserts that recognition memory comprises two underlying priming processes: self-generated priming, in which a reduction in response occurs to a stimulus has been recently experienced, and retrieval-generated priming, where reduced responding occurs towards an object that has been predicted by another stimulus through a prior association. Therefore, this thesis explored predictions related to these priming processes, generated by SOP, during spontaneous object recognition memory tasks using mice.

6.1.2 Chapter 3

Chapter 3 explored the prediction that object recognition memory may be susceptible to cue competition effects, such as blocking (Kamin, 1969), during association-based tasks such as object-in-context and object-in-place tasks (Ainge et al., 2007; Ameen-Ali et al., 2012; Dix & Aggleton, 1999; M. A. Good et al., 2007; Mumby et al., 2002; Norman & Eacott, 2005; Sep et al., 2021; Spanswick & Dyck, 2012; Spanswick & Sutherland, 2010; D. I. Wilson et al., 2013), which are thought to be tests of associative recognition (Aggleton & Nelson, 2020). The prediction was based on the SOP assumption that such associative recognition is dependent on retrieval-generated priming which uses associative context-dependent information (Brandon et al., 2003; Mazur & Wagner, 1982; Vogel et al., 2020; Wagner, 1981). Therefore, retrieval-generated priming should be susceptible to cue competition effects, and these should be observed during association-based object memory tasks. To test this prediction, we used variants of the object-in-context task in which objects were paired with cues (e.g., p→A, q→B) during sample phases and then paired with blocking cues and a second cue during compound phases (e.g., pX→A, pX→B). These were followed by tests with the

objects presented with the second cue (e.g., XA, XB). We failed to provide evidence of blocking during any of the experiments undertaken in Chapter 3.

6.1.3 Chapter 4

Chapter 4 further explored SOP predictions based on retrieval-generated priming using an indirect object recognition task, that has been previously used in rats (Whitt et al., 2012), and a blocking design based on that task. Again, we failed to provide evidence of a blocking effect but did successfully replicate the results of Whitt et al. (2012) and demonstrated indirect object recognition memory in mice. That is, mice were exposed to objects P and Q sequentially in contexts X and Y respectively (X→P, Y→Q), and then exposed to X alone. During a test that followed, mice directed less exploration towards P, which we suggested had been primed by X via retrieval-generated priming (X→P), and more exploration towards Q, which we suggested had not been primed because it had no association with X (Y→Q). In a second replication, using APP/PS1 mice and their wild type littermates, we observed a reverse effect. That is, during the test mice directed more exploration towards P than Q, which we suggested may have been due to an attentional deficit, based on the discussions of previous work related to hippocampal damage and SOP (Honey & Good, 2000a, 2000b). In a further three replications of the indirect object recognition memory task, using C57BL/6J mice, we were unable to replicate the effect observed during the initial experiment. We speculated that this may have been due to the low levels of object exploration that may have resulted in low levels of associative strength that were insufficient to reach asymptote where they would be effective (e.g., Jennings & Bonardi, 2017; Jones & Haselgrove, 2013; Sanderson et al., 2016).

6.1.4 Chapter 5

Chapter 5 explored further predictions of SOP that were based on the theory's nonassociative self-generated priming process, in which responding towards a stimulus is reduced through multiple exposures, within a short timeframe, which results in short term habituation to the stimulus (Mazur & Wagner, 1982;

Sanderson & Bannerman, 2011; Wagner, 1981). We used variants of the relative recency spontaneous object recognition task (Mitchell & Laiacona, 1998) to test these predictions, as the task maps well onto self-generated priming and because its configuration minimises the likelyhood of retrieval-generated priming contributing to test performance (Robinson & Bonardi, 2015; Sanderson et al., 2011). In this task, two objects are sequentially presented over two sample phases (A then B), followed by a test with both objects in which animals typically explore the less recent object A, more than the more recent object B. SOP explains test performance in this task by suggesting that self-generated priming reduced responding to B relative to A. Our predictions were based around potential distractor effects which may affect habituation to target objects, and thus may impact upon recency discrimination performance during variants of the relative recency task.

The first experiment tested the prediction that a distractor object presented between A and B, in the sample-sample interval, would disrupt habituation to A by accelerating the decay of A's elements from A2. Thus, during the test, A would be explored more than in a task where no distractor was presented between A and B. Our data were consistent with the SOP prediction and showed exactly this effect. We then replicated this experiment, using APP/PS1 mice and their wild type littermates, but we observed no significant distractor effect. In the next experiment, we tested the prediction that moving the distractor closer to A would disrupt habituation to A more than moving the distractor closer to B, because A's elements would be subjected to accelerated decay over a longer time. However, we found no evidence to support this hypothesis. Following this, we tested the SOP prediction that a more salient distractor (novel object) would disrupt habituation to A more than a less salient distractor (familiar object) because a more salient distractor would compete more for space in memory, specifically A2, than a less salient distractor. Unfortunately, we failed to establish a more and a less salient distractor, as was evidenced by no differences in exploration between them. We would have expected the mice to have explored the novel distractor object significantly more than the familiar distractor object. Therefore, the results of no

difference between the two distractor tasks were unsurprising – although we did observe inconsistent discrimination between the tasks which provoked the idea for the final experiment. In this experiment, we compared the effect of placing a distractor either prior to A or between A and B, and compared these with a standard recency task where no distractor objects were used. We found no significant differences between the three tasks, but the individual task data did suggest that discrimination performance may have been enhanced when a distractor followed A, and reduced when a distractor was presented prior to A.

6.2 Blocking

6.2.1 Blocking in object memory tasks

Retrieval-generated priming is a theoretical process that SOP uses to explain performance during association-based learning and memory tasks (Mazur & Wagner, 1982; Wagner, 1981). SOP uses this associative priming mechanism to explain many instances of learning (e.g., Brandon et al., 2003; Sanderson & Bannerman, 2011; Uribe-Bahamonde et al., 2019; Vogel et al., 2020; Vogel et al., 2019) and suggests that the same principles also apply to memory formation. Therefore, SOP predicts that cue competition effects, such as blocking, should also occur during association-based object memory tasks if it is assumed that the object is equivalent to an unconditioned stimulus (US), that the unconditioned response is object exploration (Robinson & Bonardi, 2015; Tam et al., 2014), and that the US needs to be surprising or unexpected to support learning (Rescorla & Wagner, 1972). Then under certain conditions, SOP predicts that blocking should occur. For example, if objects A and B were initially paired with two different cues p and q (p→A, q→B), followed by both objects presented in compound with p and a third cue X (pX→A, pX→B), then X would acquire less associative strength for A, because A would already be predicted by p. In contrast, B would be unexpected, thus able to form a stronger association with X. Therefore, if A and B were now presented with X, exploration of A would be greater than B because B would now be predicted by X and A would not. That is, X would have provoked elements of B

directly into A2 via retrieval-generated priming, because of their prior association, which consequently would have reduced responding to B. A on the other hand would not be predicted by X because association formation between A and X would have been reduced by A's prior association with p. Therefore, A would have all its elements available to enter A1 and generate stronger responding. During this report, we used variations of this example to test whether blocking effects occurred during spontaneous object recognition memory tasks.

6.2.2 Modulators of blocking

Inconsistent with SOP, we failed to provide any evidence that blocking occurred during the series of association-based memory tasks that we employed. However, an absence of evidence is not evidence of absence and there are many variables which may influence the blocking effect. Although blocking is considered to be a well-established phenomenon, the effect has not always been consistently replicated. For example, Glautier (2002) used a computer simulated card game in which humans had to learn which cards produced the best payouts. Subjects were presented with a competitor cue and an outcome during an initial stage and then, during a second stage, were presented with the competitor paired with the outcome and an additional target cue. The cues were colours and symbols that appeared on the backs of the cards. The authors reported that blocking occurred when the target and competitor cues appeared on different cards, but blocking did not occur when the cues appeared on the same card. Thus, the authors concluded that blocking effects may only occur under certain conditions. Other authors have also suggested that blocking effects may be parameter-dependent (e.g., Maes et al., 2016; Soto, 2018; Urcelay, 2017). For example, Soto (2018) reported that contemporary associative learning theories, and simulations of different models, predict that stimuli from the same modality, used during compound phases, should produce a weaker blocking effect and stimuli coming from different modalities should produce stronger blocking. In our later blocking experiments (Experiment 6 and 8), it is likely that the mice primarily used tactile information about the stimuli used (Brecht, 2007; Carandini & Churchland, 2013; Wu et al., 2013), therefore

predominantly processed them through the same modality. This could have reduced the likelihood of observing the blocking effect although these theoretical predictions are not well supported by empirical research (Maes et al., 2018).

Cue salience has also been suggested as a parameter that could influence the blocking effect (Maes et al., 2018; Soto, 2018). For example, Heckler et al. (2006) reported that differential cue salience significantly affected blocking in humans. They found that if the initially learned cue (A) was more salient than the blocked cue (B) then blocking was enhanced but when B was more salient than A, blocking was greatly reduced or eliminated. If A was of high salience, then it would have become strongly associated with the outcome during initial training and been able to be an effective blocking cue during compound training when it was paired with B. In contrast, if A was of low salience, then it would have only become weakly associated with the outcome during initial training, and this association may have extinguished prior to compound training. During compound training, if B was of high salience, then it would have been able to become strongly associated with the outcome particularly if A's association with the outcome had been extinguished. Although it is possible that the blocked cues we used (arena wall coverings) were more salient than the initially learned cues (patterned inserts, textured floors), we have no evidence that our cues had differential salience. Furthermore, the textured floors we used were likely more salient to the mice than the arena wall coverings, due to their tactile properties (Brecht, 2007; Carandini & Churchland, 2013; Wu et al., 2013), which should have enhanced any blocking effect. Therefore, cue salience is unlikely to account for our failure to produce a blocking effect.

Another variable which appears to be a necessary determinant for cue competition to be observed is the contiguity between events (Herrera et al., 2022). Temporal contiguity refers to the closeness between two events in time, where one event can be followed closely by another (e.g., milliseconds or seconds) or occur much later (e.g., hours or days). This has been empirically assessed in rats using a fear conditioning paradigm, where it was reported that when compound stimuli were

immediately followed by the outcome (strong contiguity), a tone overshadowed a clicker, but when the stimuli and outcome were separated by 10s, no overshadowing was observed (Urcelay & Miller, 2009). The authors suggested that these results may have occurred because the animals encoded the AX compound either elementally or configurally depending on contiguity with the outcome. When the contiguity was strong (i.e., no trace), the animals encoded the AX compound elementally and overshadowing occurred and when contiguity was weaker (i.e., 10s trace), the animals encoded AX configurally, and no overshadowing was observed. Further work in rodents and humans has also concluded that whether cue competition is observed or not is determined by contiguity, with strong contiguity producing consistent effects and weaker contiguity resulting in an absence of effect (Herrera et al., 2022; Urcelay, 2017). Urcelay and Miller (2009) used a tone and clicker as their conditioned stimuli (CSs) and a mild Fooshock as their unconditioned stimulus (US). In our experiments, if the blocking cues (e.g., textured floors) and blocked cues (e.g., arena wall coverings) are assumed to be equivalent to the CSs used by Urcelay and Miller (2009) and the junk objects equivalent to their US, then it is possible that the temporal contiguity between the CSs and the US were too weak to produce a blocking effect. Using the initial sample phase as an example, if the mice attended to the CS and then engaged in other activities (e.g., grooming or arena exploration) before attending to the US, then the temporal contiguity between the two events would be weak. Due to the low levels of object exploration by the mice in our experiments, this is likely to have occurred throughout all our blocking tasks and could explain why we failed to observe any blocking effects. In fact, we observed no evidence that any associative change had occurred, during our analyses of the compound phases, in the processing of the US, which suggested that the CS and US had not formed a strong association and may also suggest that contiguity was weak between them.

This hypothesis is consistent with SOPs priming processes and how they may interact and compete with one another (e.g., Sanderson & Bannerman, 2011). For example, during initial sample phases where mice were presented with the

blocking cue (p) and object (A), mice could have directed a significant amount of attention towards p and A at separate times, engaging in other behaviours in between, such as exploring the arena or grooming. Therefore, the temporal contiguity between p and A would be weak. Additionally, both p and A would now have elements in A2, via self-generated priming, and have fewer elements available to enter A1 at any given time. Before retrieval-generated priming can occur, stimuli need to become associated through concurrent A1 activity, which would now be reduced because of the A2 activity that both stimuli would now have. In contrast, with a strong temporal contiguity between p and A, mice exploring both items one after the other multiple times, there would be far more occasions for simultaneous A1 activity and elements and far more opportunity for a strong association to form between p and A.

Where modulators of blocking have been directly investigated in mice, it has been reported that both the stimulus distribution (Jennings & Bonardi, 2017) and the amount of training with the blocking cue (Sanderson et al., 2016) determine the blocking effect. For example, Jennings and Bonardi (2017) explored whether cues presented for fixed durations would produce better blocking than variable duration cues. During their blocking group, rats were initially trained with fixed and variable duration cues (F and V respectively) that were both reinforced. To equate rats experience in the blocking group with that of the control group, they were trained with a second pair of fixed and variable duration cues that were nonreinforced (Fc and Vc). Then rats in the blocking group experienced compound training where FX and VY were reinforced. This was followed by a test for rats responding to X and Y. They reported that blocking had occurred with X, which had been conditioned during compound training using a fixed duration cue, but not with Y, which had been conditioned during compound training using a variable duration cue. The authors suggested that these results may reflect that fixed cues reach a higher, more stable asymptotic level of associative strength than variable cues. During our blocking experiments, the cues used were present for the entire duration of each phase, although the time that mice spent attending to these was highly variable. It would be difficult to establish the exact durations, at any given

time, and it is unlikely that the mice attended to the cues for consistent durations throughout our experiments. Therefore, it is likely that the cues used during our blocking tasks more closely paralleled a variable duration as opposed to a fixed duration, which could explain why we failed to observe a blocking effect.

Sanderson et al. (2016) investigated the parameters required to obtain blocking in mice by testing the impact that the amount of prior blocking cue training had on producing blocking of appetitive conditioning. Over two experiments, they compared blocking cue training, prior to compound conditioning, where mice received either 80 or 200 trials. In the first experiment they used a visual blocking cue to block conditioning to an auditory cue and during the second experiment this was reversed, with an auditory cue blocking a visual cue. They chose 80 trials because they had previously found that this number of trials generally resulted in conditioned responding reaching asymptote. They were interested to see what effect continuing blocking cue training way beyond asymptotic levels of conditioned responding would have on blocking and whether this was influenced by the modalities of the cues used. They found that a visual cue only blocked conditioning of an auditory cue when mice received 200 trials with the blocking cue and not when they received 80, whereas an auditory cue blocked conditioning of a visual cue when mice received both 80 and 200 trials. Therefore, the modality of the blocking cue and the extent of exposure to this that mice receive determine the strength of blocking. In our blocking tasks, we used textured floors and patterned inserts as blocking cues which could be considered as visual in modality, although it is more likely that mice processed the textured floors primarily using tactile information (Brecht, 2007; Carandini & Churchland, 2013; Wu et al., 2013). Therefore, using the parameters defined by Sanderson et al. (2016), mice may have needed the equivalent of 200 trials to obtain a strong blocking effect. During Experiment 6, we employed two 10-minute sample phases that were equivalent to blocking cue training, although it was not possible to decipher exactly how many times mice attended to the blocking cue (p) and object (A) together, as would be the equivalent of one training trial. However, we observed a slight reduction in responding to A during the compound phase as would be

expected if the blocking cue had been well established (p→A). Although this was not a statistically significant reduction, it may have indicated that p had not acquired enough associative strength with A to reach asymptote. This would then suggest that the number of times mice attended to p and A together, during the two sample phases, was likely equivalent to less than 80 training trials, a number that has been shown to produce asymptotic levels of conditioned responding in mice (Sanderson et al., 2016). Therefore, if this were the case, then it is unsurprising that we did not observe a blocking effect.

6.2.3 Conclusion

In summary, there appear to be many modulators of cue competition that have been proposed theoretically and demonstrated empirically (Heckler et al., 2006; Herrera et al., 2022; Jennings & Bonardi, 2017; Sanderson et al., 2016; Soto, 2018; Urcelay, 2017; Urcelay & Miller, 2009). However, many of these parameters are difficult to manipulate using variants of the spontaneous object recognition task (Ennaceur & Delacour, 1988). In particular, the ability to control the number of CS-US (equivalent) pairings that occur and in ensuring that the contiguity between the CS and US are always strong. These parameters are relatively easy to control using typical rodent operant chamber training setups but difficult to control using spontaneous object recognition tasks. Although it is possible that blocking effects could occur during memory tasks, as SOP would predict (Brandon et al., 2003; Mazur & Wagner, 1982; Vogel et al., 2019; Wagner, 1981), perhaps demonstrating the phenomenon in a spontaneous object recognition paradigm is too ambitious due to the constraints of the task, which make it difficult to control important variables.

6.3 Indirect object recognition memory

6.3.1 Associative priming in object memory

Chapter 4 explored SOP predictions related to retrieval-generated priming using indirect recognition memory tasks. During Experiment 7, we successfully

replicated a previous study which had demonstrated indirect object recognition memory in rats (Whitt et al., 2012). In our replication, mice were presented with object P in context x (Px) and object Q in context y (Qy), during two sequential trials of a sample phase (order of Px and Qy trials were counterbalanced). Following the sample phase, mice were exposed to x with no objects present and then shortly afterwards tested for their responses towards P and Q in a third context z. During the test, mice directed more exploration towards Q than P which was consistent with the effect reported in rats (Whitt et al., 2012). Earlier studies had also reported similar effect in rats and mice (Honey & Good, 2000b; Sanderson & Bannerman, 2011).

Honey and Good (2000b) used intermixed trials in which rats were either presented with an auditory stimulus A for 10s, followed by a visual array X for 10s, or an auditory stimulus B followed by a visual array Y, for the same durations. The termination of A and B were immediately followed by the onset of X and Y respectively (A→X, B→Y). During a test phase, rats were presented with an auditory stimulus (e.g., A) followed by a compound visual array (XY) that contained one component that was consistent with training trials (e.g, X) and one that was inconsistent with them (e.g., Y). They reported that rats oriented more towards the array that was inconsistent with the training trials than to the one that was consistent. The authors suggested that this may have been because the consistent component X had been associatively primed by the auditory stimulus A and the inconsistent component Y had not. Similarly, Sanderson and Bannerman (2011) exposed mice to two pairs of maze arms (e.g., AB or CD), during training trials, and then exposed mice to three maze arms (e.g., ABD), during a test where they compared exploration levels between B and D. They reported more exploration of D and suggested that this was because B had been primed by A (A→B) and D had not.

Taken together, the results of Experiment 7 and the results of these earlier studies (Honey & Good, 2000b; Sanderson & Bannerman, 2011; Whitt et al., 2012), suggest that stimuli can be associatively primed in memory by stimuli with which

they are associated. According to SOP (Wagner, 1981), using the design by Honey and Good (2000b) as an example, during A→X and B→Y trials elements of the two stimuli (e.g., A and X) enter the A1 state and form an excitatory association. This allows later presentation of A to provoke elements of X into A2 where they have a reduced capacity to generate responding. Therefore, when A is followed by XY, Y can produce strong responding because it can generate A1 activity whereas X cannot, because A2 activity has been provoked by A. This SOP account can also explain the data from other previous reports of indirect recognition memory (e.g., Dellu et al., 1997; Dix & Aggleton, 1999; M. Eacott & Gaffan, 2005). However, the results from these studies can also be explained in terms of generalisation decrement. For example, Dix and Aggleton (1999) presented rats with object A in context x (x→A) and object B in context y (y→B), during four sequential sample phases, and then tested rats with A and B in either x or y. They reported more exploration of the object that was unrelated to the context than the object that was. These results are consistent with a priming effect but can be explained equally well by generalisation decrement. For example, in a context x test, object B may have been perceived differently because it had never been presented in x. This could have resulted in the rats treating B as more novel than A. However, this explanation does not explain the data provided during Experiment 7, because the objects were presented in a third context, during the test, in which neither object had previously been presented. Therefore, an associative priming account, such as that offered by SOP (Wagner, 1981), is more plausible.

However, this associative account of indirect object recognition in mice should be accepted with caution. In Experiment 10 we failed to replicate the findings of Experiment 7 in three replications of the task. In all three replications, mice directed similar amounts of exploration towards both test objects. The means were in the direction of Q being explored more than P across replications, but these observed differences were not significant. This suggests that the results of Experiment 7 may have been a false positive or that any apparent associative effects were very weak in mice. We speculated that this may have been due to low levels of object exploration by the mice. For example, SOP postulates that a strong

effect would require a strong x→P association and that the strength of this association would be determined by the number of elements that x and P have activated in A1 concurrently during the sample phase (Brandon et al., 2003; Mazur & Wagner, 1982; Wagner, 1981). The more elements x and P have simultaneously in A1, the stronger the excitatory association between them will be. Thus, low levels of object exploration would result in fewer elements of P being activated into A1 and fewer opportunities for this to occur simultaneously with elements of x. Consequently, x would only be able to prime a small number of P's elements into A2 due to a weak x→P association. Therefore, the relatively small number of P's elements residing in A2, during the test that followed, may not be sufficient to significantly reduce responding to P relative to Q. Moreover, Experiment 9 showed a reverse effect in transgenic mice and their wild type littermates; specifically, both genotypes explored P more than Q, indicating further variability of these effects in mice. These replication failures, the low levels of object exploration, and the results from the transgenic mice are discussed further in later sections.

6.3.2 Conclusion

We provided some evidence to support our SOP predictions during this series of indirect object recognition memory tasks, although we were unable to replicate these results. Therefore, further studies would be required before strong conclusions can be made in relation to whether associative priming effects occur in object recognition memory.

6.4 Recency-based object recognition memory

6.4.1 Distractor effects

Chapter 5 explored SOP predictions related to self-generated priming using variants of the relative recency task (Mitchell & Laiacona, 1998) which included distractor objects. In Experiment 11, mice were presented with object A and then object B, over two sequential sample phases, and then tested with A and B to measure discrimination performance based on the relative recency of the objects.

In a distractor task, a third object C was presented during the sample-sample interval between A and B, and in a standard task, a third object C was presented prior to A. Discrimination performance was significantly better in the distractor than the standard task. This was consistent with the SOP prediction that a distractor would accelerate the decay of elements already residing in the A1 and A2 states (Mazur & Wagner, 1982; Wagner, 1981). That is, responding to A would be enhanced because A would be able to generate more A1 activity because more of its elements would have decayed back to the inactive state. These results were the opposite of those predicted by theories of proactive interference, which would predict a reduction in discrimination during the distractor task, and an enhancement during the standard task (Bartko et al., 2010; Engelmann, 2009). That is, the distractor would proactively interfere with the memory of object A when it was presented prior to the target objects (A and B), and enhance recency discrimination, and when the distractor was presented between the target objects, it would proactively interfere with the memory of object B, but would not interfere with the memory of A, and would reduce recency discrimination. Experiment 12 replicated Experiment 11 using our transgenic mice and their wild type littermates. Although discrimination was good during both tasks, there was no significant difference between the tasks, or between genotypes, suggesting no distractor effect. When discrimination performance was compared to chance, performance was significant for both genotypes during the distractor task but significant only for the wild type mice during the standard task. These data and the observed means suggested that there may have been a weak distractor effect. Therefore, it is possible that the study may have been underpowered and unable to detect any effect. The performance of these mice is discussed further in a later section.

The results of Experiment 11 could also be explained by the theory that suggests that recency judgements are simply a discrimination between the relative strengths of memory traces corresponding to the objects (e.g., Ennaceur, 2010; Fortin et al., 2002; Kwok et al., 2012). For example, A would have a weaker memory trace than B because it has had more time to weaken relative to B's memory trace. Presenting a distractor after A then further weakens the strength of A's memory trace thus

enhances discrimination between A and B (Kwok et al., 2012). However, there is evidence to suggest that rodents do not use the relative strength of memory traces for recency discriminations. For example, Fortin et al. (2002) presented rats with unique series of 5 odour stimuli (e.g., A-E) and then presented them with two odours during a single choice test and rewarded the rats for selecting the odour that had appeared earlier in the series. Following training on this sequential task, half of the rats were operated on to perform hippocampal lesions and half of the rats remained unoperated as a control group. The two groups were matched for their average number of trials to reach the learning criterion during initial training. Following surgery, all rats were tested on the sequential order task and then trained and tested on a recognition task. During the sequential task test, two odours were chosen that had appeared in non-adjacent positions in the series (e.g., B and E). For the recognition task, rats were presented with a series of 5 odours and then presented with a choice test in which the animals were rewarded for selecting the odour that had not been presented in the series. During the recognition task test, one odour from the series was paired with another odour from the pool of odours that had not appeared in the unique series. The control rats performed well during the sequential task whereas the lesioned rats did not. However, during the recognition task, both groups of rats performed equally well. The authors concluded that the lesioned rats likely had normal access to the differences in relative memory trace strengths because they were able to recognise odours from the series list, and that the fact that they were not able to use this information to discriminate between any of the sequential order probes suggests that the rats were not using relative strengths of memories for temporal order judgements. Therefore, these data suggest that the mice in Experiment 11 may not have used the relative strengths of memory traces to discriminate between A and B, although the conditions of Experiment 11 were very different to those used by Fortin et al. (2002).

To be able to reject more alternative explanations for distractor effects from other theories, we tested some further predictions that were more unique to SOP, related to factors that may influence the distractor effect. Experiment 13 tested the

possibility that moving the distractor to earlier or later within the sample-sample interval would influence discrimination performance. SOP would predict that an earlier distractor would cause more disruption to habituation of the object that it followed (e.g., object A) than a later distractor, because an earlier distractor would subject A's elements to accelerated decay over a longer duration relative to a later distractor (Kwok et al., 2017; Mazur & Wagner, 1982; Wagner, 1981). Empirical data has previously reported that a distractor presented earlier disrupts habituation to the target (e.g., object A) more than a distractor presented later (Kaye, Gambini, et al., 1988). Our results did not support these findings as they showed no difference in habituation to A when the distractor was presented earlier than later. In fact, in both instances discrimination was very good which suggests that habituation to A may have been equally disrupted by both distractor placements. Therefore, it may be that for relative recency object memory, distractor placement does not modulate the distractor effect. However, it may have been that the differences in our distractor placements were too small to detect any effects. For example, the distractor placements used by Kaye, Gambini, et al. (1988) differed by 210 minutes whereas ours differed by 70 minutes. Therefore, before any strong conclusions can be made regarding distractor placement modulating its effects, the experiment would need to be repeated using greater differences between the earlier and later placements.

Experiment 14 tested the SOP prediction that the effectiveness of distractors is modulated by their salience (Mazur & Wagner, 1982; Wagner, 1981). Unfortunately, we were unable to establish distractors as more or less salient during this experiment and so were unable to confirm or reject this hypothesis. Future experiments of this nature should establish the less salient stimulus prior to the experiment with repeated exposures to this stimulus. For example, Shanks et al. (1986) tested the effects of distractor familiarity on habituation of neophobia. They gave rats a solution (e.g., vinegar) to consume and then soon after gave them a distractor solution that was either novel (e.g., sucrose) or familiar (e.g., Sal). The rats had previously been exposed to the familiar solution for 5-minutes on two consecutive days. Four hours later the rats were given the original solution (e.g.,

vinegar) and the amount they consumed was measured. The authors reported that 10-minutes of exposure time (exactly what we used) was insufficient to establish a distractor object as familiar enough to observe differences in interference between familiar and novel distractors. However, when the exposure time for the familiar distractor was increased to 40-minutes, the novel distractor disrupted habituation to the target significantly more than the familiar one. These results were consistent with our SOP prediction (Mazur & Wagner, 1982; Wagner, 1981); thus, future work should repeat this experiment and implement these changes to further explore this hypothesis.

Experiment 15 compared discrimination performance between three relative recency tasks, one with a distractor presented prior to A (CAB; pre-distractor), one with a distractor presented during the sample-sample interval (ACB; post-distractor), and one in which no distractors were used (AB; no-distractor). There were no significant differences in discrimination performance between the three tasks, although the observed means suggested that a pre-distractor had a reduction effect, and that a post-distractor had an enhancement effect, both relative to a no-distractor task. When the discrimination indices for each task were compared with chance, discrimination performance was significant for no-distractor and post-distractor tasks but not for pre-distractor, further suggesting a reduction effect of a pre-distractor. Therefore, this experiment should also be repeated in future work to establish whether such reduction and enhancement effects do occur during these types of tasks.

6.4.2 Conclusion

This series of distractor experiments have produced interesting data that should be further explored to continue to test the suitability of using SOP as a theoretical framework for studying recency-based object memory.

6.5 Transgenic mice

6.5.1 Testing specific predictions of SOP using transgenic mice

During Chapters 4 and 5, we used a transgenic mouse model of Alzheimer's disease ($APP_{swe}/PS1_{\Delta e9}$, which we refer to as APP/PS1) and their wild type littermates, at 5-months old, to further explore specific SOP predictions. At this age, APP/PS1 mice have already suffered pathological changes in the brain related to amyloid-β protein (Garcia-Alloza et al., 2006; Hong et al., 2016; Pedrós et al., 2014; Ruan et al., 2009; Zhu et al., 2017) and have begun to display some mild cognitive deficits, although their learning and behaviour generally appear to be unimpaired and they typically do not display deficits in memory tasks, such as the widely used novel object recognition task (Bonardi et al., 2011; Jardanhazi-Kurutz et al., 2010; Kelly et al., 2017; Kilgore et al., 2010). However, using variants of the object-in-place task (e.g., Ameen-Ali et al., 2012; Dix & Aggleton, 1999), which may be a test of associative recognition (Aggleton & Nelson, 2020), a selective deficit has been reported in these mice at this age (Bonardi et al., 2016, 2021). In this study, the same mice showed no deficits in the novel object (e.g., Ennaceur & Delacour, 1988) and relative recency (e.g., Mitchell & Laiacona, 1998) memory tasks. Therefore, it appears that they exhibit deficits in association-based but not in recency-based tasks at this age. This is consistent with dual process accounts of memory, such as that proposed by SOP (Wagner, 1981). In terms of SOP, it is therefore possible that the deficit reported in these mice, during the object-in-place tasks, may reflect an impairment in retrieval-generated priming while self-generated priming remains intact, and is able to compensate during the other tasks so that performance seems normal (Bonardi et al., 2016, 2021).

We explored this hypothesis further by evaluating the performance of these mice during an indirect object recognition memory task, thought to be association-based (Whitt et al., 2012), and a recency-based memory task that used variants of the relative recency task (Mitchell & Laiacona, 1998), which included distractor objects. SOP asserts that association-based memory formation is dependent on retrieval-generated priming (Brandon et al., 2003; Mazur & Wagner, 1982;

Wagner, 1981), therefore, during tasks where contextual associative information underlies performance, such as that suggested for the indirect object recognition task that we used (Whitt et al., 2012), this priming process would need to be intact. Therefore, we predicted that APP/PS1 mice would be impaired during the indirect object recognition memory task, if retrieval-generated priming was not functioning normally in these mice. SOP also postulates that recency-based memory formation is dependent on self-generated priming (Brandon et al., 2003; Mazur & Wagner, 1982; Wagner, 1981), and this process may underly performance in tasks that require a discrimination between the recency of two objects, such as our distractor task. Therefore, we predicted no differences in performance, during the distractor task, between APP/PS1 mice and their wild type controls, if self-generated priming was intact in the APP/PS1 mice.

We tested these predictions during Chapters 4 and 5 respectively and found no significant differences in performance between APP/PS1 mice and their wild type controls in either task. However, during the indirect object recognition task (Experiment 9), both genotypes of mice displayed a reverse priming effect relative to the C57BL/6J mice used during Experiment 7. This effect appeared to be specific to the transgenic mice as when we replicated the experiment in C57BL/6J mice of the same age (Experiment 10), we observed no such effect – specifically C57BL/6J mice displayed no priming effect in either direction in these replications. In the distractor experiment (Experiment 12), there were no significant differences in discrimination performance between the two tasks (with and without a distractor) for either genotype.

6.5.2 Performance in relation to hippocampal damage present in these mice

It is possible that the differences in performance of APP/PS1 mice, when compared with the C57BL/6J mice of Experiments 7, 10 and 11, may have been due to the hippocampal damage present at this age (Garcia-Alloza et al., 2006; Hong et al., 2016; Pedrós et al., 2014; Ruan et al., 2009; Zhu et al., 2017). Previous studies have reported performance differences in these types of tasks between humans and rodents, with or without hippocampal damage imposed by

excitotoxic hippocampal lesions (Honey & Good, 2000b), hypoxia (R. O. Hopkins et al., 1995), or Alzheimer's disease (Madsen & Kesner, 1995). For example, Honey and Good (2000b) exposed rats to trials in which auditory stimuli were preceded by visual arrays. In A→X trials, A (e.g., a tone) was followed by X (e.g., constant illumination of left and right panel lights), and in B→Y trials, B (e.g., a series of clicks) was followed by Y (e.g., pulsed operation of left and right panel lights), and these two trial types were intermixed. After three days of training, rats were exposed to A followed by XY and their orienting responses towards X and Y were recorded. Healthy rats oriented more towards Y than X, which the authors suggested may have been because X was primed by A and Y was not (A→X). The lesioned rats displayed a reverse priming effect, similarly to our APP/PS1 mice, in that they oriented more towards X than Y. The authors suggested that the reverse priming effect may have been due to the hippocampal damage causing a disruption to attentional processes. The pathological burden present in the hippocampus of the APP/PS1 mice could have led to such an attentional deficit. Their object exploration levels were certainly lower, during Experiment 12, than the object exploration levels of the C57BL/6J mice from Experiment 11, which suggests that this may have been the case. However, this does not explain why the wild type mice displayed similar performance during these tasks.

6.5.3 Performance of wild type littermates

It is possible that the wild type mice could have altered their behaviour to mimic that of their littermates and consequently directed less attention towards the objects. For example, in a study by Kalbassi et al. (2017), it was reported that mice with a deletion in Neuroligin-3 (Nlgn3) exhibited deficits in social behaviour and that their wild type littermates also displayed a similar deficit. However, when Nlgn3 was re-expressed in the knockout mice they displayed normalised social behaviour and their wild type littermates modified their behaviour to mimic this – specifically they also displayed normalised social behaviour. Therefore, the behaviour of wild type mice can be influenced by the behaviour exhibited by their genetically modified littermates.

For this reason it has been suggested that wild type littermates should be compared to the relevant background strain to establish that behavioural responses do not significantly differ between wild type mice and the strain they are representative of (Bailey et al., 2006). Such differences can arise from background strains used to generate transgenic mice as they often exhibit different behaviours in tests of interest (e.g., Holmes, Wrenn, Harris, Thayer, & Crawley, 2002; Owen, Logue, Rasmussen, & Wehner, 1997). Our transgenic mice were created and then maintained by crossing transgenic mice to B6C3F1/J mice, which is a cross between C57BL/6J females (B6) and C3H/HeJ males (C3), and then transgenic mice were backcrossed to C57BL/6J over multiple generations (For more information see: The Jackson Laboratory, 2022). This mixed genetic background could have introduced variability which resulted in the observed differences between the behavioural responses of the wild type littermates and the standard C57BL/6J used across experiments (Bailey et al., 2006). For these reasons it has been suggested that non littermate mice may provide a more suitable control (Kalbassi et al., 2017). Certainly, studies using APP/PS1 mice have used this approach previously during object recognition memory tasks (Pedrós et al., 2014; Shen et al., 2017). Therefore, for future studies using this mouse model it may be useful to include a control group of C57BL/6J mice so that their performance could be compared with that of APP/PS1 mice and their wild type littermates.

6.5.4 Conclusion

In summary, the APP/PS1mice did not perform significantly different to their wild type littermates in either the association-based indirect object recognition task (Experiment 9), as we had predicted, or in the recency-based distractor task (Experiment 12). In both tasks, their performance differed from that of the C57BL/6J mice used in the other replications of these tasks (Experiments 7, 10, and 11). This suggests that the differences observed were likely strain dependent. For future studies it may be a good idea to include a third control group of C57BL/6J mice. This would control for any behavioural differences between wild type mice and the C57BL/6J mice of which they are representative.

6.6 Replication failures

6.6.1 Replication crisis

Psychology has been said to be facing a replication crisis because of failures to replicate past studies (e.g., Lilienfeld, 2017; Maxwell, Lau, & Howard, 2015; Shrout & Rodgers, 2018) and this crisis may extend to comparative psychology and closely related fields (e.g., Beran, 2018; B. G. Farrar, Boeckle, & Clayton, 2020; Stevens, 2017). Failures to replicate and reproduce past studies have been attributed to many factors including small sample sizes (B. Farrar & Ostojic, 2019; Stevens, 2017), which may lead to low statistical power in single replication studies (Maxwell et al., 2015), overestimated published effect sizes with *p*-values close to significance level, which lead to inconsistent replication (B. G. Farrar et al., 2020), publication bias (B. Farrar & Ostojic, 2019; Maes et al., 2016), and differences between species and cross site comparisons (Tecwyn, 2021). In rodents, strain differences have often been reported and these could impact replication studies that use different strains to those used during the original studies. For example, mice have been shown to exhibit strain-dependent differences in levels of object exploration in a novel object recognition task (Şık, van Nieuwehuyzen, Prickaerts, & Blokland, 2003), and in spatial learning performance and rates of exploration, in a Barnes maze task (O'Leary, Savoie, & Brown, 2011).

6.6.2 Our replication failures

During Chapter 4, we were unable to replicate the results of the indirect object recognition task that we obtained during Experiment 7, in three further replications of the task. Similarly, during Chapter 5, we were unable to replicate the distractor effect of Experiment 11 during Experiment 15. Because we used the same strain of mice at similar ages, the same experimental setup in the same lab, and the experiments were run by the same experimenter, many of the potential replication confounds should not apply. However, small sample sizes may have been a factor. When we combined the results of Experiments 11 and 15, we observed a highly

significant difference which may suggest that a sample size of 32 would be more appropriate than 16, for testing these distractor effects in mice. When we combined the data from Experiment 7 and the three replications of this, which should have been a large enough sample size to have enough power to identify solid effects (Maxwell et al., 2015), we still observed no statistical effect overall, although the observed means were suggestive of this. This may indicate that the first replication was a false positive. However, Whitt et al. (2012) reported a statistical effect during two replications of this task with rats. Therefore, the failure to replicate consistently may be due to species differences between rats and mice (e.g., Frick, Stillner, & Berger-Sweeney, 2000; Netser et al., 2020) or differences in object exploration levels, which was discussed separately in the following section of this report.

6.6.3 Intrastrain behavioural variability

Another factor that may have contributed to the replication failures was variability between the mice used for each replication. For example, a study by Crabbe, Wahlsten, and Dudek (1999) tested several strains of mice, obtained from the same breeders, using a behavioural test battery in three sperate laboratories. They reported that even though the conditions were replicated as closely as possible between three test sites (e.g., test apparatus, test protocols and animal husbandry), the behaviour of each strain differed across laboratories. The authors reported that one key variable that could have contributed to differences in behaviour was that each laboratory had specific experimenters performing the testing and these were unique to each lab. Certainly, the manipulation of animals prior to testing (e.g., transporting from holding to test room or habituation to handling) and the specific test conditions (e.g., aversiveness of the environment or handling techniques) can both influence the behavioural responses of rodents (Hogg, 1996). Nonetheless, this was unlikely to have contributed to the variation in behaviour observed during the current set of experiments, reported here, as the experimenter and test conditions were consistent throughout all replications. However, intrastrain variability could have contributed to the behavioural differences observed. For

example, Karp et al. (2014) demonstrated that behavioural phenotypes can fluctuate unexpectedly between batches of mice and that this can be problematic when only a small number of batches are tested, such as in the experiments reported here. Although intrastrain differences have seldom been directly investigated, where they have, both rats and mice show intrastrain variation in behaviour and it has been suggested this variability may be a consequence of stimulating or aversive early life experiences (Schellinck, Cyr, & Brown, 2010; Theilmann et al., 2016). Although we obtained all of our C57BL/6J mice from the same supplier (Charles River, UK), we could not control any variability that may have occurred during the early life experience of the mice that we used. Therefore, this could have contributed to the variation in behaviour that we observed between the different batches of mice that we used. However, our statistical analyses should have allowed for this so instead it may have been that the variation between batches was because the different batches came from different statistical populations.

6.6.4 Variation in social factors and aggressive characteristics

Additionally, social factors can exert a significant influence on the behavioural responses of mice (Ferrari et al., 1998). For example, laboratory mice establish dominance hierarchies which are highly variable and which are maintained through intimidation from the dominant mouse to the subordinate mice in the group (Wang et al., 2011). Therefore, individuals differ in their social history and psychosocial stress (Ferrari et al., 1998) and this likely influences anxiety levels in submissive animals (Sapolsky, 2005). This variation in social factors can lead to differences across various behavioural tests. For example, mice that differ in their social status (dominant or subordinate) and aggressive characteristics respond differently to novelty, with less aggressive and subordinate mice exhibiting lower levels of exploration than dominant individuals (Ferrari et al., 1998). Differences related to social status and interactions have also been shown between dominant and subordinate mice in other behvioural tasks including the swim test, hole board, and plus-maze (Hilakivi-Clarke & Lister, 1992; Rodgers & Cole, 1993). Therefore,

variability in social factors could have contributed to the differences in behaviour that we observed during our replications. Anecdotally, we observed differences in the levels of aggression and conflict between mice both between and within the different batches that we used. Unfortunately, such factors are difficult to control for.

6.6.5 Conclusion

In summary, our replication failures, during Chapter 4 and 5, may have been partly due to intrastrain behavioural differences which would be difficult to address and control for during further work. However, these differences are unlikely to influence the outcomes to such a degree that would result in consistent multiple failures to replicate, such as in Chapter 4. The sample sizes used (N=16) could have been too small to produce reliable effects, as was suggested during Chapter 5 when we pooled the data from two replications (N=32). However, this may have been specific to distractor effects because when we pooled the data for the four replications (N=64) of the indirect object recognition memory task (Whitt et al., 2012), we saw no significant effect. Whitt et al. (2012) reported significant effects in rats using two replications of 16 animals per replication, which suggests that 16 may be sufficient. Therefore, it is more likely that our replication failures of this task were due to other factors. The main difference between our data and that reported by Whitt et al. (2012) was the species used and the object exploration levels by the animals. Therefore, it is likely that this may have been the primary factor that contributed to our replication failures. This was discussed in the following section.

6.7 Low levels of object exploration by mice

6.7.1 Object exploration levels

Throughout these experiments we have consistently observed low levels of object exploration by the mice. During our replications of the study by Whitt et al. (2012), our mice directed far less exploration towards the objects, during the

sample and test phases, than did their rats. For example, our mice only spent around 7-8 percent of the total object exposure time exploring the objects during the sample phases, and around 12 percent during the test. In contrast, their rats explored the objects for approximately 20-25 percent the total object exposure time during the sample phases, and approximately 50 percent during the test. This was surprising, as during object memory tasks mice have been shown to direct similar levels of exploration towards the objects as rats. For example, Stranahan (2011) exposed rats and mice to two identical junk objects and then following various delays, tested their responses towards a copy of the familiar object and a novel object. Both rats and mice directed more exploration towards the novel object and there was no significant difference between the two species in this novelty bias. Importantly, there was also no significant difference in the amount of time that mice and rats spent exploring the objects. This suggests that the differences in object exploration levels, observed during our study and that by Whitt et al. (2012), were more likely due to experimental parameters rather than a difference between the two species. Our setup was identical to theirs except that we used much smaller objects that were more appropriate for mice. Because rats are significantly larger than mice, and the objects used for rats are also substantially larger, both would have resulted in less floor space for rats to explore in between object exploration, relative to the floor space available for our mice. Therefore, our mice would have had significantly more space to occupy and explore relative to the rats which may have resulted in reduced object exploration.

6.7.2 Issues with low object exploration levels in terms of SOP

In terms of our SOP analysis (Mazur & Wagner, 1982; Wagner, 1981), a certain level of object exploration would have been required for the association-based and recency-based effects, that we were investigating, to have had occurred. For example, in our distractor tasks we hypothesised that a distractor object (e.g., C), experienced after a target object (e.g., A), would compete for elemental space in A1 and A2 and would consequently cause elements already residing in these states (e.g., elements of A) to decay more rapidly. If mice directed low levels of

exploration towards objects A and C, then both objects would have very few elements enter the activation states. Therefore, there would be less competition for elemental space in A1 and A2 between the elements of A and C, so fewer of A's elements would suffer accelerated decay and the distractor effect would be reduced.

In our indirect object recognition tasks, we hypothesised that object P could be associatively primed by context x and that this would reduce responses to P in the test that followed. For P to have been associatively primed would have required a prior excitatory x→P association to have formed (Brandon et al., 2003; Mazur & Wagner, 1982; Wagner, 1981). For this, P and x would have needed to have had elements concurrently activated into A1 multiple times during the sample phase. The number of elements that x and P would have had simultaneously activated in A1 during this phase would have determined the strength of the x→P association. Low levels of object exploration would have resulted in fewer elements of x and P occupying A1 concurrently, thus reducing associative strength between them. For x to have effectively associatively primed P, x would have needed to have acquired enough associative strength with P to reach asymptotic levels of conditioning (e.g., Jennings & Bonardi, 2017; Jones & Haselgrove, 2013; Sanderson et al., 2016). Therefore, low levels of object exploration may have resulted in a weak x→P association that was insufficient to produce a strong priming effect.

6.7.3 Recommendations to increase object exploration levels in mice

For these reasons, future work should implement measures to increase object exploration levels, with the aim of obtaining more reliable recency and associative memory effects using mice. To achieve this, the floor space should be significantly reduced so that mice are more often in close proximity with the objects. This would also reduce the amount of time that mice spend exploring the arena, rather than the objects, as there would be less space for them to explore. In addition, the objects should be moved nearer to the corners to further increase object exploration. Both manipulations have been shown to be effective in increasing

stimulus contact time in mice (Pacchiarini, 2019). For example, Pacchiarini (2019) performed two novel object recognition tasks, using C57BL/6J mice, which differed from one another in terms of floor space and placement of the stimuli. The first task had an arena floor space of 60 x 60cm (width x depth) and the stimuli were placed in the middle of the arena (similarly to our tasks). In the second task they reduced the floor space to 50 x 50cm (width x depth) and moved the stimuli nearer to the corners of the arena. During the first task, mice spent very little time exploring the stimuli and spent most of their time around the arena perimeter. During the second task, they reported a marked increase in exploration of the stimuli –exploration levels had more than doubled. Moving objects nearer to the corners would also improve temporal contiguity between the objects and contexts (e.g., arena wall coverings) used which may strengthen context→object associations. Certainly, excitatory associative strength between them would be more likely to incrementally increase at a faster rate due to more concurrent A1 activation of the objects and contexts (Brandon et al., 2003; Vogel et al., 2020), and associative strength could be measured directly to confirm this.

6.7.4 Conclusion

These modifications would be relatively easy to implement and could improve the reliability of obtaining recency and associative memory effects using mice. Therefore, for future work using mice to explore SOP predictions, implementing these adjustments would be highly recommended.

6.8 Final conclusion

During this thesis, I have used variants of the spontaneous object recognition task (Ennaceur & Delacour, 1988) to test predictions related to object recognition memory, generated using the Sometimes Opponent Process (SOP) theory of associative learning and memory (Brandon et al., 2003; Wagner, 1981). I have explored the suitability of using this alternative approach to study recognition memory because it sidesteps many of the complexities associated with other methods used and is translatable to humans (e.g., Fagan, 1970; Nitka et al., 2020).

I have provided some evidence to support the use of this approach to study recognition memory during association-based and recency-based memory tasks. Further work is required to validate and develop these findings to establish this method as a suitable general framework for studying recognition memory. For future work using this method with mice, I have made practical recommendations which could improve the reliability of the data collected.

Appendix A

A.1 Habituation data C57BL/6J mice

A.1.1 Batch 1

The mice from batch 1 completed Experiment 1.

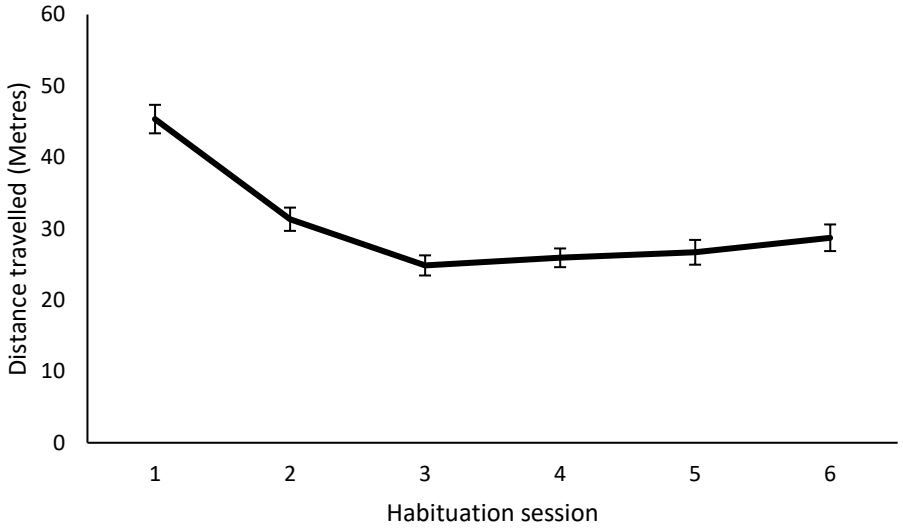


Figure 40. Mean (±SEM) distance travelled by the mice from batch 1, during each 10-minute habituation session.

A.1.2 Batch 2

The mice from batch 2 completed Experiments 2, 3 and 4.

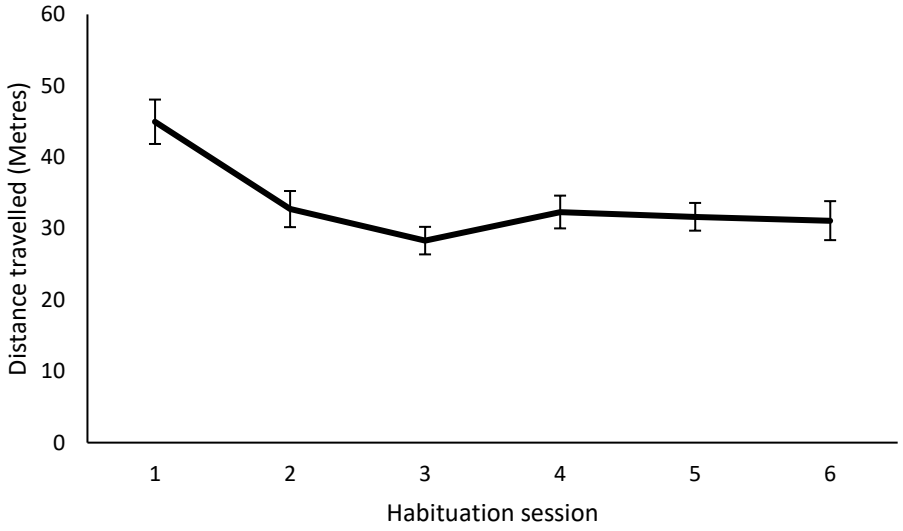


Figure 41. Mean (±SEM) distance travelled by the mice from batch 2, during each 10-minute habituation session.

A.1.3 Batch 3

The mice from batch 3 completed Experiments 5, 6 and 11.

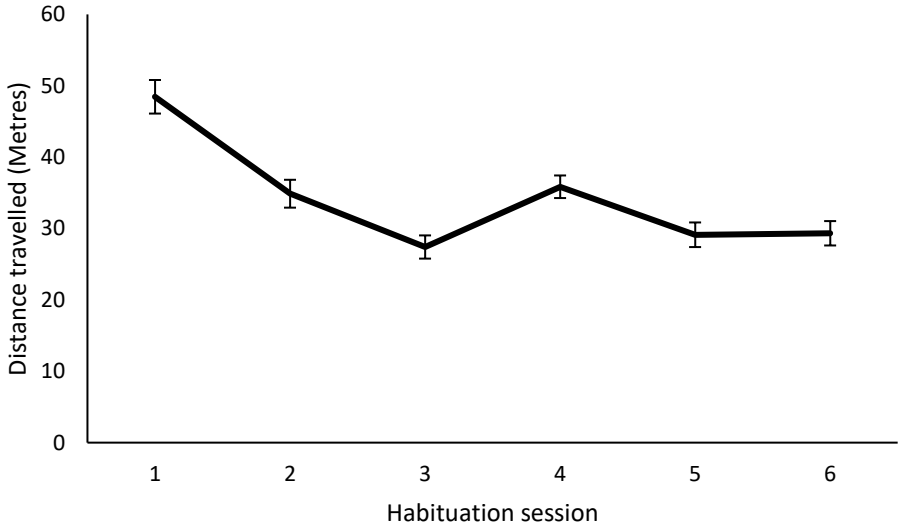


Figure 42. Mean (±SEM) distance travelled by the mice from batch 3, during each 10-minute habituation session.

A.1.4 Batch 4

The mice from batch 4 completed Experiment 7.

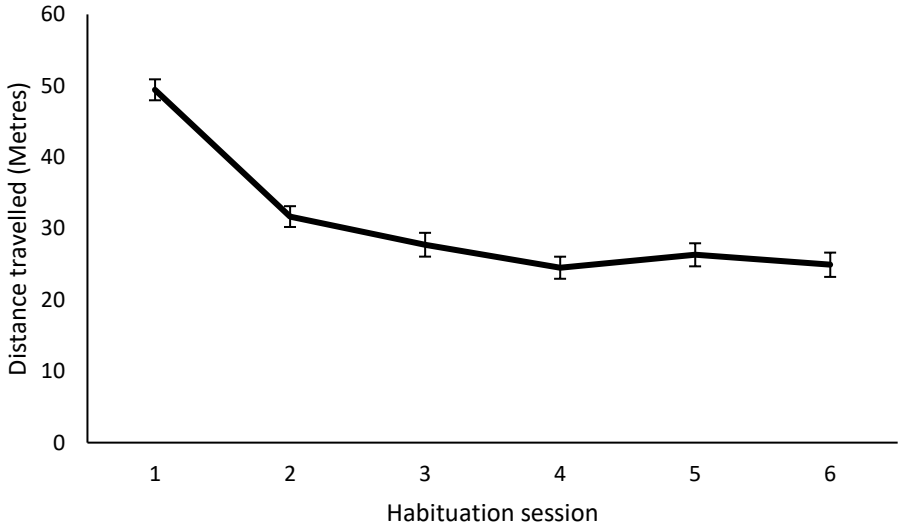


Figure 43. Mean (±SEM) distance travelled by the mice from batch 4, during each 10-minute habituation session.

A.1.5 Batch 5

The mice from batch 5 completed Experiments 8 and 13, and a pilot study reported in Appendix D.

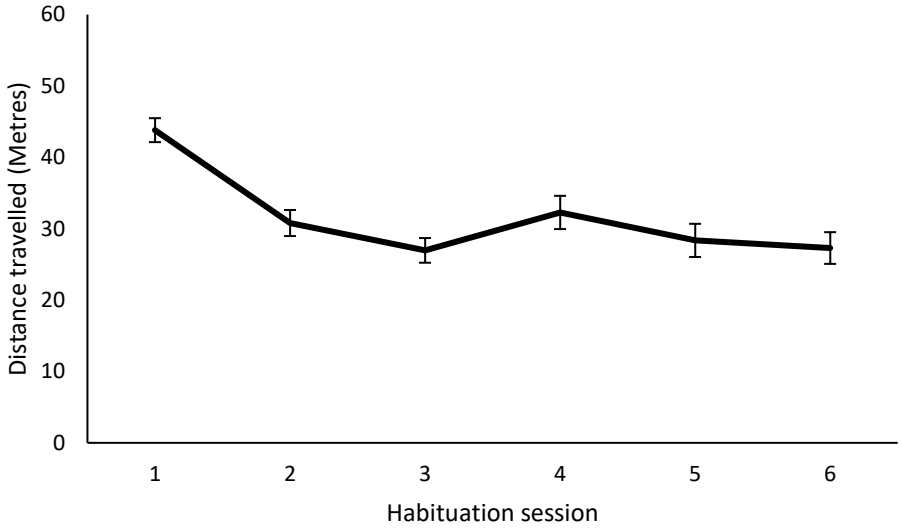


Figure 44. Mean (±SEM) distance travelled by the mice from batch 5, during each 10-minute habituation session.

A.1.6 Batch 6

The mice from batch 6 completed the second replication of Experiment 8.

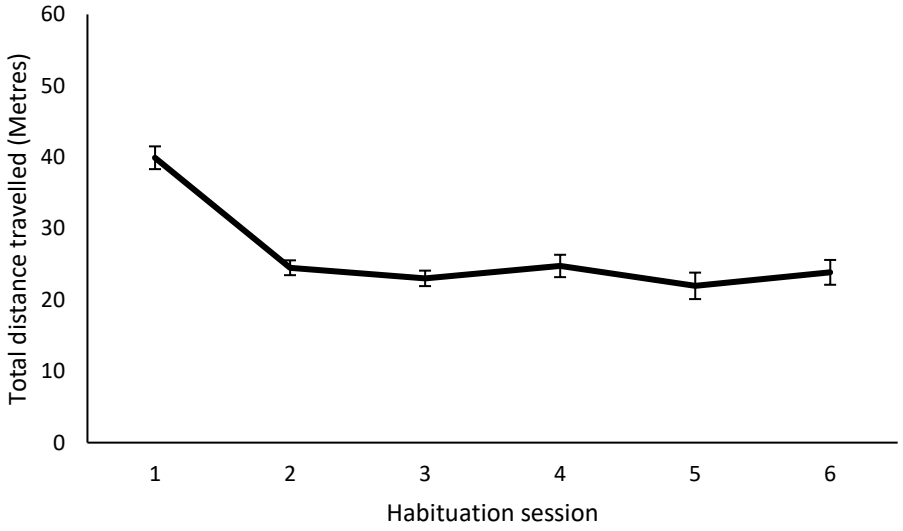


Figure 45. Mean (±SEM) distance travelled by the mice from batch 6, during each 10-minute habituation session.

A.1.7 Batch 7

The mice from batch 7 completed the first replication of Experiment 10.

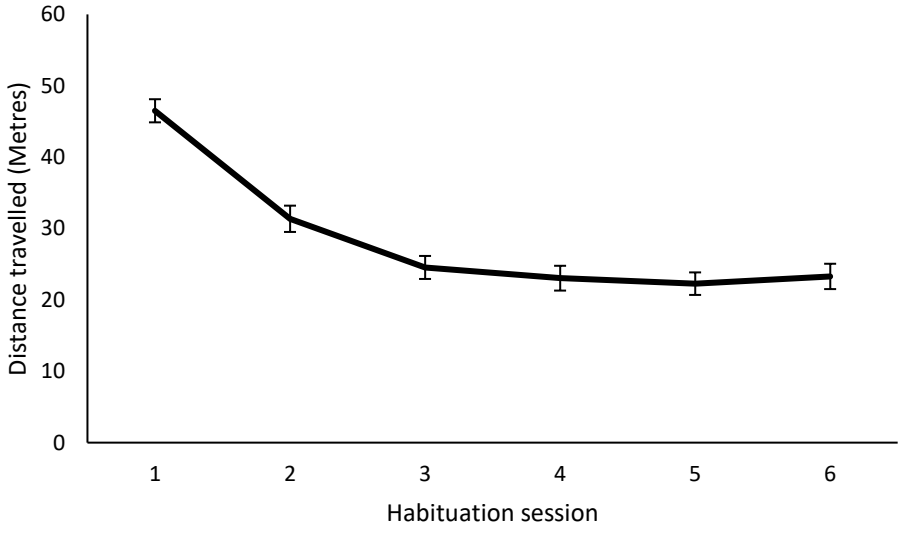


Figure 46. Mean (±SEM) distance travelled by the mice from batch 7, during each 10-minute habituation session.

A.1.8 Batch 8

The mice from batch 8 completed the second replication of Experiment 10.

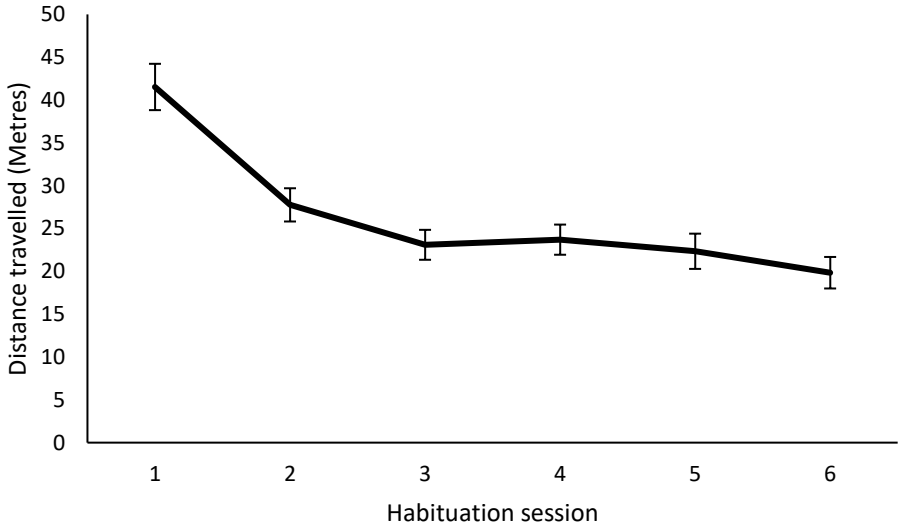


Figure 47. Mean (±SEM) distance travelled by the mice from batch 8, during each 10-minute habituation session.

A.1.9 Batch 9

The mice from batch 9 completed Experiment 14.

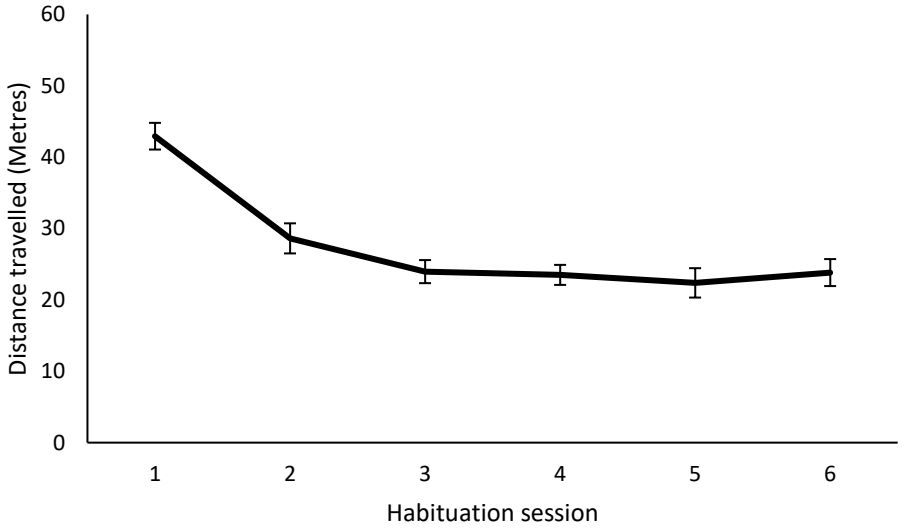


Figure 48. Mean (±SEM) distance travelled by the mice from batch 9, during each 10-minute habituation session.

A.1.10 Batch 10

The mice from batch 10 completed the third replication of Experiment 10 and Experiment 15.

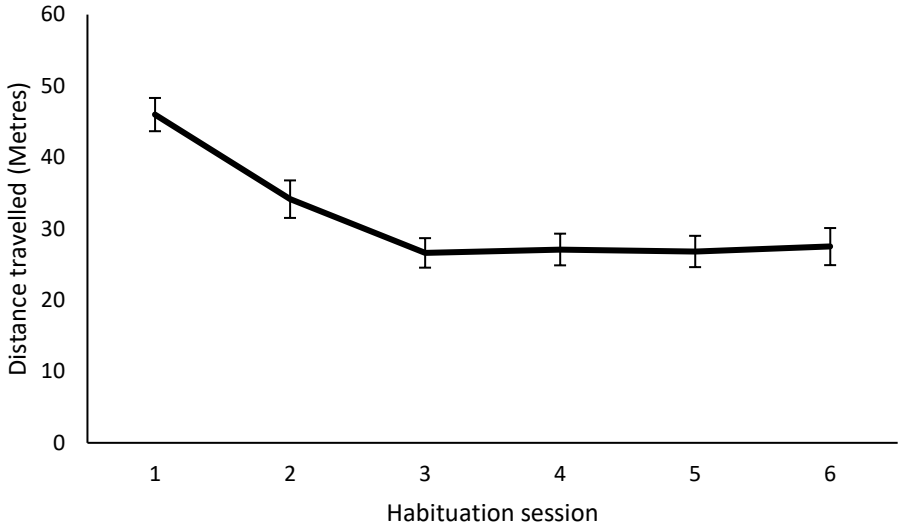


Figure 49. Mean (±SEM) distance travelled by the mice from batch 10, during each 10-minute habituation session.

A.2 Habituation data APPswe/PS1dE9 mice

A.2.1 Batch 11

The mice from batch 11 completed the first replication of Experiment 12.

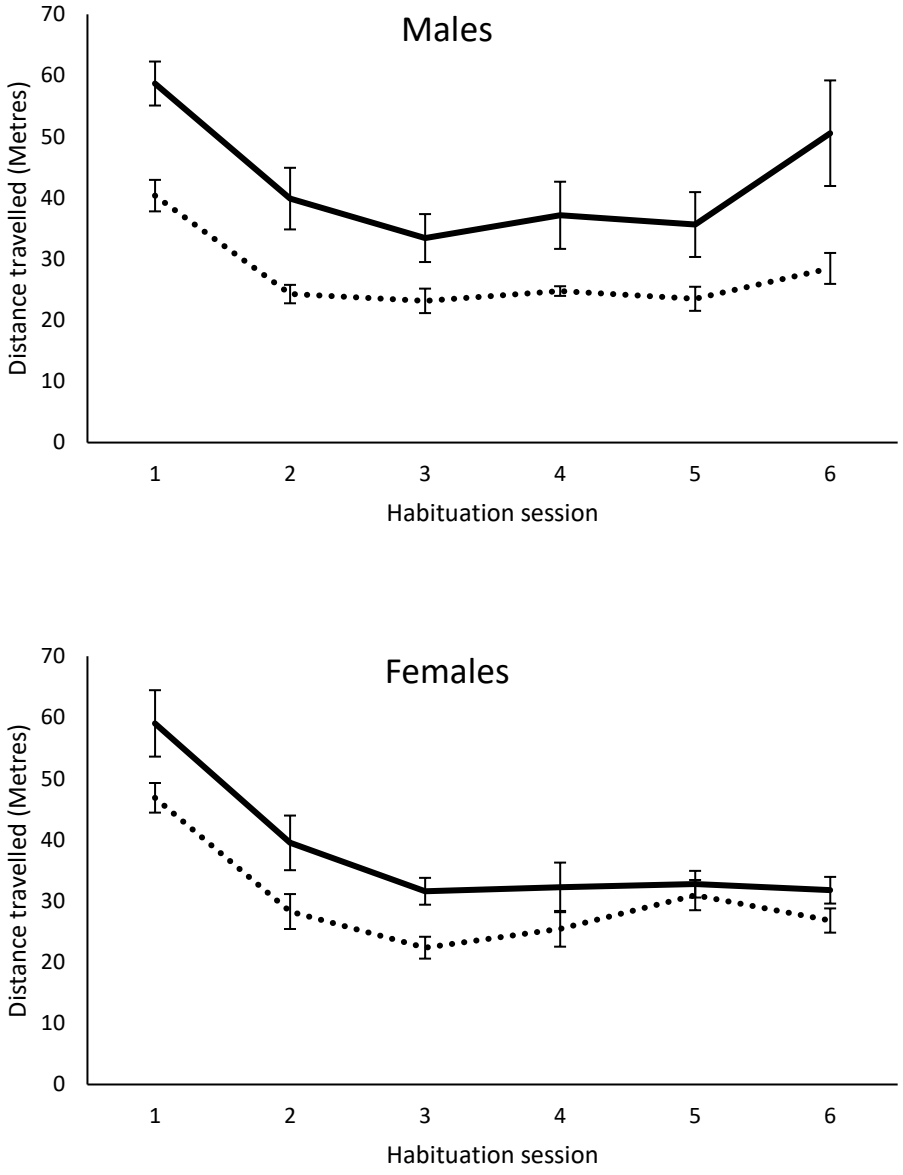


Figure 50. Mean (±SEM) distance travelled by the mice from batch 11, during each 10-minute habituation session. The solid line depicts APPswe/PS1dE9 mice, and the dotted line depicts their wild type littermates.

A.2.2 Batch 12

The mice from batch 12 completed Experiment 9 and the second replication of Experiment 12.

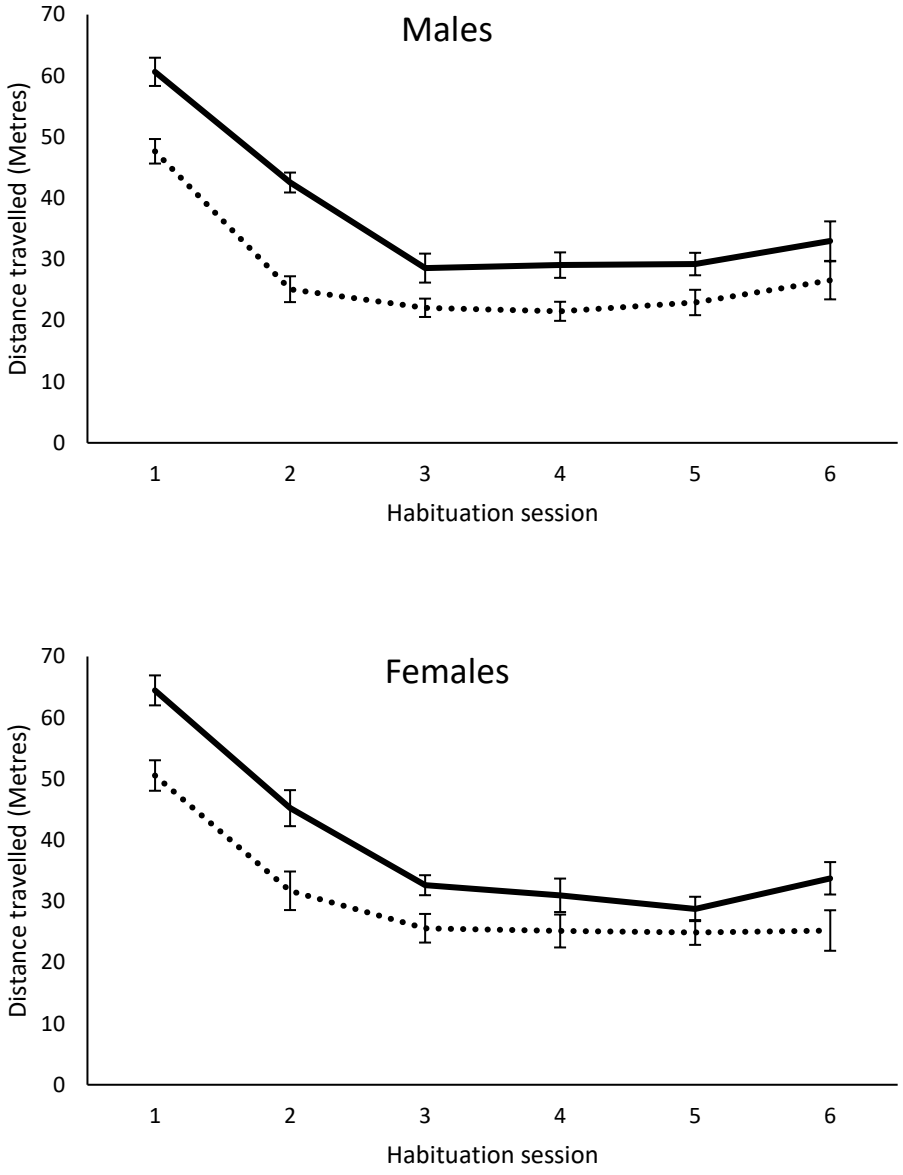


Figure 51. Mean (±SEM) distance travelled by the mice from batch 12, during each 10-minute habituation session. The solid line depicts APPswe/PS1dE9 mice, and the dotted line depicts their wild type littermates.

Appendix B

B.1 Pretraining data C57BL/6J mice

B.1.1 Batch 1

The mice from batch 1 completed Experiment 1.

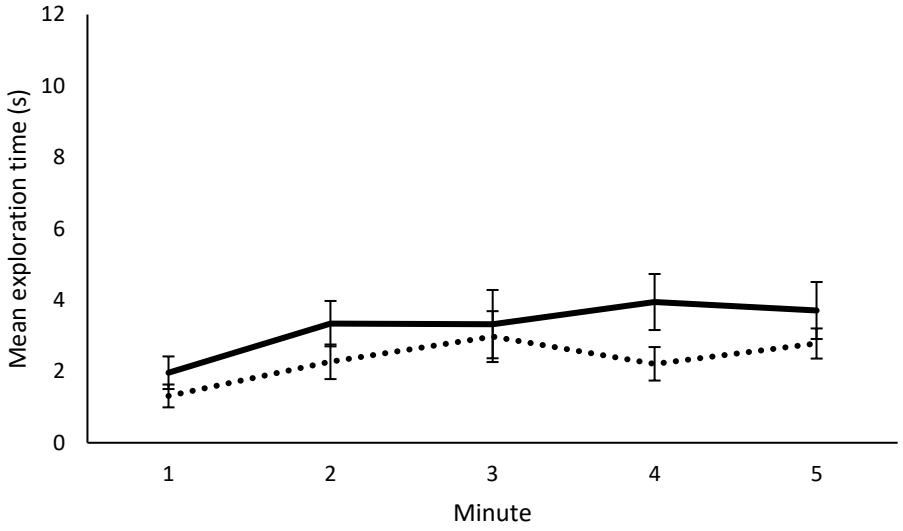


Figure 52. Mean (±SEM) exploration time of the novel and of the familiar object, during pretraining for batch 1. The solid line depicts the novel object, and the dotted line denotes the familiar object.

B.1.2 Batch 2

The mice from batch 2 completed Experiments 2, 3 and 4.

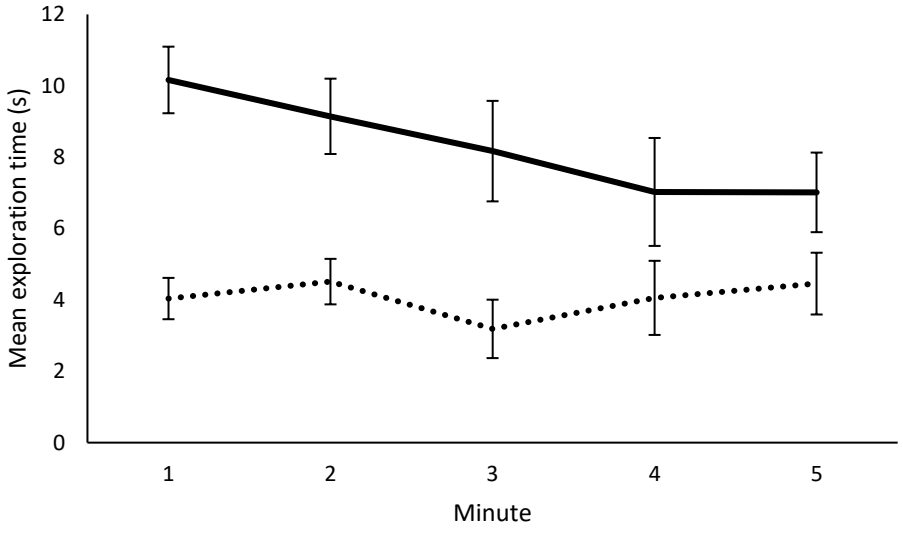


Figure 53. Mean (±SEM) exploration time of the novel and of the familiar object, during pretraining for batch 2. The solid line depicts the novel object, and the dotted line denotes the familiar object.

B.1.3 Batch 3

The mice from batch 3 completed Experiments 5, 6 and 11. These mice did not undertake the pretraining task.

B.1.4 Batch 4

The mice from batch 4 completed Experiment 7.

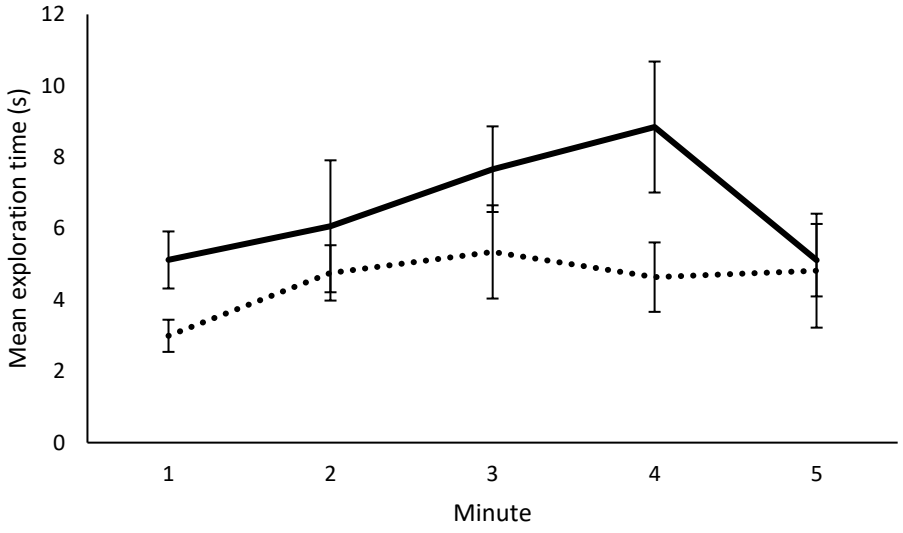


Figure 54. Mean (±SEM) exploration time of the novel and of the familiar object, during pretraining for batch 4. The solid line depicts the novel object, and the dotted line denotes the familiar object.

B.1.5 Batch 5

The mice from batch 5 completed Experiments 8 and 13, and a pilot study reported in Appendix D.

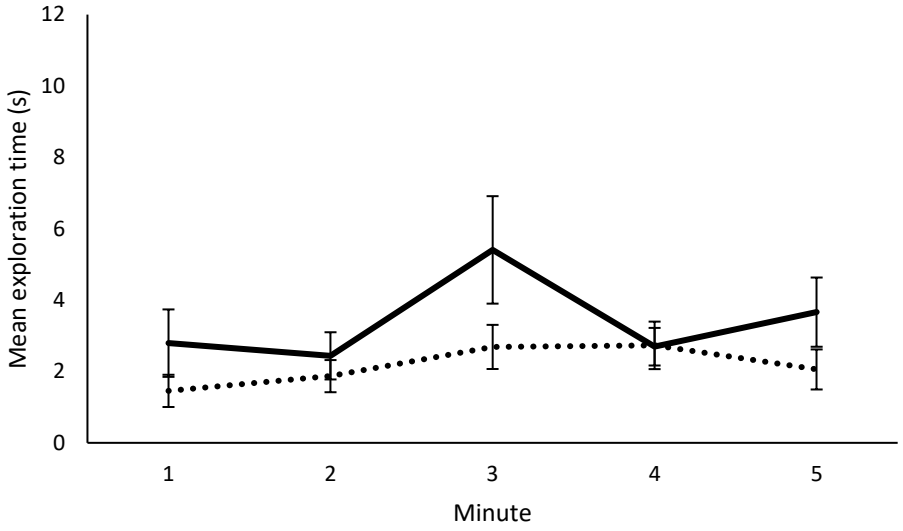


Figure 55. Mean (±SEM) exploration time of the novel and of the familiar object, during pretraining for batch 5. The solid line depicts the novel object, and the dotted line denotes the familiar object.

B.1.6 Batch 6

The mice from batch 6 completed the second replication of Experiment 8.

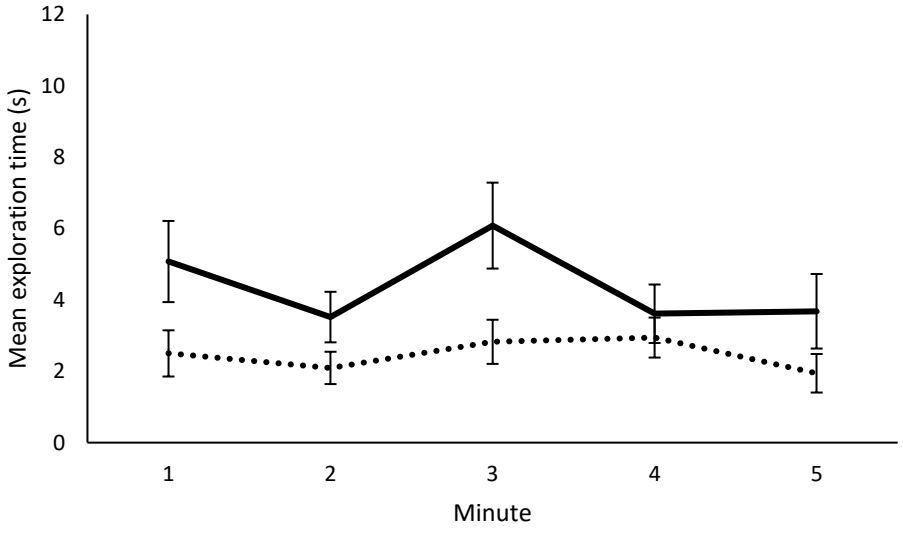


Figure 56. Mean (±SEM) exploration time of the novel and of the familiar object, during pretraining for batch 6. The solid line depicts the novel object, and the dotted line denotes the familiar object.

B.1.7 Batch 7

The mice from batch 7 completed the first replication of Experiment 10.

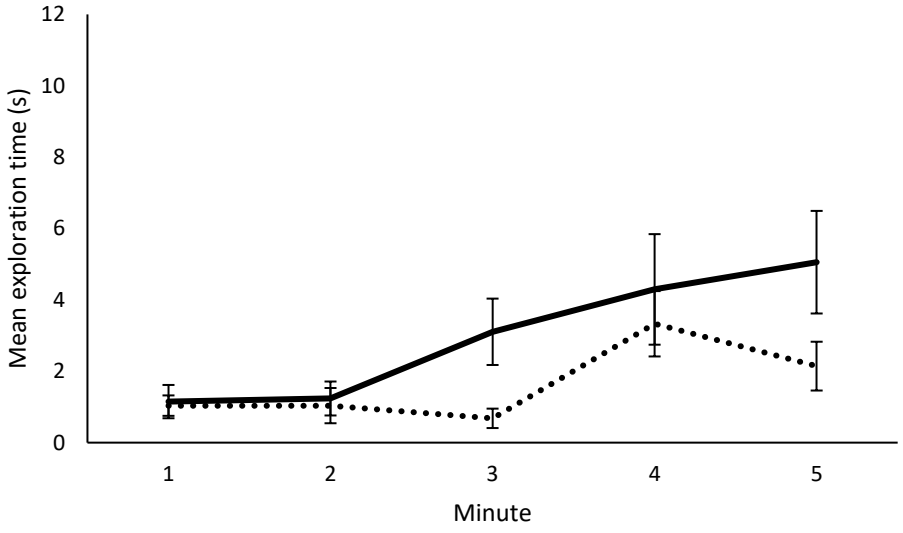


Figure 57. Mean (±SEM) exploration time of the novel and of the familiar object, during pretraining for batch 7. The solid line depicts the novel object, and the dotted line denotes the familiar object.

B.1.8 Batch 8

The mice from batch 8 completed the second replication of Experiment 10.

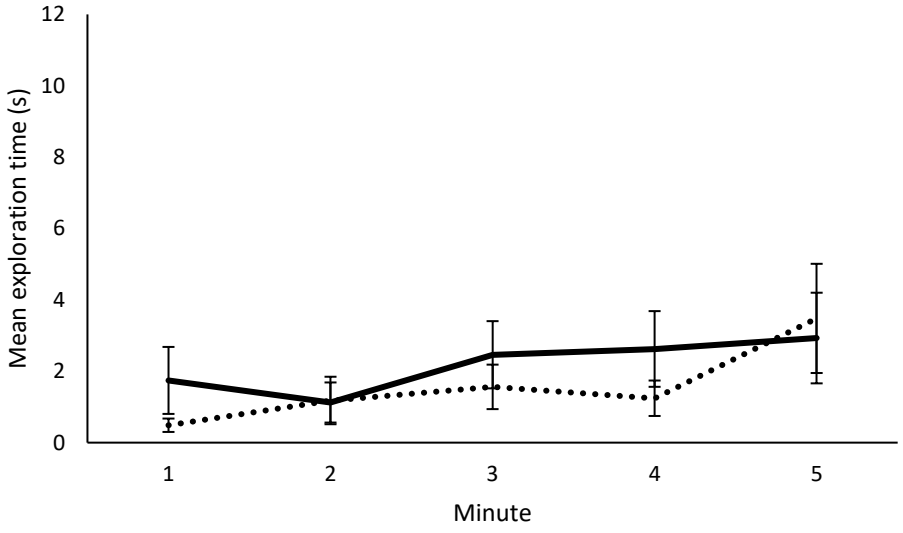


Figure 58. Mean (±SEM) exploration time of the novel and of the familiar object, during pretraining for batch 8. The solid line depicts the novel object, and the dotted line denotes the familiar object.

B.1.9 Batch 9

The mice from batch 9 completed Experiment 14.

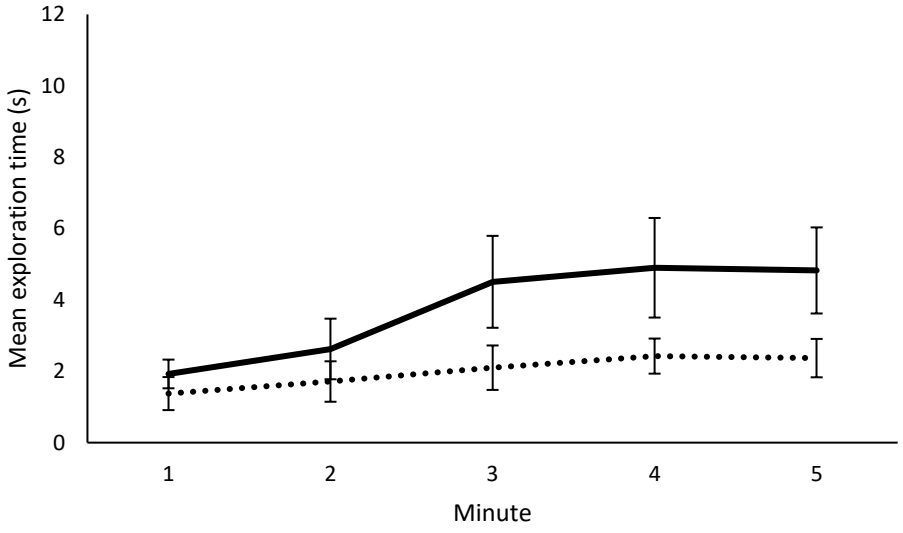


Figure 59. Mean (±SEM) exploration time of the novel and of the familiar object, during pretraining for batch 9. The solid line depicts the novel object, and the dotted line denotes the familiar object.

B.1.10 Batch 10

The mice from batch 10 completed the third replication of Experiment 10 and Experiment 15.

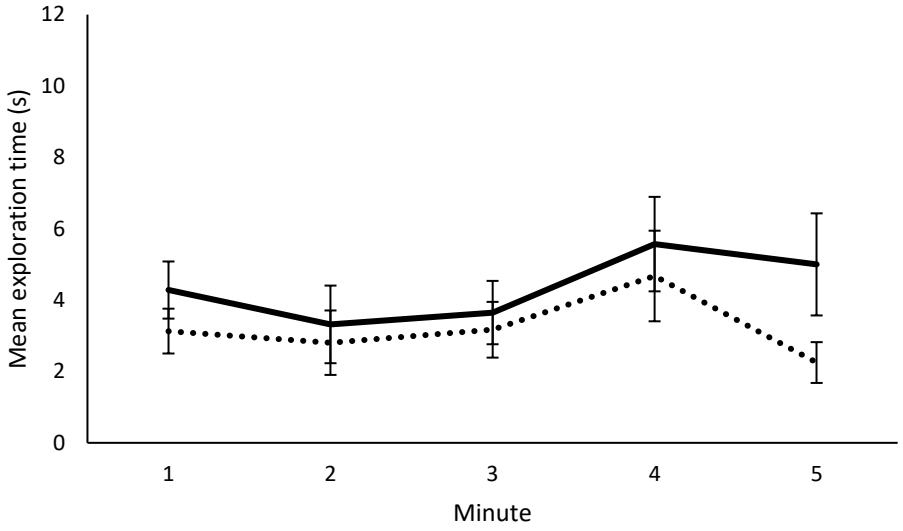
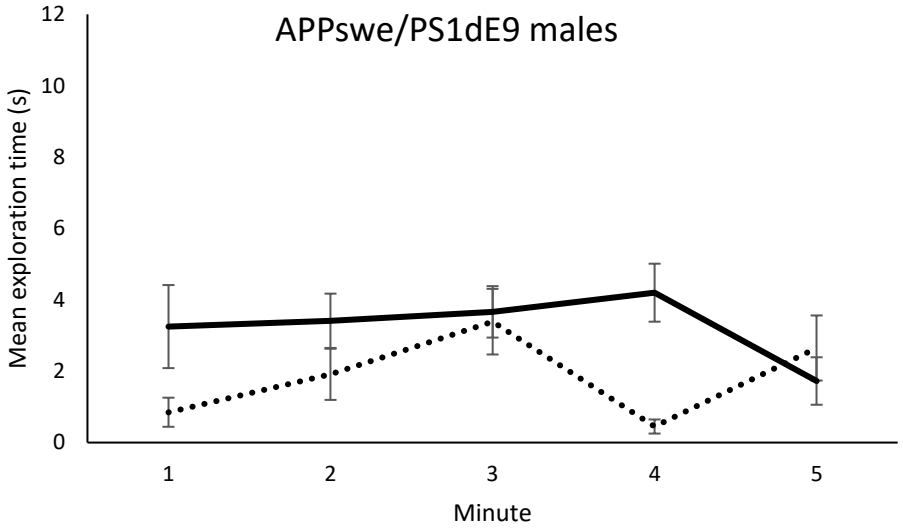


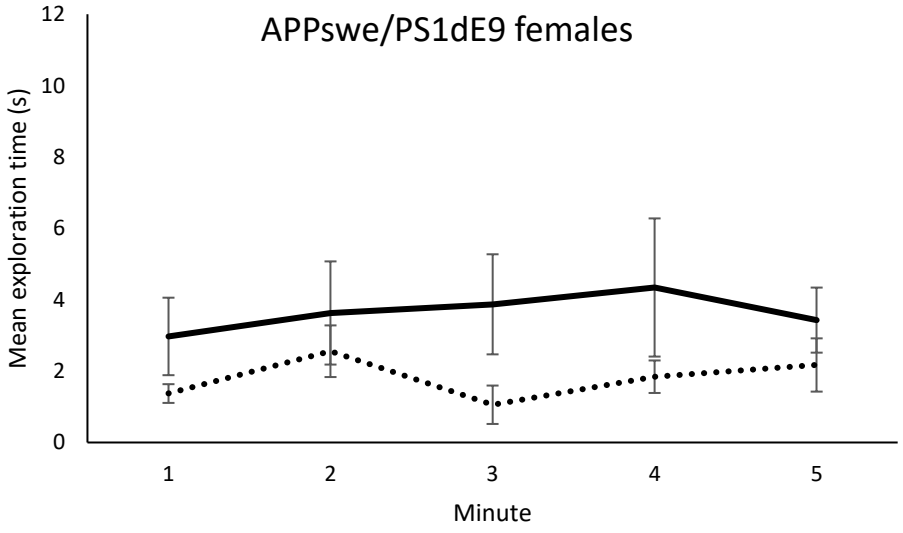
Figure 60. Mean (±SEM) exploration time of the novel and of the familiar object, during pretraining for batch 10. The solid line depicts the novel object, and the dotted line denotes the familiar object.

B.2 Pretraining data APPswe/PS1dE9 mice

B.2.1 Batch 11

The mice from batch 11 completed the first replication of Experiment 12.





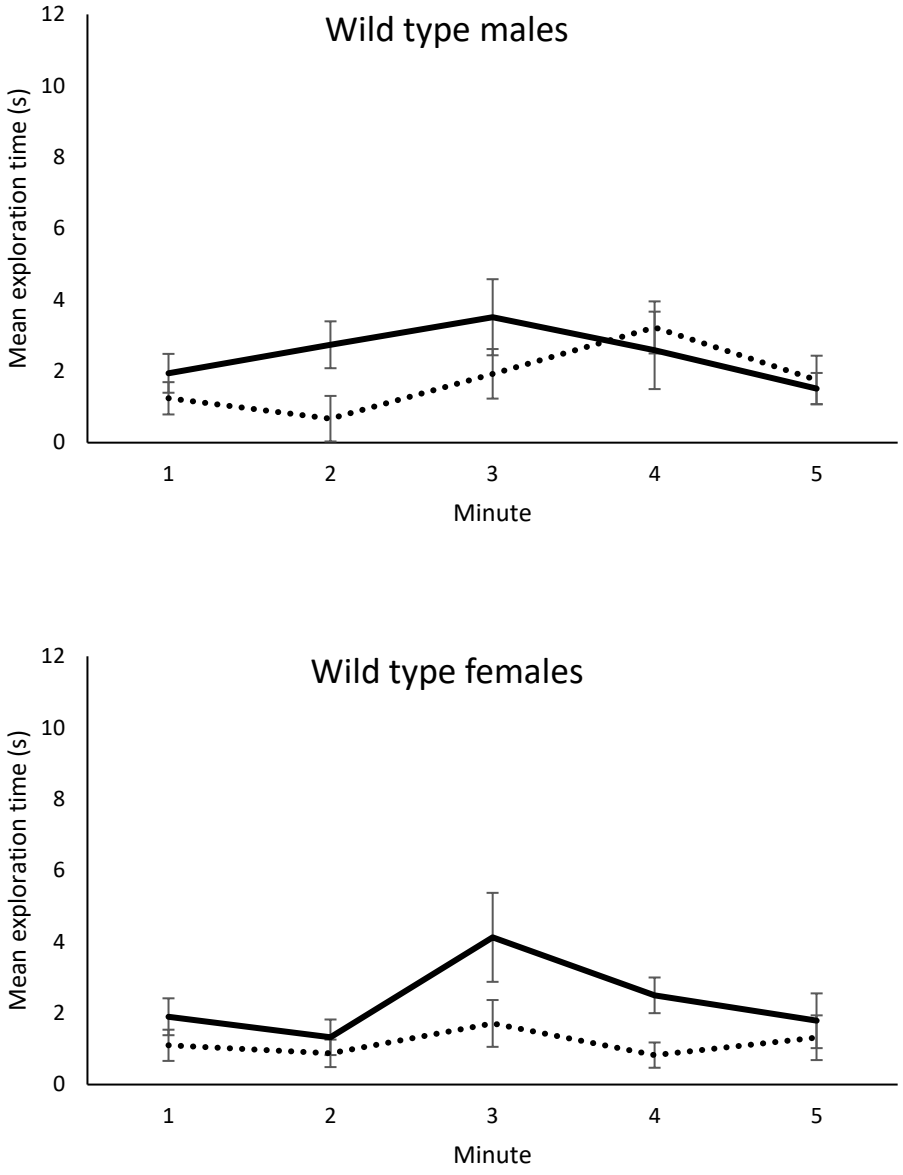
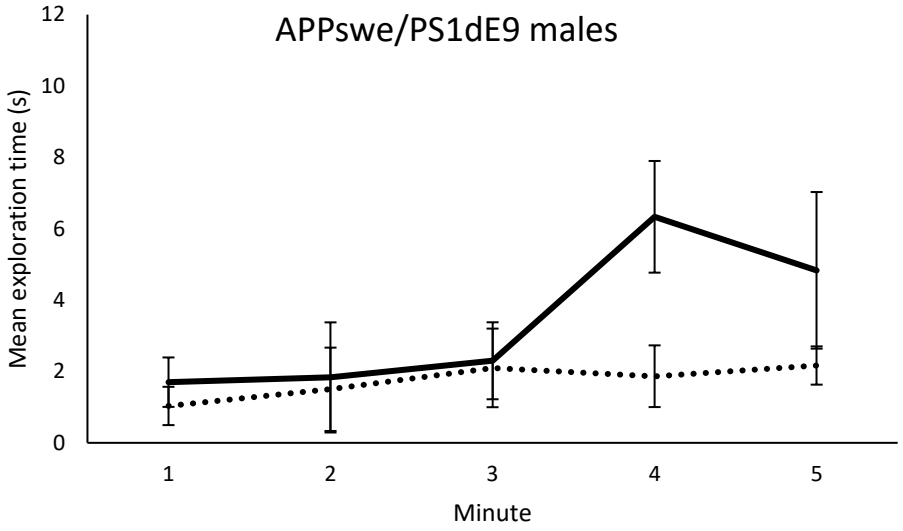
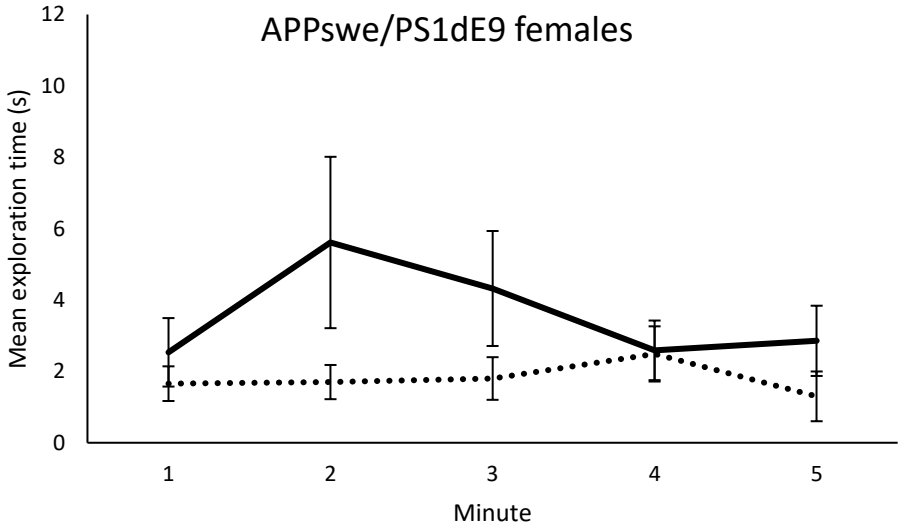


Figure 61. Mean (±SEM) exploration time of the novel and of the familiar object, during pretraining for batch 11. The solid line depicts the novel object, and the dotted line denotes the familiar object.

B.2.2 Batch 12

The mice from batch 12 completed Experiment 9 and the second replication of Experiment 12.





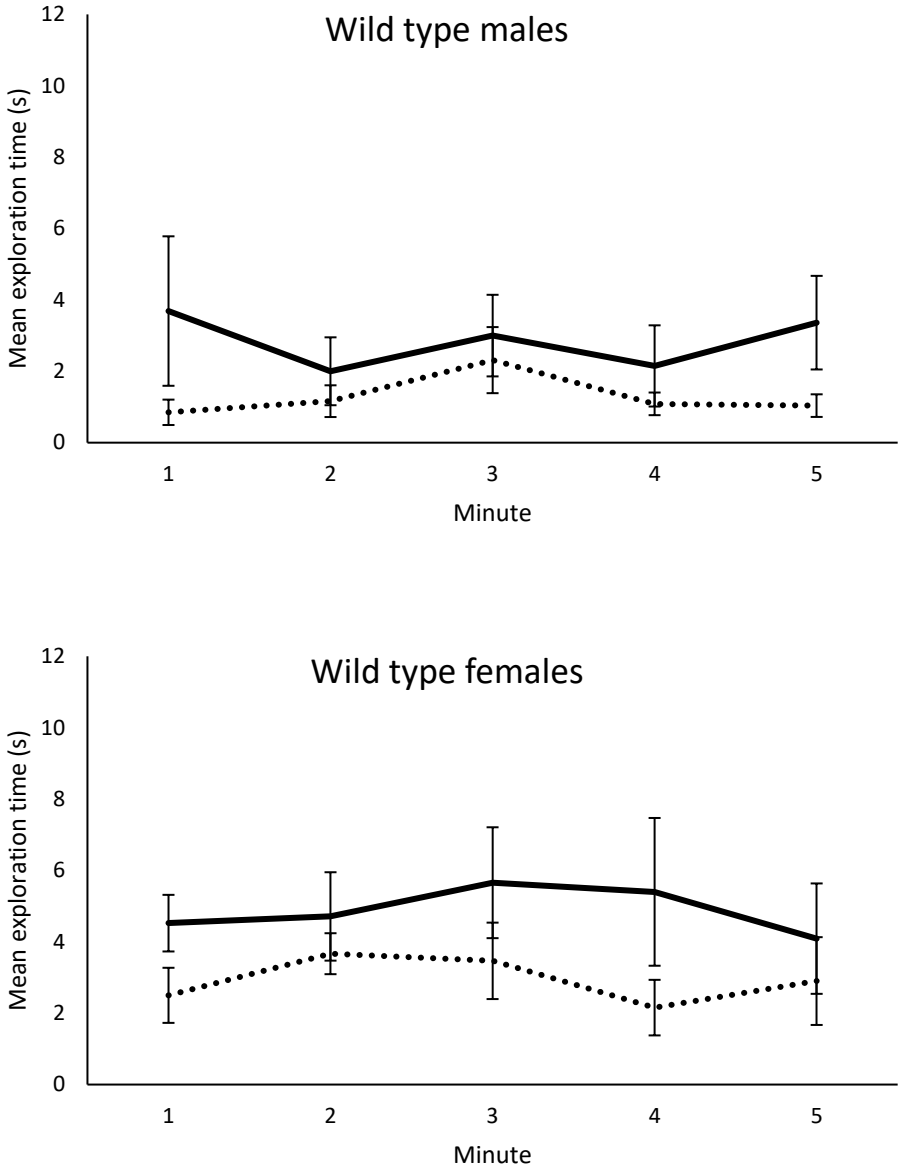


Figure 62. Mean (±SEM) exploration time of the novel and of the familiar object, during pretraining for batch 12. The solid line depicts the novel object, and the dotted line denotes the familiar object.

Appendix C

C.1 Chapter 3 experimental test data over 5-minutes

C.1.1 Experiment 1

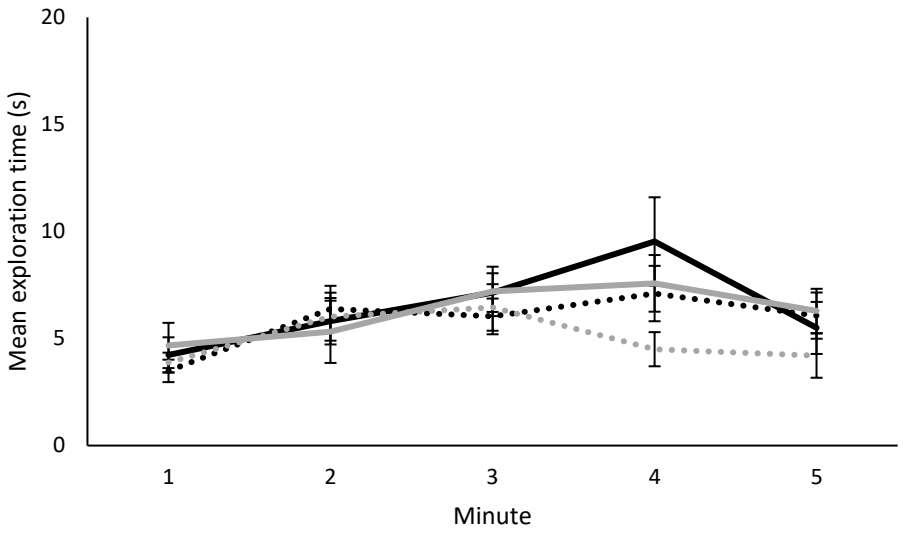


Figure 63. Mean (±SEM) exploration time of the novel and of the familiar compound and component objects during 5-minutes of test of Experiment 1. The solid black line and dotted black line depict the novel and familiar compound objects respectively. The solid grey line and dotted grey line denote the novel and familiar component objects respectively.

C.1.2 Experiment 2

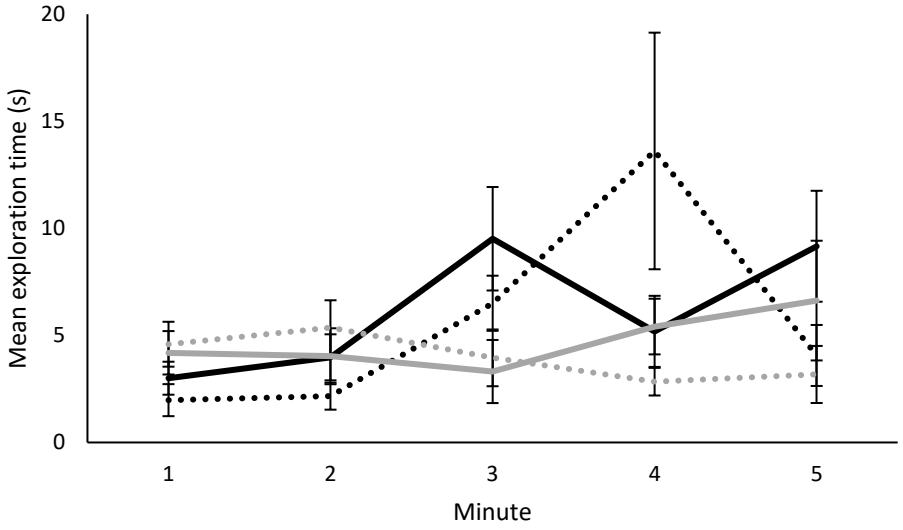


Figure 64. Mean (±SEM) exploration time of the novel and of the familiar compound and component objects during 5-minutes of test of Experiment 2. The solid black line and dotted black line depict the novel and familiar compound objects respectively. The solid grey line and dotted grey line denote the novel and familiar component objects respectively.

C.1.3 Experiment 3

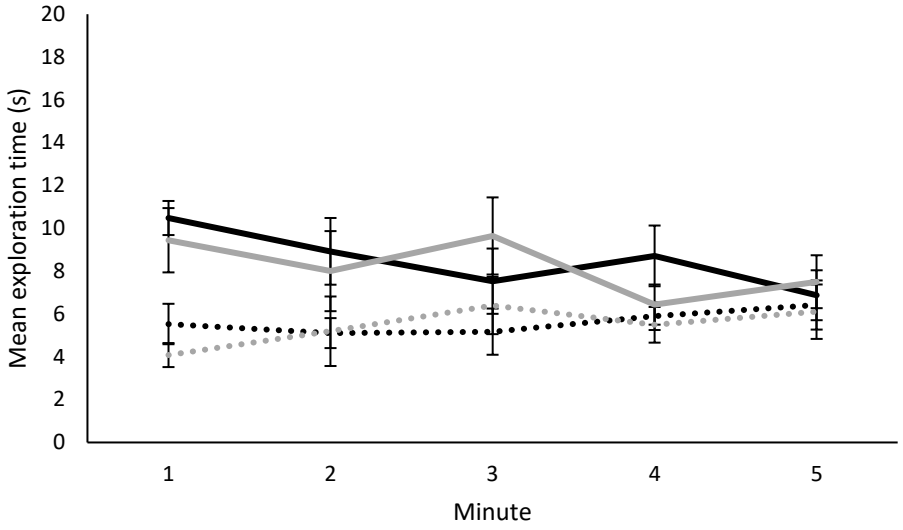


Figure 65. Mean (±SEM) exploration time of the novel and of the familiar objects for the blocking and control tasks, during 5-minutes of test, of Experiment 3. The solid black line and dotted black line depict the novel and familiar objects for the blocking task respectively. The solid grey line and dotted grey line denote the novel and familiar objects for the control task respectively.

C.1.4 Experiment 4

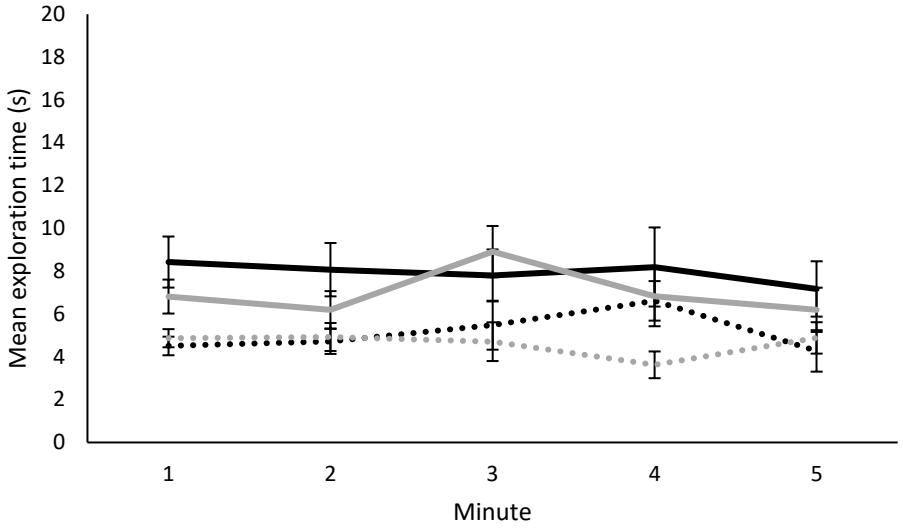


Figure 66. Mean (±SEM) exploration time of the novel and of the familiar objects for the blocking and control tasks, during 5-minutes of test, of Experiment 4. The solid black line and dotted black line depict the novel and familiar objects for the blocking task respectively. The solid grey line and dotted grey line denote the novel and familiar objects for the control task respectively.

C.1.5 Experiment 5

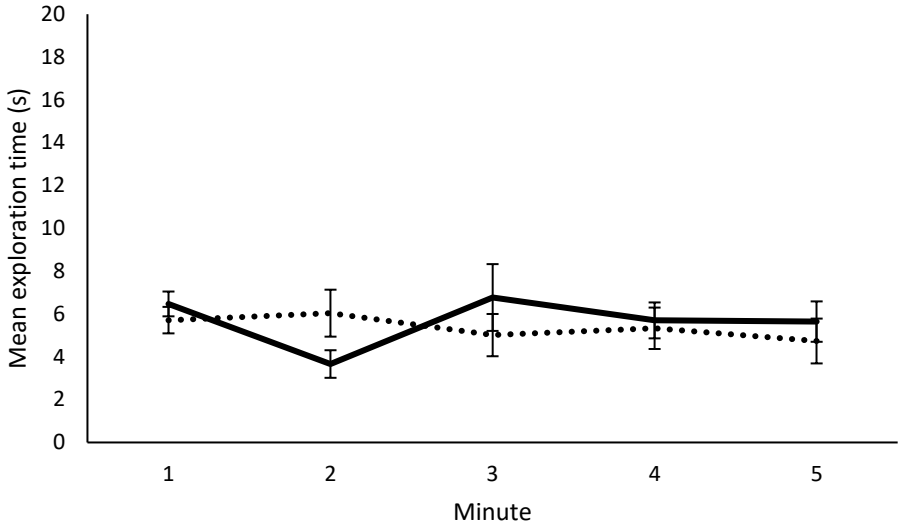


Figure 67. Mean (±SEM) exploration time of the novel and of the familiar objects during 5-minutes of test for Experiment 5. The solid black line depicts the novel object, and the black dotted line denotes the familiar object.

C.1.6 Experiment 6

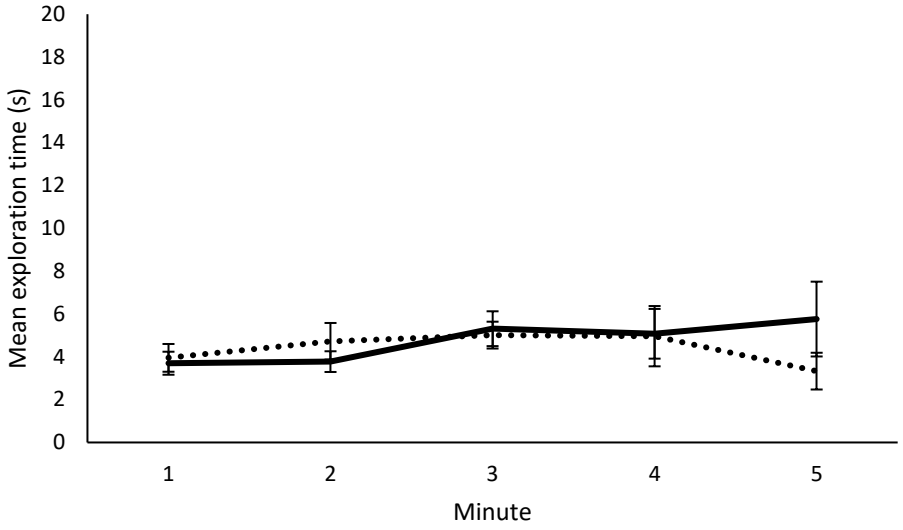


Figure 68. Mean (±SEM) exploration time of the novel and of the familiar objects during 5-minutes of test for Experiment 6. The solid black line depicts the novel object, and the black dotted line denotes the familiar object.

C.2 Chapter 4 experimental test data over 5-minutes

C.2.1 Experiment 7

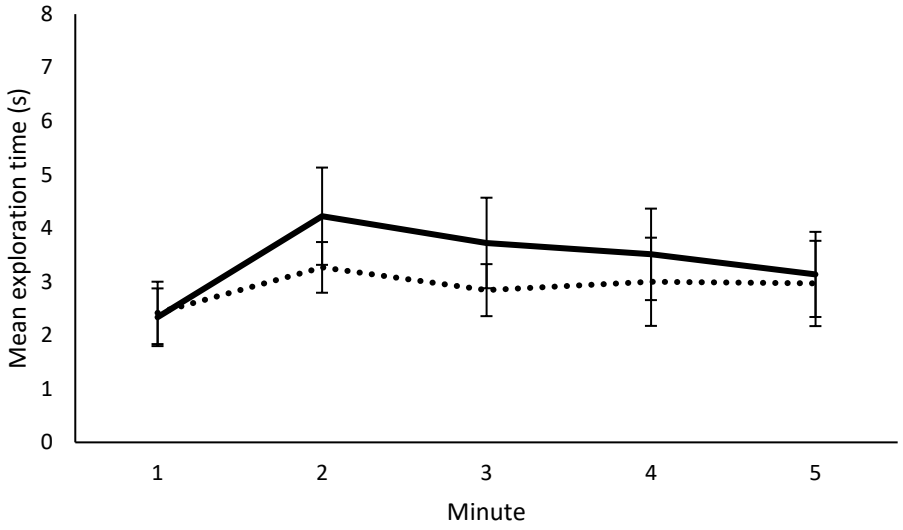


Figure 69. Mean (±SEM) exploration time of objects P and Q during 5-minutes of test for Experiment 7. The solid black line depicts object Q, and the black dotted line denotes object P.

C.2.2 Experiment 8

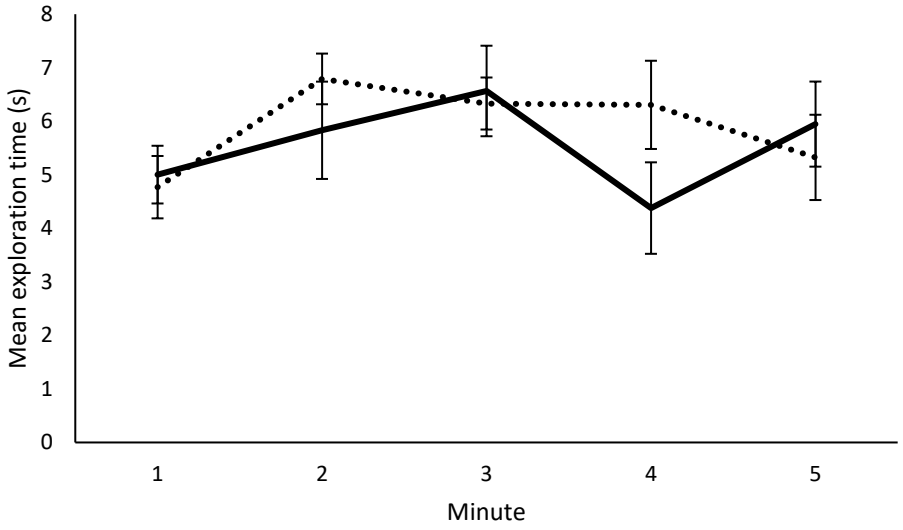


Figure 70. Mean (±SEM) exploration time of objects P and Q during 5-minutes of test for Experiment 8. The solid black line depicts object Q, and the black dotted line denotes object P.

C.2.3 Experiment 9

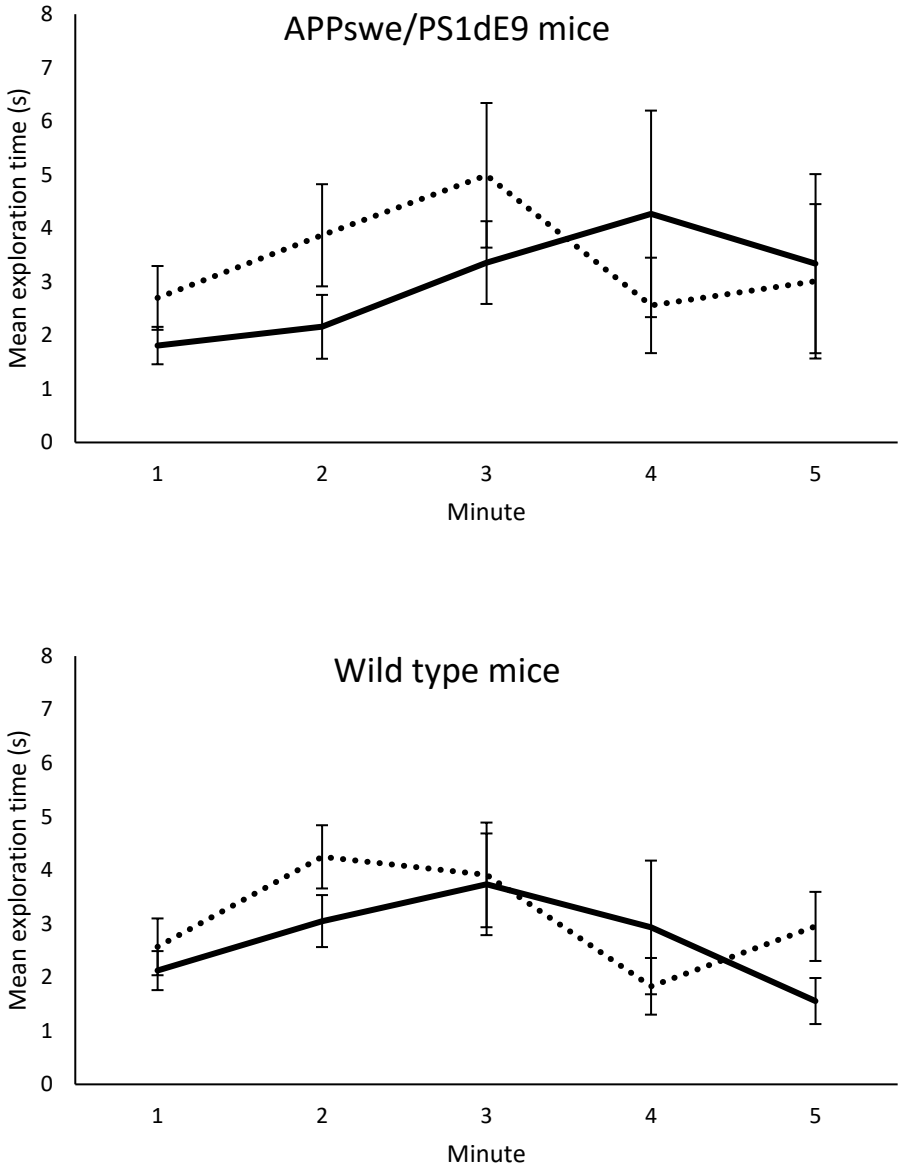


Figure 71. Mean (±SEM) exploration time of objects P and Q during 5-minutes of test for Experiment 9. The solid black lines depict object Q, and the black dotted lines denote object P.

C.2.4 Experiment 10

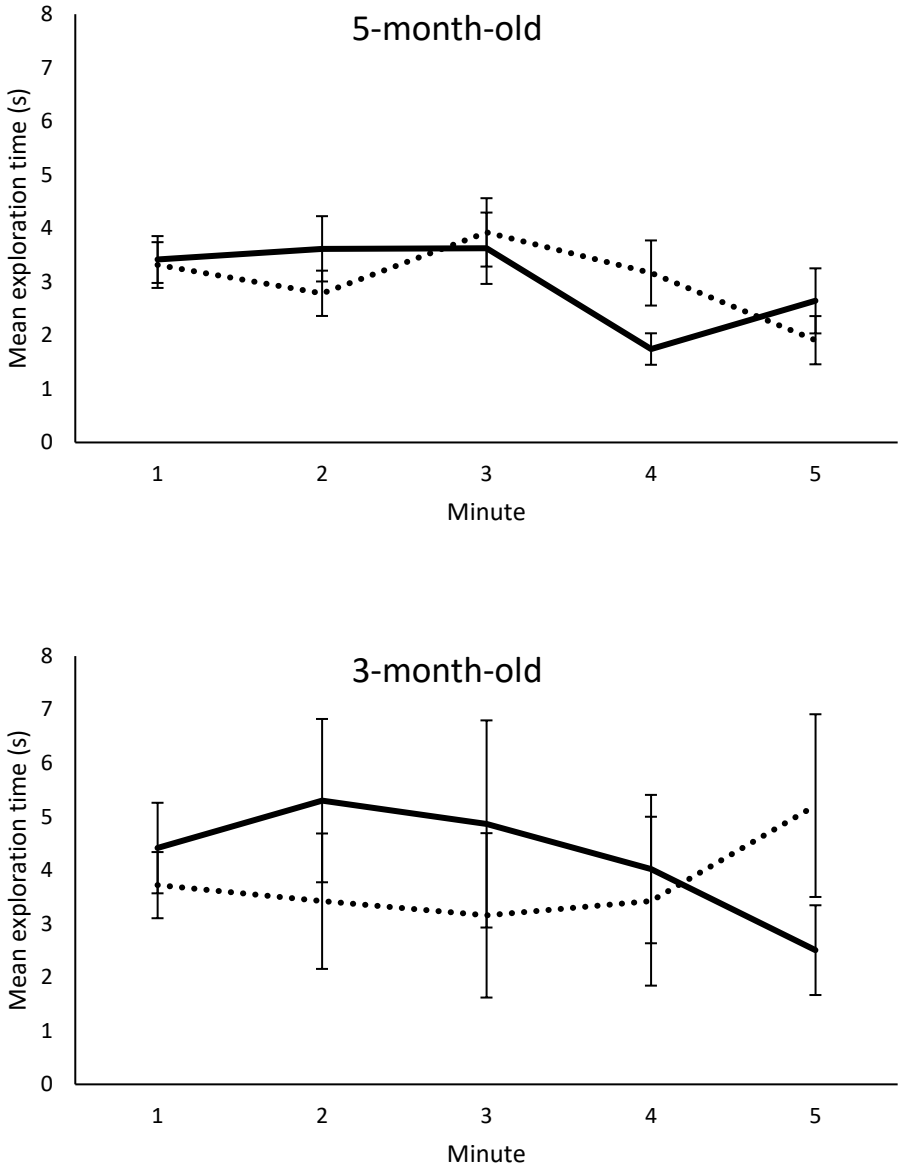


Figure 72. Mean (±SEM) exploration time of objects P and Q during 5-minutes of test for Experiment 10. The solid black lines depict object Q, and the black dotted lines denote object P.

C.3 Chapter 5 experimental test data over 5-minutes

C.3.1 Experiment 11

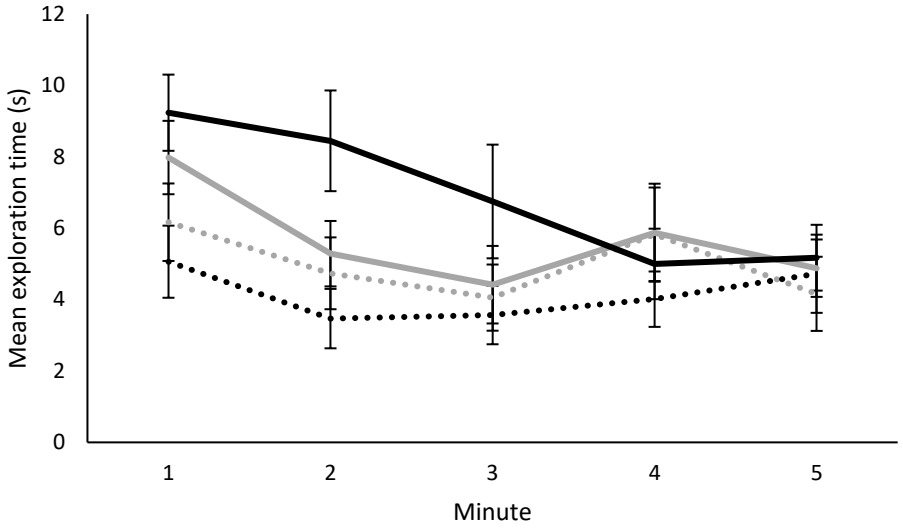


Figure 73. Mean (±SEM) exploration time of objects A and B during 5-minutes of test for Experiment 11. The solid black line and dotted black line depict objects A and B respectively for the distractor task. The solid grey line and dotted grey line denote objects A and B respectively for the standard task.

C.3.2 Experiment 12

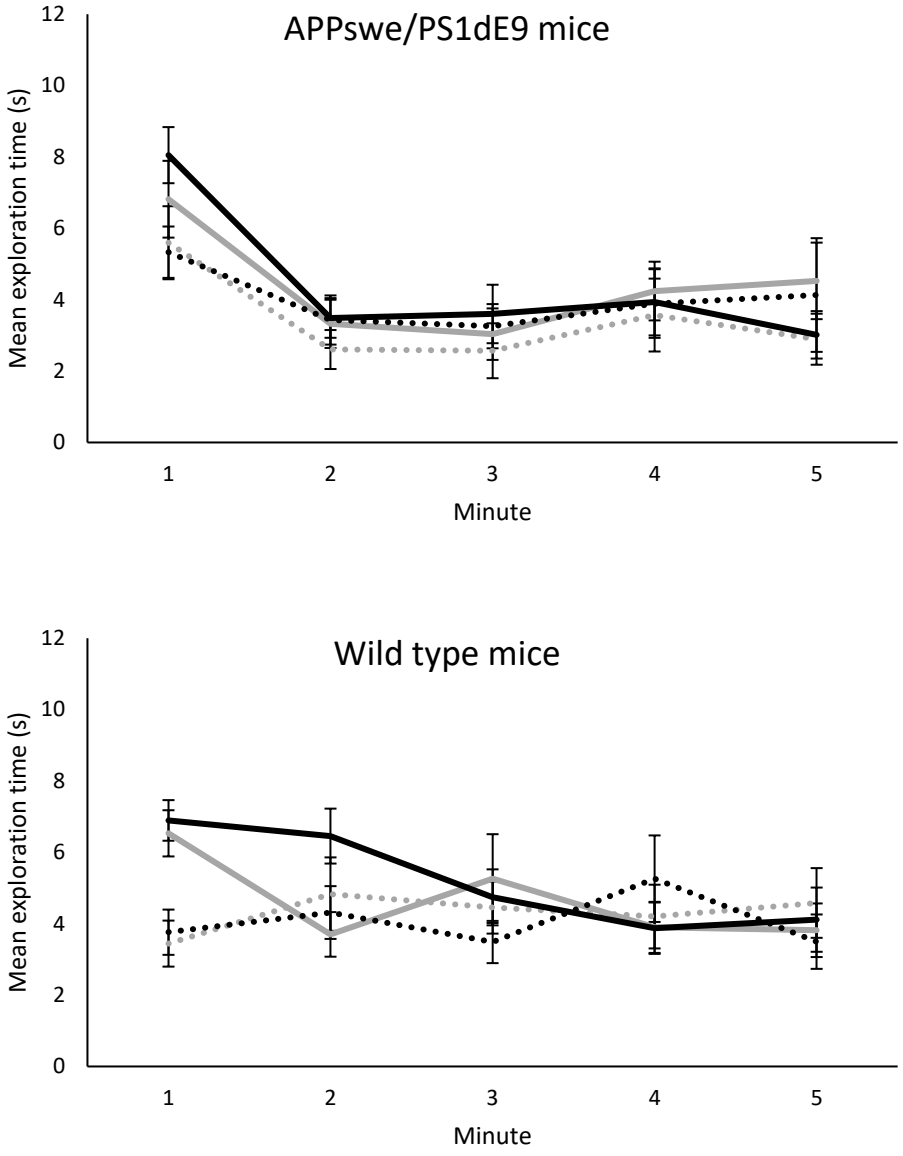


Figure 74. Mean (±SEM) exploration time of objects A and B during 5-minutes of test for Experiment 12. The solid black line and dotted black line depict objects A and B respectively for the distractor task. The solid grey line and dotted grey line denote objects A and B respectively for the standard task.

C.3.3 Experiment 13

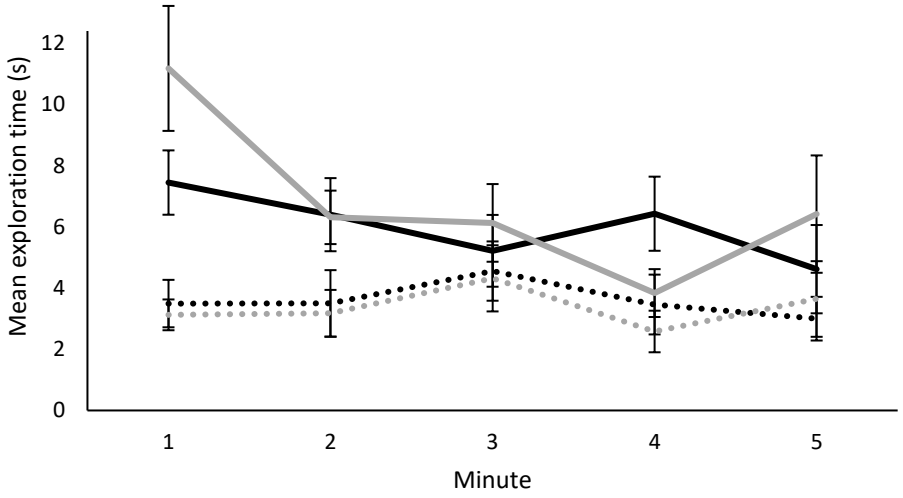


Figure 75. Mean (±SEM) exploration time of objects A and B during 5-minutes of test for Experiment 13. The solid black line and dotted black line depict objects A and B respectively for the proximal task. The solid grey line and dotted grey line denote objects A and B respectively for the distal task.

C.3.4 Experiment 14

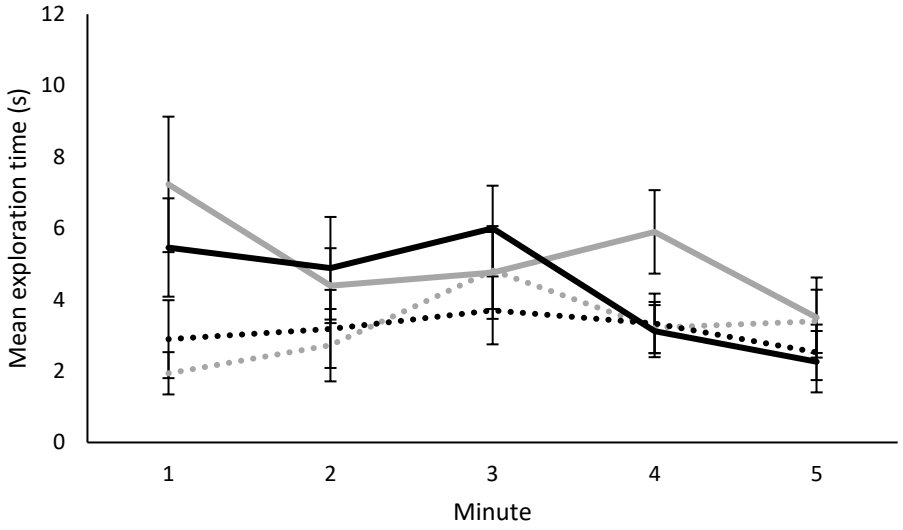
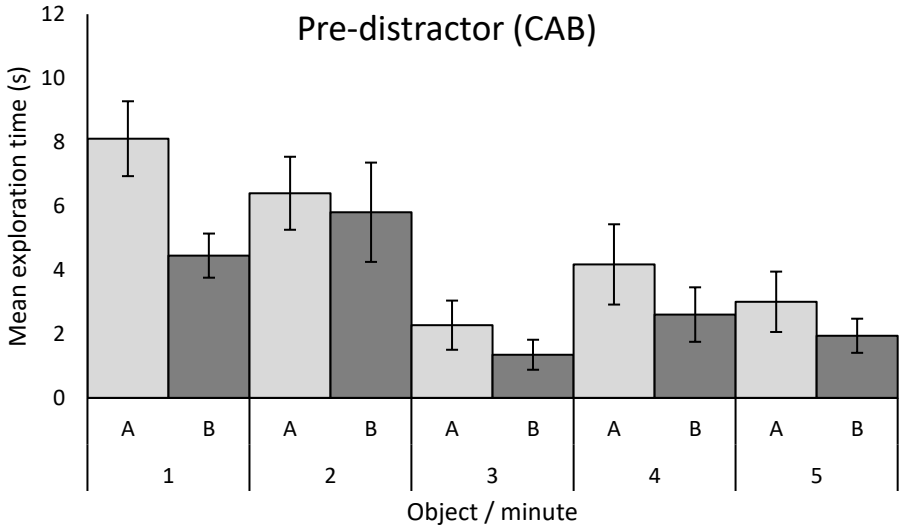
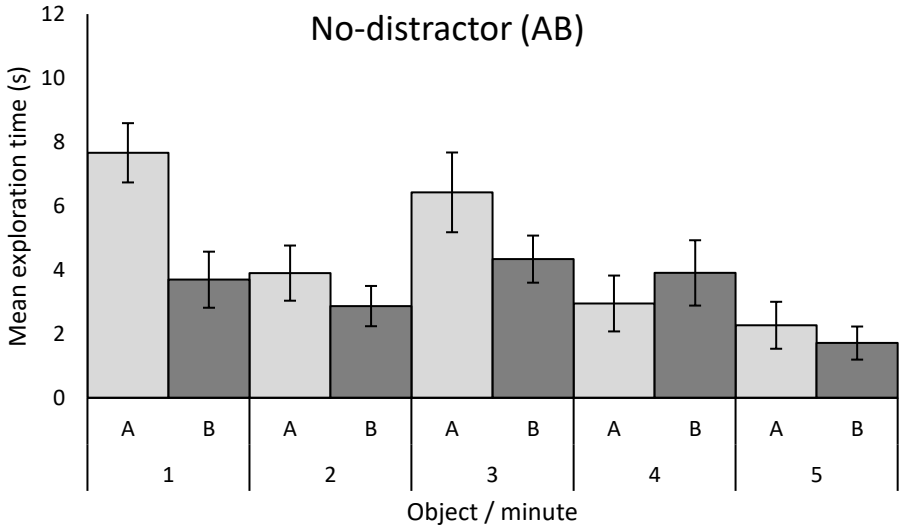


Figure 76. Mean (±SEM) exploration time of objects A and B during 5-minutes of test for Experiment 14. The solid black line and dotted black line depict objects A and B respectively for the more salient (DACB) task. The solid grey line and dotted grey line denote objects A and B respectively for the less salient (CACB) task.

C.3.5 Experiment 15





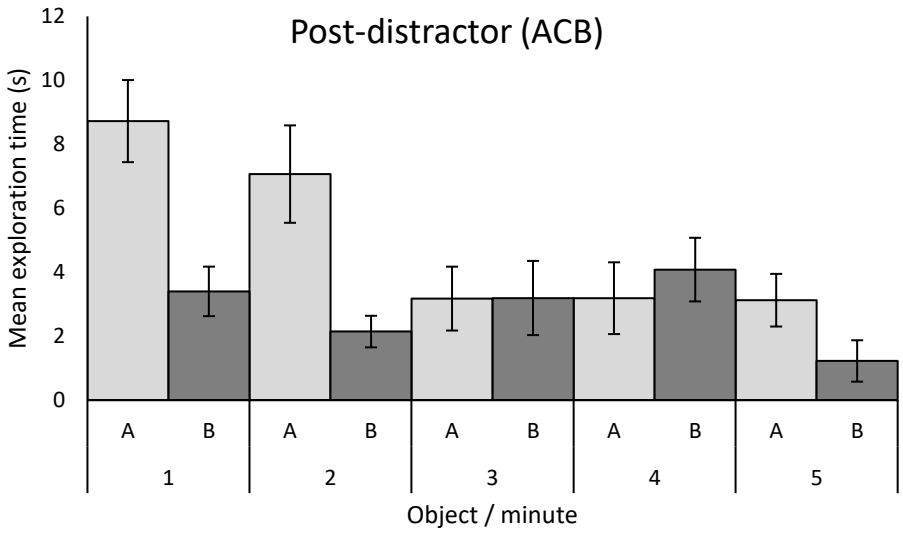


Figure 76. Mean (±SEM) exploration time of objects A and B during 5-minutes of test for Experiment 15, for each of the three distractor tasks used.

Appendix D

D.1 Pilot study for the blocking stimuli used for Experiment 8

D.1.1 Introduction

Chapter 4 generally focused on the retrieval-based aspect of recognition that may underlie performance during object-in-place and object-in-context tasks. Experiment 7 provided some evidence that recognition memory may have underlying associative processes, as previously suggested by other studies of recognition memory in rodents (e.g., M. A. Good et al., 2007; Sanderson & Bannerman, 2011; Spanswick & Dyck, 2012; Whitt et al., 2012). During Chapter 3, we explored SOPs associative priming theory using experimental designs based on Kamin (1969) blocking, a learning phenomenon that SOPs process of retrieval-generated priming should be susceptible to. We were unable to demonstrate a blocking effect and speculated that one possibility for this may have been that the animals were unable to discriminate between the blocking cues used. Based on previous literature (Wu et al., 2013), the textured floors used in Experiment 6 should have been discriminable by mice using our parameters. However, as our stimuli differed from theirs, and because we did not specifically test whether our mice could discriminate between the stimuli we used, we could not confirm this. For blocking to occur, the blocking cues need to form strong associations with the objects, that is, conditioning needs to reach asymptote to establish effective blocking (Jennings & Bonardi, 2017; Jones & Haselgrove, 2013; Sanderson et al., 2016). So far, we have not found any evidence to suggest that this had occurred. Therefore, before we continued to undertake further blocking experiments, we wanted to confirm that mice could reliably discriminate between the stimuli that we would use for future experiments under the parameters used.

Thus, this pilot study investigated the suitability of using various textured floor coverings for future blocking designs. Mice were tested using three replications of a modified novel object recognition task (Wu et al., 2013) where animals were exposed to two copies of textured floor A and then tested with a copy of A and a

novel textured floor B. The purpose of these tasks was to establish which pairs of textured floors the mice could readily discriminate between. The first pair were a rubber mat and a textured draw liner which we had previously used but never directly compared their discriminability. The second pair were two different grades of sandpaper which rats and mice have been shown to be able to discriminate between when trained to do so (Montuori & Honey, 2016) and when using a modified novel object recognition task (Wu et al., 2013). The third pair combined sandpaper and wire mesh.

D.1.2 Materials and method

D.1.2.1 Subjects

The subjects were 16 naive male C57BL/6J mice that were 3 months old (N=16) at the start of the experiment.

D.1.2.2 Procedure

Mice were exposed to two copies of textured floor A, during a sample phase which lasted 10-minutes. Following a 2-hour interval, they were then presented with a copy of A and a novel textured floor B and allowed to explore these freely for 5-minutes (Figure 77). Mice completed three replications of this task each using different stimuli as described below.

D.1.2.3 Textured floor stimuli

Floor discrimination task

The textured floors used were a 4mm thick black rubber mat with a textured square design and the other was a 2mm thick grey ethylene vinyl acetate (EVA) draw liner with a textured convex polka dot design. These were identical to the ones described during Chapter 3, Experiment 6 (Figure 19), except that they were reduced in size to 25cm x 11.5cm to match the size of the floors used during the other two discrimination tasks described below.

Sandpaper discrimination task

Two different types of aluminium oxide sandpaper were used as textured floors. One was a very coarse grade (240 grit) and the other was a very fine grade (40 grit).

Sandpaper/mesh discrimination

One of the textured floors used was medium grade (120 grit) aluminium oxide sandpaper and the other was galvanized steel square design wire mesh.

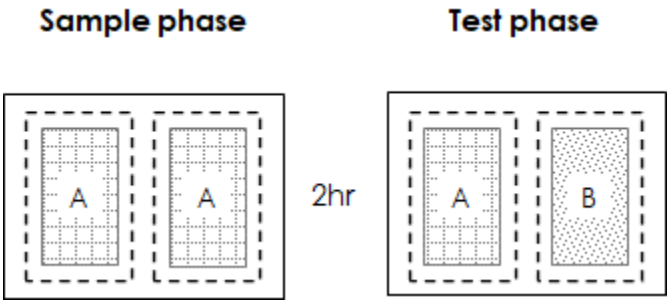


Figure 77. Schematic of the experimental design used for the pilot study, textured floor discrimination tasks. A represents the familiar textured floor, B depicts the novel textured floor, and the dashed line denotes the superimposed zones used for data collection, which measured the same as the textured floors.

D.1.3 Results and Discussion

During the sample phases of all three tasks, there were no side bias effects observed during the floor and sandpaper/mesh discrimination tasks but there was a left side bias during the sandpaper task. This was confirmed using three Student's t-tests which compared the mean total time (s) that mice spent within the left and the right superimposed zones (Figure 77) around the floor items used. These revealed no difference between the time spent in the left and right zones for the floor task ($T(15) = 1.626$, $p = .125$) or the sandpaper/mesh task ($T(15) = .448$, $p = .661$), but did show a left side bias during the sandpaper task ($T(15) = 4.540$, $p < .001$; Figure 78). There were no differences in overall exploration between the three tasks during the sample phases. This was confirmed with an ANOVA which

compared the mean total time (s) spent within the two superimposed zones during the sample phases of each task. This showed no statistical difference in the total exploration of floor items between the three tasks ($F(2, 45) = 2.782$, $p = .073$, $MSe = 14136.086$, $\eta_p^2 = .110$).

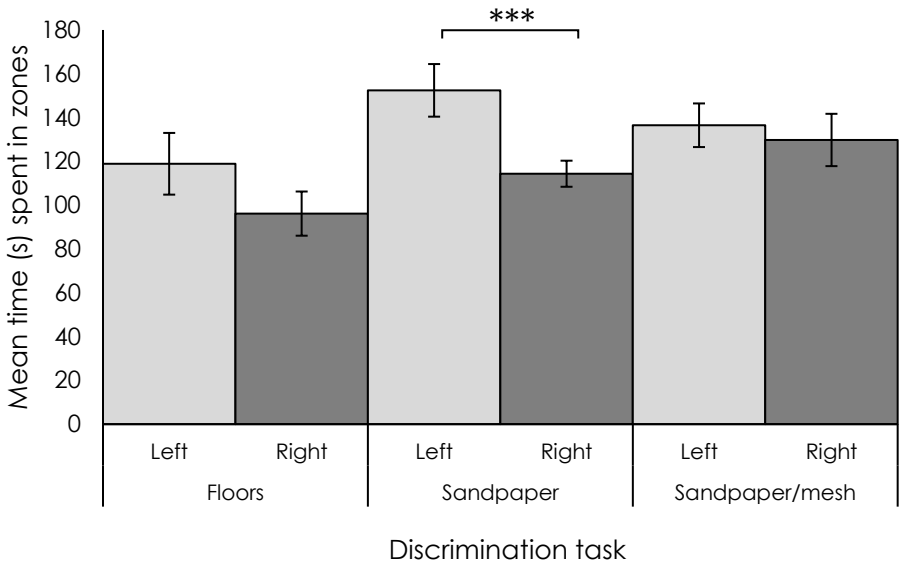


Figure 78. Mean (± SEM) time (s) spent in the left and right item zones during the sample phases of the discrimination tasks during the pilot study. *** above a bar denotes $p < .001$.

During the tests of the floor discrimination and the sandpaper discrimination tasks, mice were unable to discriminate between the novel and familiar items. Instead, they directed similar amounts of exploration towards both items. However, during the sandpaper/mesh discrimination task, mice successfully discriminated between the novel and familiar items and directed relatively more exploration towards the novel item. This was explored using three Student's t-tests which each compared the mean time (s) that mice spent in the superimposed zones (Figure 77) that surrounded the novel and the familiar items. These revealed no differences for the floor discrimination task ($T(15) = .194$, $p = .848$) or the sandpaper discrimination task ($T(15) = .520$, $p = .611$), but did show a difference during the

sandpaper/mesh discrimination task (T(15) = 2.175, p = .046; Figure 79). Because the mice had displayed a left side bias during the sampling phase of the sandpaper discrimination task, we also compared their left and right exploration data during the test for this task. A left side bias during the test phase would have compromised the test results. However, a Student's t-test, which compared the mean time (s) that mice spent in the left and right item zones, revealed no difference between left and right item exploration (T(15) = -.359, p = .725).

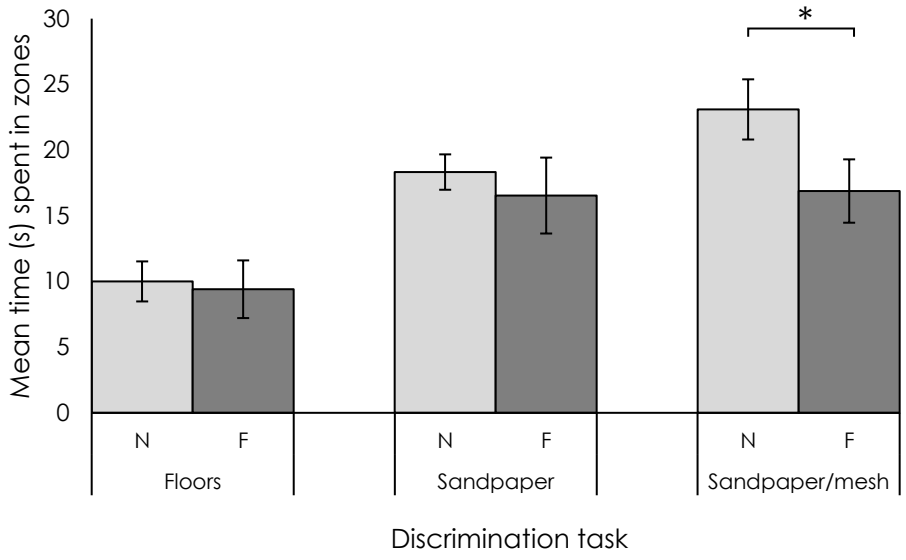


Figure 79. The mean time (±SEM) that mice spent in the zones during each of the three floor discrimination tasks, during the first three minutes of the test, during the pilot study.

During this pilot study we demonstrated that mice could discriminate between sandpaper and wire mesh textured floors during a modified novel object recognition task. However, during the same task, mice were unable to discriminate between the rubber mat and textured draw liner. These stimuli were used during Experiment 6, which may explain why we failed to obtain a blocking effect. Such cues need to be discriminable if they are to become an effective blocking cue. Unexpectedly, mice were also unable to discriminate between two different grades of aluminium oxide sandpaper. This was surprising as rats can be trained to

discriminate between textures which include sandpaper (Montuori & Honey, 2016; Wu et al., 2013) and mice have been shown to discriminate between several grades of sandpaper during a modified novel object recognition task (Wu et al., 2013). Furthermore, the study by Wu et al. (2013) used grades of sandpaper that were closer together (80, 100, 120, 220 grit) than the ones we used (40 and 240 grit) and their stimuli were smaller (7.5cm x 4cm) than the ones used here (25cm x 11.5cm), both which should have made the task relatively more difficult. One possibility for this disparity in results is that we used a new piece of sandpaper each time a mouse was placed in the arena, which would have eliminated olfactory cues, whereas Wu et al. (2013) used the same three pieces of sandpaper throughout their study, so it is possible that mice used olfactory cues during the discrimination. Although Wu et al. (2013) cleaned their stimuli between sessions and between animals, this may not have been sufficient to eliminate odour cues as mice have an extremely high sensitivity to odours. For example, different mice strains have been shown to be able to detect ethyl acetate at concentrations as low as 0.00005% in CF-1 mice (Bodyak & Slotnick, 1999) and 0.00003% in C57Bl/6J mice (Patel & Larson, 2009).

Nonetheless, we were able to achieve our aim of confirming a pair of stimuli that mice were able to successfully discriminate and that we could use during a blocking design for the next experiment.

Appendix E

E.1 PIPS reflective statement

I completed my placement with The Academy of Medical Sciences where I undertook the role of a Grants and Programmes Officer. I worked on many different projects which covered multiple aspects of the processes involved with the Academy's grant and programme schemes.

For grant schemes my tasks included, eligibility checking of applications, identifying peer reviewers for clinical grant applications, collating application scoring from the peer review stage, taking minutes at award decision panel meetings, and writing up the minutes and redacting peer reviews in preparation for applicant feedback. For programme schemes my tasks included, recruiting mentors for the mentoring scheme, preparing surveys for post event feedback, training colleagues to create surveys using SurveyMonkey, collecting information from colleagues and using it to create two newsletters for two different awardee schemes, and helPin to run various events that were either in-person, online only, or hybrid.

My largest task was taking the lead on running a scheme called INSPIRE which is designed to engage medical, dental and veterinary undergraduates with research. This is achieved through the provision of funding for medical, dental, and veterinary schools, which allows them to deliver locally designed activities aimed at informing and exciting students about the benefits and potential of a career in research. For this task I was responsible for, helPin to run the online award decision panel meeting, writing up the minutes ready for applicant feedback, preparing and sending out decision letters, obtaining prior approval for the letters from the panel chair and the Academy director, respond to awardee questions and provide further support to them, distribute various forms to awardees, update system with submitted forms, and create a payment control sheet for the awards.

During my placement I learned many new skills and further developed several of my existing skillset. A key new skill I learned was how to complete a mailmerge in Word which is used for producing mass emails and letters to multiple recipients. I also was heavily involved with event management which incorporated both planning and running events which was something I have not really done prior to my placement. I learned how to grant management software (Flexigrant) which could be very useful if I pursue a career related to research management. I also sourced awardee information for a professorship scheme and used this information to update the Academy's webpage relating to the scheme. I imagine that these new skills will be very useful in applying for jobs and during future employment as they have application in many different job roles. I also further developed my skills in time management and flexibility as I was faced with multiple deadlines and an ever-changing list of tasks to be completed. This was a very different experience from my PhD project which has a fixed list of tasks and only a few deadlines for these. In contrast during my placement, some tasks would be presented and need to be completed on a single day whereas others would be larger time commitments within a relatively short timeframe but with very strict completion deadlines. This resulted in me having to continuously rearrange the order in which I completed tasks and required me to monitor and prioritise new and existing tasks constantly.

My placement with The Academy of Medical Sciences has given me many ideas about possible career pathways once I graduate from my PhD. Prior to my placement, I felt that my only career option was to pursue a post-doctoral position, however, I now have ideas of careers outside of academia that I may find interesting and rewarding that would allow me to use the skills that I have developed throughout my PhD and I now feel that I would be very competitive when applying for these types of job roles.

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