# *A Continual Trials Approach to Recognition Memory in Mice*

CHAN, MICHELE,SWEE,YEE

# A Continual Trials Approach to Recognition Memory in Mice

Durham University, Department of Psychology

**CHAN, MICHELE S.Y.**

**12/1/2017**


Thesis submitted for the degree of Doctor of Philosophy

## ABSTRACT

This thesis sought to address and improve resolve some issues surrounding tests of recognition memory in animals. Since these spontaneous object recognition memory tasks are widely used, especially in mice, there is a need to develop a recognition task that would reduce the variability, extend and translate the task to potential areas of neuroscience research.

Study 1 sought to validate the continual trials approach that was originally designed for rats to mice and replicate the findings of Ameen-Ali et al., (2012) in the spontaneous object recognition and object-location task. Study 1 found that performance of mice was comparable to previous studies of object recognition and object location memory, and statistically meaningful results were obtained with approximately 30 – 50 % fewer mice than typically used in the standard one trial a day version of the spontaneous object recognition tasks. Study 2 sought to extend the continual trials apparatus to establish the age-related changes of object recognition and object-location memory in normal ageing mice; and found that ageing mice showed no age-related decline of recognition memory. Study 3 found no evidence of age-related changes of object recognition and object-location memory in a transgenic mouse model of Alzheimer's Disease, TASTPM mice. In study 4, the continual trials apparatus was adapted to incorporate variable retention delays (by blocking the sample and test phases) and found no evidence of delay-dependent effect on object recognition memory. Study 5 provided novel evidence that NMDA blockade using the MK-801 drug had no effect on object recognition memory in mice when controlled for state-dependency of memory. The key findings of this thesis include the successful validation of the continual trials apparatus in mice and the evidence that studies using reduced number of mice can nonetheless provide valid results in object recognition memory tasks.

**TABLE OF CONTENTS**

## LIST OF FIGURES

## LIST OF TABLES

Summary of the findings and results of all experiments from the current study which assessed the effects of MK-801 administration on long-term and short-term memory in the multiple trials version of the spontaneous object recognition task Table 7.2 (p. 207)

Summary of performance levels of mice within the testing session of experiment 1, 2, 3 and 4.

## DECLARATION

I confirm that no part of the material contained in this thesis has previously been submitted for a degree in this or any other University. Any work presented in this thesis that was generated through collaborative research clearly acknowledged the work of others.

## COPYRIGHT

## ACKNOWLEDGEMENTS

First and foremost, I'd like to thank my supervisors, Dr. Alex Easton, Dr. David Sanderson and Professor Madeline Eacott. I am grateful for their unwavering support and faith in my ability to complete this work. I would also like to thank NC3Rs for funding this project and GlaxoSmithKline for providing the animals that make up a large part of the work in this thesis.

I thank my family, especially Ethel and Christon (who does not like being left out) for their support, encouragement, and light-hearted banter that made the whole process bearable at its worst. I would like to thank my friends, Natalia, Sabrina and Barbara-Anne for entertaining my need to exchange ideas, for providing support and being there for me. My biggest thanks go to Francesca, who provided refuge when I needed it and went on an adventure with me that one night in Leuven.

I would also like to thank Sam, for his unwavering support, especially during my viva and corrections. For his patience and understanding when I was stressed out and high strung. Oh! And carrot cake. Thanks for the carrot cake. You're the best-est.

Finally, I would like to thank everyone, especially Greg, who was there for me during the last month of pulling this thesis together, for believing in me and ensuring I never went hungry.

# Chapter 1

# Introduction

## 1.1 Introduction

The introductory chapter of this thesis will review the literature on tasks examining recognition memory in rodents, such as the delayed-non-matching to sample task and the spontaneous object recognition tasks and its complex variants, such as the object location task. Following this, the present chapter will introduce the shortcomings of the spontaneous object recognition task and present a solution (continual trials apparatus) that has been used to address the disadvantages of the spontaneous object recognition task in rats. However, the increasing use of mice in neuroscience literature has prompted the translation of the continual trials apparatus from rats to mice. The current chapter will also consider the physiological, behavioural, and cognitive differences between rats and mice; alongside the potential difficulties that may be encountered during the validation of the continual trials approach in mice.

## 1.2 Recognition memory – single or dual process theory?

Imagine a scenario where you are walking around in a conference room and see someone who looks vaguely familiar. When the both of you exchange greetings, you are sure you know who the person is, but for some reason you are unable to pin point the persons' name, where and how you met this person. After spending some time having a casual conversation with the person, they mention a meeting that happened last week which prompted a recall of the persons' name, where the meeting was, and some agenda discussed during the meeting. The common scenario illustrated above describes two forms of experiences which occur during recognition. The first type is familiarity, where the experience occurs rapidly and ranges from a weak intuition to a strong belief (or knowing

– Tulving, 1985). The second experience is recollection, which involves remembering associations prompted by cues critical to the memory (or 'remembering' – Tulving, 1985).

Beyond the subjective distinction of these experiences, researchers have been interested in the investigations of the underlying neural mechanisms of recognition memory. Thus far, there have been two theories involved in the debate of the underlying processes of recognition memory; the first of which stipulates that recognition memory, or familiarity and recollection occurs along a single continuum (Single-process theory; Donaldson, 1996; Dunn, 2004; Squire et al., 2004), whereas the second theory argues that recognition memory is driven by two functionally distinctive processes (Dual-process theory; Eichenbaum et al., 2007; Yonelinas, 2001; Brown and Aggleton, 2001).

The single-process theory proposed by Squire (1994; see also Squire and Zola, 1998; Squire et al., 2007) presented an argument that recognition memory tests traditionally used to distinguish familiarity from recollection vice versa was, in actuality separating strong from weak memories. This theory further argues that the perirhinal cortex and the hippocampus are equally involved in familiarity and recollection, and damage to either of the areas will result in impairments in familiarity and recollection (Squire et al., 2007; Wixted and Squire, 2010).

The dual-process theory on the other hand, proposed (Eichenbaum et al., 1994) that recognition memory is supported by two functionally distinct processes mediated by medial temporal lobe structures; the hippocampal formation, which supports the recollection of relevant associative representations and stimuli and the parahippocampal region, which supports the storage and recognition of specific items. This functional dissociation was further extended by Brown and Aggleton (2001), when they proposed that the hippocampal region is involved in the recollection and episodic memory processes, whereas the perirhinal cortex was involved in the processing of familiarity and recency of a stimuli; and while the hippocampus and perirhinal cortex interact to process recognition

memory, the contributions of these structures may be dissociable. The dual-process theory of recognition memory as proposed by Eichenbaum et al., (1994; see also Brown and Aggleton, 2001) is based on the idea that recognition memory is functionally distinct, there is still considerable debate about how recognition memory is supported by different regions of the medial temporal lobe (Eichenbaum et al., 2007). Thus far, literature on the regions involved in recognition memory have found that the perirhinal cortex and the parahippocampal region are responsible for familiarity, whereas the hippocampus and fornix plays an important role in recollection (Aggleton et al., 2005; Brown and Aggleton, 2001; Eichenbaum et al., 2007; Langston and Wood, 2010). Hence, damage to the hippocampus should impair recollection but not familiarity, whereas perirhinal cortex and parahippocampal damage would impair familiarity but not recollection (Eichenbaum et al., 2007).

Recent clinical studies have attempted to test these theories by dissociating familiarity and recollection in amnesiac patients with selective hippocampal damage. Research into clinical patients with hippocampal damage have found that impairments in recollection whilst sparing familiarity processing (Giovanello et al., 2003; Holdstock, 2005; Mayes et al., 2002; Aggleton et al., 2005); whereas other studies reported deficits in both recollection and familiarity processing following hippocampal damage in amnesiac patients (Cipolotti et al., 2006; Wais et al., 2006; Maans et al., 2003). The discrepancy in these findings could be a result of the differences between medial temporal lobe damage in patients and/or the different testing methods. However, in general, object recognition memory has been found to be impaired in human patients affected by brain injury or neurodegenerative diseases (Buffalo et al., 1998; Holdstock et al., 2005; Laatu et al., 2003; Manns and Squire, 1999; Reed and Squire, 1997).

Whilst human patient literature successfully provided insight into differentiating the role of the hippocampus from other parts of the parahippocampal gyrus in recollection,

only a limited amount of evidence were provided on the role of the perirhinal cortex on familiarity processes. To further elucidate the contributions of the hippocampus and perirhinal cortex on recognition memory, researchers have focused on the development of animal research and it has been proven to be a substantial improvement in part due to the ability to investigate the effects of selective lesions to the medial temporal lobe on recognition memory.

### 1.3 Delayed non-matching to sample task

Gaffan (1974) introduced the delayed matching to sample task with the aim to develop a parallel test of recognition memory in laboratory animals equivalent to that of human patients with anterograde amnesia. The delayed matching to sample task was a test of recognition memory that was evaluated by the ability of an animal to discriminate the familiar from the novel object. Monkeys were initially presented with object 'A' at sample; then a pair of objects 'A' and 'B' at test whereby a food reward (typically in the form of a pellet) was hidden beneath the familiar object 'A'. The monkey has to learn that the familiar object was rewarded, thus to pick the familiar object each time.

Mishkin adapted Gaffans' DMS task in 1978, by training twelve monkeys in a trial-unique multiple trial task to select the novel object at test, instead of a familiar object. The delayed non-matching to sample (DNMS) task exploits the animals' natural preference towards exploring novelty (Mishkin and Delaceur, 1975), and in turn resulted in monkeys reaching the learning criterion (90 correct out of 100 trials) in approximately one-third of the time compared to the DMS task. Following this, monkeys received lesions of the hippocampus, the amygdala or a larger lesion of the hippocampus and amygdala. Two weeks post-operation, monkeys were re-trained to learn the non-matching rule; then further tested their recognition abilities by incorporating increasing delays of 10s, 30s, 60s, then

120s between sample and test. This study found mild impairments in monkeys with lesion of the hippocampus or amygdala; but severe impairment (especially in longer delays) of recognition memory in monkeys with the combination of hippocampal and amygdala lesion. The D(N)MS task was widely used in recognition tests in monkeys (Mishkin and Delaceur, 1975; Mishkin, 1978; Eacott et al., 1994) and in humans (Holdstock et al., 2000) to investigate the neural basis of recognition memory. Whilst lesions to the rhinal cortex yielded impairments to the DMS and DNMS task (Eacott et al., 1994; Munier et al., 1993; Zola-Morgan et al., 1989), studies investigating the effects of hippocampal lesions on performance in the DNMS task have yielded conflicting findings, with some studies reporting intact performance in the task (Nemanic et al., 2004; Murray and Mishkin, 1998), whilst other studies reported impairments following hippocampal lesions (Alvarez-Royo et al., 1991; Zola-Morgan et al., 1994; Zola et al., 2000). Although the role of the hippocampus on performance in the DNMS task continues to be examined, there is a consensus that the development of the DNMS task laid the foundation for an animal model of human medial temporal lobe amnesia.

Tasks of D(N)MS were most widely used in investigations of memory in non-human primates (Gaffan, 1974; Mishkin & Delaceur, 1975; Mishkin, 1978; Eacott et al., 1994), which typically uses a small sample of animals and runs on multiple trials within a training session, which is advantageous. Also, the D(N)MS task enables the use of varying retention delays (Mishkin, 1978). Apart from testing recognition memory in non-human primates, the D(N)MS task has been modified to test rodent recognition memory in objects (Aggleton,1985; Kesner et al., 1993; Mumby et al., 1990; Rothbalt & Heyes, 1987) and odours (Otto and Eichenbaum, 1992; Winters et al., 2000). There are however, issues surrounding the use of DNMS task in rats. A major issue includes, the requirement to undergo considerable training sessions prior to test to ensure the acquisition of task rules (matching or non-matching). Furthermore, performance deficits could be attributed to

other reasons apart from memory impairment, such as the failure of learning the rules to the task or changes in motivation (as a result of baiting novel objects with food reward). Despite the advantages that come with the DNMS task, the issues tied to the task prompted researchers to develop a simpler task to assess recognition memory in rodents.

### 1.4 Spontaneous object recognition task

The spontaneous object recognition task (figure 1.1) was an adaptation of the DNMS task developed by Ennaceur and Delaceur (1988), which capitalises on an animals' preference to explore novelty. The spontaneous object recognition task is a simple test of recognition memory which addressed the weaknesses of the DNMS task, such as extensive training, rule learning and food reward. The spontaneous object recognition task is typically performed within an open field and a trial of the task consists of two phases: a sample and test (acquisition and retrieval respectively). At sample, which normally lasts between 2-10 minutes, an individual animal is allowed to freely explore a pair of identical objects in the open field. After a retention delay, which could range between minutes, hours or even days, the animal is then returned into the open field to further explore a novel object and a familiar object that was previously seen in the sample phase. The animal is said to demonstrate a memory of the familiar object previously encountered in the sample phase by preferentially exploring the novel object presented in the test phase. Because animals are able to actively explore the objects, preferential exploration is not only driven by visual representations, but also the tactile and olfactory properties of an object (Clark and Squire, 2010).

Performance levels in the object recognition task is driven by several factors, stimuli salience and properties, an animals' motivation to explore, amongst other things. Between lab procedural differences such as (a) lighting conditions during test in which

some are conducted in brightly lighted room (Whitt and Robinson, 2013; Clark et al., 2000; Nanfaro et al., 2010; Broadbent et al., 2010) and others in darkened rooms (Ameen-Ali et al., 2013; Seel et al., 2017; Silvers et al., 2007; Clarke et al., 2010); (b) experiments conducted in silence (Whitt and Robinson, 2013) or with the presence of white noise (Ameen-Ali et al., 2012; Seel et al., 2017; Ennaceur and Delaceur, 1988); (c) an animal receives single trial testing (Dere et al., 2005) or single trials over several days (Whitt and Robinson, 2013; Norman and Eacott, 2004); and (d) differences in exploration criterion whereby animals are given a fixed duration to explore objects (Dix and Aggleton, 1999; Langston and Wood, 2010; Pezze et al., 2017; Norman and Eacott, 2004; Barker and Warburton, 2011) or by reaching a certain level of exploration (eg. 15s of object exploration or 10 minutes in the open field; Kim and Frick, 2017; Zhao et al., 2012; Winters et al., 2004; Ainge et al., 2006). The extension of the sample phase, which increases the chances an animal comes in close contact with the object (active exploration), have been found to improve performance in the spontaneous object recognition task. Work by Albasser et al., (2009) found a positive correlation between the time spent actively exploring the objects and the success in novelty preference. Furthermore, the length of the test phases are equally important, as reported by Dix and Aggleton (1999), where majority of active exploration by rats occurred within the first 2 minutes.

### 1.5 Applications of the spontaneous object recognition task

The spontaneous object recognition task allows for variable retention intervals, ranging from minutes (Dix and Aggleton, 1999; Norman and Eacott, 2004; Langston and Wood, 2010; Hale and Good, 2005), to hours (Winters and Bussey, 2005; Sik et al., 2003; King et al., 2004; de Lima et al., 2005; Scullion et al, 2011), even days (Ennaceur and Delaceur, 1988; Frick and Gresack, 2003) between sample and test. The memory strength of the familiar stimuli is dependent on the duration of delay between the presentation of

stimuli at sample and the retrieval at test. Some studies of recognition memory in rodents have found a delay-dependent effect of memory (Sik et al., 2003; Dodart et al., 1997; Winters and Bussey, 2005); but not in other studies (Hammond et al., 2004; Jessberger et al., 2009; Winters et al., 2004; de Bruin and Pouzet, 2006; de Lima et al., 2006; Hall et al., 2016; Taglialatela et al., 2009).

Apart from retention intervals, the features of a stimuli also play a role in spontaneous object recognition task performance. For example, Norman and Eacott (2004) found that stimuli feature ambiguity affected performance in the spontaneous object recognition task. When rats were tested in the spontaneous object recognition task with junk objects (e.g., vases, bottles and candlesticks) comprising of different materials, shapes and sizes, the animals were able to successfully discriminate the novel from the familiar objects with retention delays of up to 24 hours. However, when objects were made out of Duplo, which enabled the configuration of different levels of overlapping features between stimuli, discrimination between novel and familiar objects were successful at retention delays of up to 15 minutes in the control animals. Recent work by Heyser and Chemero (2012) investigated the effects of object affordances on mice exploration and discrimination using the spontaneous object recognition task. They compared mice' interaction with two types of objects: (a) objects that were able to support the weight of the animals and has a surface parallel to the ground; and (b) objects that could only be touched. The study found that animals spent more time exploring objects that could be climbed compared to objects that could only be touched; also, discrimination ratios were higher in objects that could be climbed. Providing further support that object features and affordances affects spontaneous object recognition task performance.

Lesion studies have been particularly useful in elucidating the underlying neural mechanisms related to the spontaneous object recognition task. There is a broad agreement that lesions to the perirhinal cortex disrupts recognition memory capabilities in animals

(Norman and Eacott, 2004; Olarte-Sanchez et al., 2015; Barker et al., 2007; Winters et al., 2008; Warburton and Brown, 2015; Ennaceur and Aggleton, 1997; Ennaceur et al., 1996). Although the perirhinal cortex plays a crucial role in performance in the spontaneous object recognition task, there is still a debate of the role of hippocampus in recognition memory. Whilst many studies report of hippocampal and fornix lesions not affecting performance in the spontaneous object recognition task (Langston and Wood, 2010; Good et al., 2007; Winters et al., 2004; Barker and Warburton, 2011), other studies report impairments in spontaneous object recognition task performance (Baker and Kim, 2002; Clark et al., 2000, Hammond et al., 2004). However, despite reports of impairments in the hippocampal lesioned animals, it is often less severe than animals with perirhinal lesions (Winters et al., 2008) and occurs over long delays (Clark et al., 2000; Hammond et al., 2004). The discrepancy in these findings were addressed in studies involving rats dissociating the functions of the hippocampus and perirhinal cortex. Research found that rats with hippocampal lesions were impaired in spatial tasks but spared in the spontaneous object recognition; whereas perirhinal lesioned rats were found to be spared in tasks requiring spatial memory but not in the spontaneous object recognition task (Winters et al., 2004; Ennaceur et al., 1996). Findings from double dissociative studies suggests that the hippocampus does not play a critical role in the recognition memory of the spontaneous object recognition task.

The spontaneous object recognition task and the advantages associated with the task have contributed to its widespread use in the investigation of recognition memory in rodents. Research have also found that, in comparison to the DNMS task, the spontaneous object recognition task was a more sensitive measure in the detection of recognition memory deficits (Clark and Squire, 2010). Furthermore, the relative ease of administration allowed for the widespread use of the spontaneous object recognition task across different

fields and performance in the task have been consistent across species (Clark and Martin, 2005).

### 1.6 Issues surrounding the spontaneous object recognition task

Despite the advantages that come with the spontaneous object recognition task, there are methodological issues in relation to the task. First, because the exploration of objects in this task is driven by the animals' spontaneous exploratory behaviour, the unpredictability often results in high between animal variance, and this effect especially pronounced when the animals are tested over a relatively low number of trials. This in turn decreases statistical power, and normally would be solved by running a higher number of trials or increasing the animal numbers in the experiment. The issue of high variance within the animals is further exacerbated by varying levels of exploration driven by object salience. When objects of different degrees of salience are paired together, animals may explore the objects that are more salient and possibly skewing discrimination levels. Careful consideration should be taken when pairing objects, to ensure that objects with similar salience levels are paired together and proper counterbalancing between and within animals, to minimise biased exploration of objects driven by salience (Ameen-Ali et al., 2015).

Second, because discrimination in the spontaneous object recognition task is measured by differential exploration between the novel and familiar object, there is a need to define what is meant by 'exploration', and the criteria that could be adequately be described as exploratory behaviour. Also, the methods in which discrimination is measured in the spontaneous object recognition task plays a role in reducing the variance associated with the task. Typically, discrimination levels in spontaneous tasks are measured using the D1 and D2 ratios (Ennaceur and Delaceur, 1988). The D1 is the difference in time spent exploring the novel and familiar objects, whereas the D2 is the difference between exploration of the novel and familiar object divided by the total time spent exploring the

novel and familiar object. The D2 ratio is generally thought to be a more reliable indicator of discrimination compared to D1, because it corrects for total exploratory activity (Ennaceur and Delaceur, 1988). The resulting discriminatory ratio from the D2 calculation will fall within the range of -1 and +1, with +1 being absolute preference for the novel object and -1 being absolute preference for the familiar object.

Also, because the spontaneous object recognition task is driven by natural exploratory behaviour, task performance is highly reliant on the animals' state during testing. To illustrate this, when animals are repeatedly handled during the task; where the animals are constantly being placed into and taken out of the open field during the start of sample, during the retention interval and test (total of 4 times each trial). The stress caused by repeated handling may influence exploration and disrupt performance (Yuan et al., 2009). To illustrate, when an animal is repeatedly taken in and out of an arena, stressed induced neophobia (Ennaceur et al., 2009) may drive exploration away from the novel object, thus masking the animals' recognition capabilities. Hurst and West (2010) provided evidence for this, showing that the standardised method of handling mice (by the tail) induced higher stress response in the elevated plus maze task compared to using the tunnel or cup methods.

The spontaneous object recognition task have been instrumental in the investigations of object recognition memory in rodents, but this advantage was further extended when the task was developed to test other more complex forms of recognition memory. Variants of the spontaneous object recognition task that included the investigations of locations, contexts and combinations of location and contextual representation have further contributed to understanding the underlying neural mechanisms of recognition memory (Dix and Aggleton, 1999; Eacott and Norman, 2004; Langston and Wood, 2010; Norman and Eacott, 2004).

### 1.7 Object location task (object-place)

The object recognition task was modified to test spatial location memory in animals (Ennaceur et al., 1997; Dix and Aggleton, 1999). There are several versions of the task that test spatial location in recognition memory; in the version by Ennaceur and colleagues (1997) a pair of identical objects ('A1' and 'A2') were placed in the top left and top right corner of the arena. After a retention interval, the objects are replaced with an identical copy of object 'A' ('Af' and 'An'; where 'f' represents the familiar location and 'n' is representative of the novel location) where 'Af' is placed in a location previously seen by the animal (top left) and 'An' in a new location (bottom right); as a result, the animal should spend more time exploring object 'An' which is in a novel location (figure 1.1). In a different version of the spatial location task (figure 1.1), the object-in-place task (Dix and Aggleton, 1999), rats were presented with four different objects ('A1', 'B1', 'C1', 'D1'), each located in four different corners of the arena. Following a retention delay, the animals were then reintroduced to the arena containing copies of the objects presented at sample, except the location of two objects were swapped. The animals are predicted to preferentially explore the objects that had swapped locations compared to objects that were in the same locations.

This task was later simplified (Ameen-Ali et al., 2012; Davis et al., 2013; Eacott and Norman, 2004), presenting only two objects at sample ('A' and 'B'), then two copies of the familiar object at test ('Af' and 'An'). Object An would be in a novel location (where object B was previously seen) and the animal would be driven to explore the object in a novel location (An) over the object in the familiar location (Af), figure 1.1.

The extent to which the object-location task relies on the hippocampus have been called into question, with some studies reporting performance deficits after hippocampal/fornix lesions (Ennaceur et al., 1997; Mumby et al., 2002; Save et al., 1992), but other studies reported successful performance in the task (Langston and Wood, 2010; Eacott and Norman, 2004). Langston and Wood (2010) proposed that the conflicting

findings may be a result of procedural differences. To examine this, they compared performance of animals in an allocentric and egocentric version of the object location task. Rats with hippocampal lesions were impaired when the task required allocentric strategy; but had intact performance when the task required egocentric strategies. They further explained that animals may have used egocentric representation to solve the task (whereby the animal discriminated the left-right locations of the objects based on the animals starting point) which is not hippocampal dependent (Eichenbaum et al., 1990); and when the animal is required to use allocentric strategies (due to having different start points at sample and test), the object-location task then becomes hippocampal dependent.

### 1.8 Cellular representation of the object recognition and object location task

Electrophysiological recording studies in monkeys (Miller et al., 1996; Ringo, 1996; Brown and Xiang, 1998; Brown et al., 2010) and rats (Zhu and Brown, 1995; Zhu et al., 1995) have found the presence of a population of neurons in the perirhinal cortex which is involved in visual recognition memory. These neurons (up to 25%) have been shown to respond less following subsequent presentation of the previously encountered novel stimuli (Brown et al., 1987; Fahy et al., 1993; Li et al., 1993; Miller et al., 1993; Sobotka & Ringo, 1993; Xiang and Brown, 1998), thus indicate their suitability for making familiarity judgements. The reduction of neuronal response following repeated presentation of stimuli have been shown to be maintained for more than 24 hours (Xiang and Brown, 1998; Miller et al., 1993) and is selective to the previously seen stimuli, thus indicating that these neurons carry information crucial to recognition memory (Brown and Xiang, 1998).

To date, no electrophysiological study examined the role of the CA1 hippocampal subfield on the object-location task used to investigate the memory of object location in this thesis. However, studies examining firing response of hippocampal neurons in rats

have found that the firing rates of neurons in the CA1 altered when objects were displaced to a novel location (Lee and Park, 2013; Kim et al., 2011; Larkin et al., 2014) and selective inactivation of CA1 have resulted in memory impairments in tasks of spatial novelty detection (Barbosa et al., 2012; Lee et al., 2005). Aside from the hippocampus, studies have examined the role played by the anterior cingulate cortex (Weible et al., 2009) and lateral entorhinal cortex (Deshmukh and Knierim, 2011) on object-location memory.

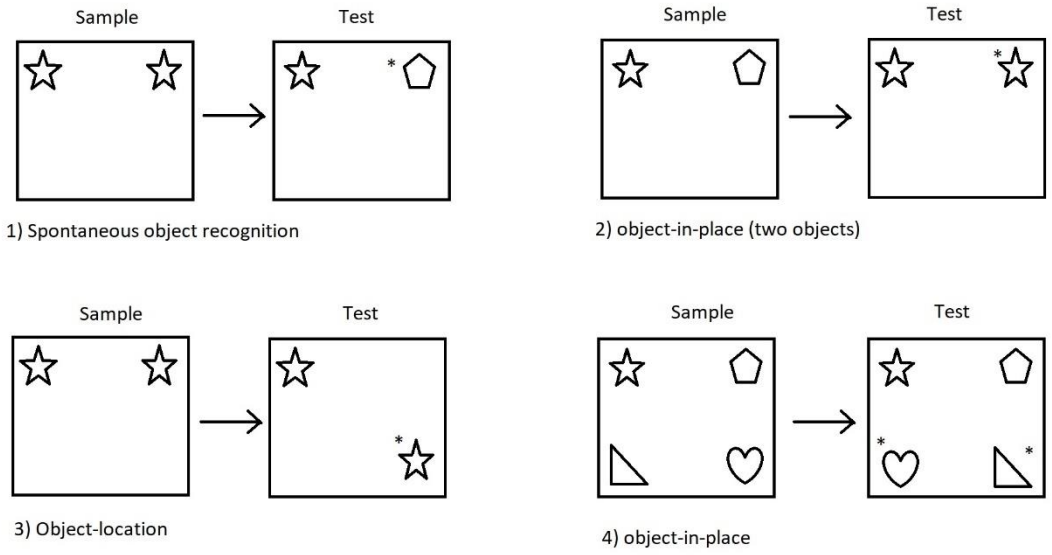



*Figure 1.1* represents the four different spontaneous object recognition tasks in the open field. The figures represent a single trial, which consists of a single sample and test phase, separated by a retention delay. The asterisk represents the novel object or the novel location configuration of an object in the test phase which the animal should preferentially explore. 1) Spontaneous object recognition (SOR) where the familiar object is swapped with a novel one; 2) Object in place with two objects; 3) object-location, where an object is placed in a novel location in the test phase; and 4) object-in-place with four objects, where the locations of two objects are swapped during the test phase.

### 1.9 Current solution to spontaneous object recognition task and its complex variants

The need to address weaknesses of the spontaneous object recognition task has prompted Albasser and colleagues to introduce the bow-tie maze in 2010 (figure 1.2). The bow-tie maze was developed to primarily address two shortcomings of the spontaneous object recognition task: 1) the time-consuming data collection when proper counterbalancing is considered; and 2) the repeated handling rodents receive during the task. This task combines the multiple trials of the DNMS task and the spontaneous free exploration of the spontaneous object recognition task. The apparatus is shaped like a bowtie consisting of two compartments separated by a sliding door. The animal is initially placed in one compartment containing object A. After one minute, the door opened, and the animal is allowed to shuttle into the opposite compartment containing object A (familiar) and object B (novel). The animal should demonstrate preferential exploration towards the novel object B. The door reopens after 1 minute and the animal returns to the initial compartment, now containing object B and object C. Object B now acts as the familiar object and object C is novel. Food rewards are placed in a well hidden under the objects and rats are required to displace the objects to obtain food rewards.

Research using the bow-tie maze has brought improvements in investigations using the spontaneous object recognition task. For example, this apparatus was used in investigations into the mechanisms of perirhinal cortex (Albasser et al., 2011; Albasser et al., 2015), proactive interference (Albasser et al., 2015), different lighting conditions (Albasser et al., 2011) of recognition memory. Aside from the spontaneous recognition task, the bow-tie maze was also used in investigations of spatial (object-in-place; Nelson and Vann, 2014) and temporal order/recency memory (Olarte-Sanchez et al., 2014; Kinnavane et al., 2014). The novel object of trial 1, served as the familiar object of trial 2. The time taken to run a single trial, with 1-minute trial length and short retention delays, meant that the time to complete a 16-trial session would take approximately 17 minutes.

Also, rats do not learn the non-matching to sample rule in this maze because both objects are baited with food reward. Despite the advantages of the bow-tie maze, it is difficult to run tasks that incorporate spatial representations, such as the location-context task (Easton et al., 2011) and episodic-like memory task examining the memory of object, location and context (Eacott and Norman, 2004).

So, to address the weaknesses of the bow-tie maze, Ameen-Ali et al., (2012) developed an E-shaped maze (figure 1.3) distinct from the bow-tie maze by having two chambers (arenas) which served different purposes: a holding arena and an object arena. The separation of the testing and holding chamber would make it easier to incorporate tasks requiring spatial and/or contextual information. Unlike in the bow-tie maze, animals are able to apply egocentric or allocentric strategies to spatial tasks. Furthermore, the apparatus was designed with a rotatable object arena containing four distinctive contexts, allowing tests which rely on contextual cues. Both compartments are separated by three doors, a central door and two side arm doors.

At the beginning of the testing session, the rat is placed in the holding arena, the central door opened and the rat shuttled into the object arena containing a pair of identical objects. After two minutes of exploration, the side arm doors open to allow the rat to return to the holding area for 1 minute. During this time, the experimenter would swap the objects around in the object arena to prepare for test. The central door opened and the rat is now presented with a copy of the familiar object and a novel object. At the end of the test phase, the animal returns to the holding area via the side arm doors. During the 1-minute inter-trial interval, the experimenter sets up another set of objects to prepare for the next trial. Two food pellets were placed close to the familiar and novel objects as motivation to encourage rats to actively explore objects. The continual trials apparatus provided a method to directly compare the object location task with previous studies using the task (Davis et al., 2013; Dix and Aggleton, 1999). Thus far, the continual trials apparatus was

successful in validation of the spontaneous object recognition, object-location and object-context task (Ameen-Ali et al., 2012). Recently, Seel et al., (2017) developed a variant of the continual trials apparatus (figure 1.4) which was used to examine the cholinergic pathways in the hippocampus and its effects on the what-where-which (episodic-like memory) task and where-which (location-context) task.

The ability to run multiple trials within a testing session without the repeated handling resulted in a methodology with increased sensitivity and power (Ameen-Ali et al., 2012). Because the task is run in a similar way to an open field, whereby testing takes place in a single compartment, performance of animals in the continual trials apparatus could be compared to findings from previous studies, and Ameen-Ali found that performance of rats in the continual trials apparatus was comparable to performance of rats from Norman and Eacott (2005) study. The development of the continual trials apparatus has been shown to reduce the number of rats used in spontaneous tasks by 50% whilst maintaining statistical power comparable to previous studies running one trial a day (Ameen-Ali et al., 2012).

The continual trials approach to testing spontaneous recognition tasks has been proven to be a useful improvement to the investigations of spontaneous object recognition task and its complex variants (Ameen-Ali et al., 2012; Seel et al., 2017). However, to fully capitalise on the advantages of the continual trials apparatus, especially in the field of neuroscience research, the continual trials task should be extended to test spontaneous object recognition in mice.

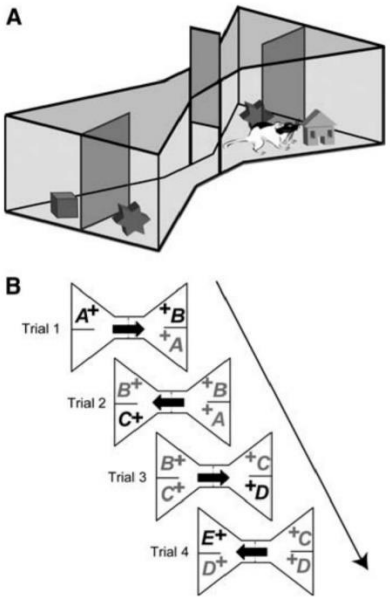



*Figure 1.2* depicts the bow-tie maze and the general procedure of task. The animal was initially placed in a compartment containing object 'A' (sample phase) and after a retention delay, objects 'A' and 'B' (test phase) and this procedure is continued until the end of the testing session. The arrow within the bow-tie maze represents the movement of the animal in between compartments. Image obtained from Albasser et al., 2009.

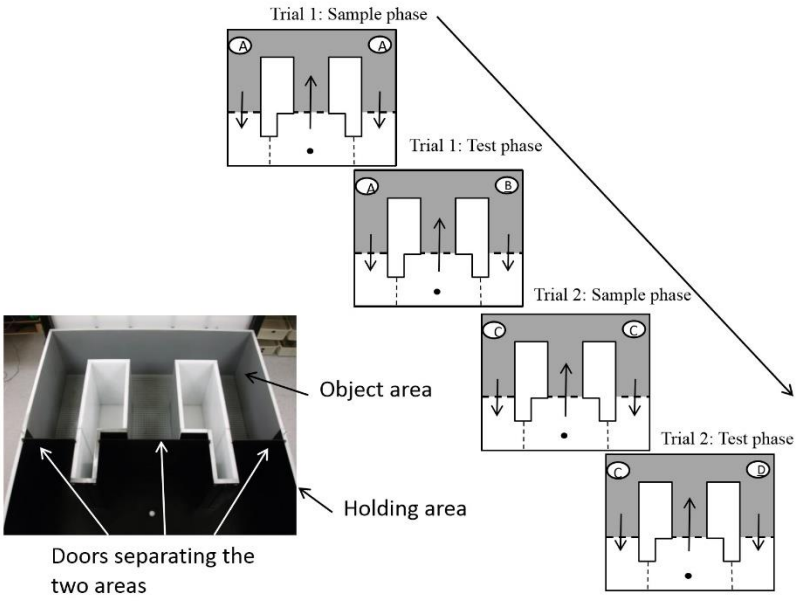



*Figure 1.3* represents the photograph and schematic diagram of E shaped continual trials apparatus. The animal starts the trial in the holding area (white area in the diagram and black area in the photograph), the central door opened, and the animal shuttled into the object area (grey area) which contained a pair of identical object. After exploring the objects, the side arm doors opened, and the animal returned to the holding area, while the experimenter swapped the objects in the object area to prepare for the test phase. The central door opened once more, and the animal shuttled into the object area. This time the animal is exposed to two objects, the familiar object from the sample phase and a novel object. The arrow within the continual trials apparatus represents the movement of the animals between the holding and object area. The letters represent the objects that are encountered by animals during the testing session. The image is obtained from Ameen-Ali et al., (2012).

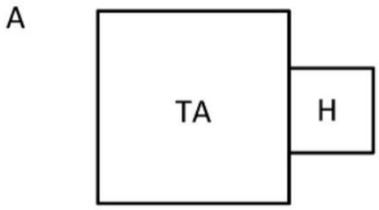



*Figure 1.4* depiction of the continual trials apparatus developed by Seel et al., (2017). The left side of the apparatus (TA) represents the test area, whereas the right side if the image (H) represents the holding area. The objects are placed in the test area.

### 1.10 How different are mice from rats?

Rodents (mice and rats) have been the most widely used models in animal research for several decades. However, the advancement in molecular and genetic research in the manipulation of mouse genome have seen the shift of biomedical research in favour of mice. Initially, rats have been the rodent of choice when studying cognition (Morris, 1984; Ennaceur and Delaceur, 1988; Save et al., 1992; Norman and Eacott, 2004). The increase in the use of rats in neuroscience research was partially due to the low cost relative to non-human primates and the efficiency involved in conducting physiological and behavioural in rodents (Jaramillo and Zador, 2014). However, in recent years, there has been an increase in the use of mice as an experimental model from 20% in the 1970's and 1980's to 50% in recent years (Ellenbroek and Youn, 2016) and was propelled by the introduction of genetic manipulation techniques in mice and the availability of hundreds of transgenic lines that target genes (Madisen et al., 2010; Taniguchi et al., 2011; Gerfen et al., 2013); which plays a critical role in understanding the neural underpinnings of behaviour and cognition. However, the rapid increase in the use of mice in behavioural research have led to the situation where mice are tested in behavioural paradigms which were originally designed for rats with little consideration about the differences between both species and using them as though they are interchangeable (Hok et al., 2016; Frick et al., 2000; Wishaw, 1995; Wishaw and Tomie, 1996; Stranahan, 2011).

One of the most common feature of found in psychiatric and neurological diseases is the impairments of cognitive abilities. Cognition however, encompasses a broad area comprising of different components and one such aspect is short-term and long-term memory. Thus far, the most commonly used paradigm to study spatial learning and memory is the Morris water maze (Morris, 1984). This behavioural assay involves the placement of an animal in a circular pool of water, and they are required to search for an invisible platform hidden underwater. A variant of the task involved placing the animal at

different start positions within the pool so they learn to use external cues to navigate and find the hidden platform. Wishaw and Tomie (1996; see also Frick et al. 2000; Stranahan, 2011) have found that when performance of mice (C57BL/6J) and rats (Long-Evans) were compared in the Morris water maze task, mice had more difficulties in learning to find the platform compared to rats. They proposed that the differences in performance was due to species differences, where rats inhabit burrow systems, which result in the competency in mazes, but also well adapted to water, which prepares rats to water-based tasks (Wishaw and Tomie, 1996). This idea was further supported when performance of mice and rats were similar on dry-land mazes; suggesting that rats and mice do not differ in spatial abilities but differences in performance were caused by non-spatial differences. In a more detailed analysis by Lipp and Wolfer (1998), it was found that mice performance is largely influenced by the extent to which mice swim at the outer walls of the pool instead of the development of a spatial learning strategy. It should be noted however, that there are strains of mice that are able to find the hidden platform better than other strains, suggesting strain-by-strain differences (Vorhees and Williams, 2015). These findings indicate that, while mice may be able to locate the hidden platform, they use different strategies in order to complete the task, hence showing that the Morris water maze task may not be a suitable test of spatial learning and memory in mice.

Aside from the performance in the Morris water maze task, there are other differences in learning exist between rats and mice, such as habituation and length of training sessions. Though not systematically studied, a small body of research have pointed out that mice take a substantially longer time to habituate to the task and often need lengthy training sessions to learn the task (Colaccico et al., 2002; Jaramillo and Zador, 2014; Prusky et al., 2000) and experience higher levels of stress and anxiety. Colaccico and colleagues (2002) reported that, in a task measuring higher-cognitive function in mice,

performance of mice over time in the task became more erratic compared to rats, hence the need to extend the trial length to one hour, with training spanning over several days.

The use of rodents (mice and rats) in research was to model aspects of human function and physiology, and most importantly to further the understanding of human diseases. However, the availability of mouse transgenic lines have greatly increased the use of mice as a model of disease in neuroscience research. With the cognitive and behavioural differences highlighted above, consideration have to be taken when translating a behavioural task initially designed for rats to mice.

### 1.11 Conclusions

The spontaneous object recognition tasks have been proven to be instrumental in the understanding of the neurobiological underpinnings of recognition memory in animals. While there are methodological issues surrounding the task (Ennaceur, 2010), the spontaneous object recognition tasks are relatively easy to administer, require no pre-training or reinforcement. This allowed the task to be administered without the concern of motivation or learning of rules.

The studies reviewed in this chapter clearly indicate that the perirhinal cortex is crucial to object recognition memory and plays some role in the representations of objects and their locations. The role of the hippocampus in object recognition memory, however is unclear, but evidence suggest that the hippocampus is not important for familiarity-based recognition.

The present chapter also reviewed the shortcomings of the spontaneous object recognition tasks and presented potential solutions to those disadvantages. The solutions presented incorporated the multiple trials feature of the delayed-non-matching to sample task and the spontaneous exploration of the object recognition task that have been

developed in rats. The continual trials approach presented in the present chapter provided a more reliable and sensitive task, by reducing the variance of animal behaviour often associated with spontaneous object recognition tasks. The advantages provided by the continual trials paradigm could be further extended to test mice, which is crucial because recognition memory is often impaired in models of diseases and the translation of the continual trials to mice would provide a more reliable method of testing recognition memory in mice, especially in diseased models and pharmacology. A further advantage of the continual trials method was that it would allow for significant improvement in terms of the 3Rs (Replacement, Reduction and Refinement), and this is important in animal research, as the continual trials approach provided a refined method of assessing recognition memory in rats whilst reducing the number of animals typically used in the task by 50% (Ameen-Ali et al., 2012). The following chapters of this thesis will present findings of validation of the continual trials approach in mice.

## 1.12 The aims and hypothesis of this thesis

The primary objective of this thesis was to improve the methodology of recognition memory tests in humans and animals. The aim was to address methodological issues often found in spontaneous recognition tasks in mice and to take an existing continual trials approach originally developed in rats and translate it in mice. The continual trials apparatus, as shown by Ameen-Ali and colleagues (2012), is a highly reliable method of assessing recognition memory in rats. This is due to the running of multiple trials within the session, which reduces the variance associated with spontaneous tasks, and this in turn reduces the number of animals used to obtain statistically meaningful results. Despite the advantages of the continual trials approach, careful considerations must be taken to successfully translate the continual trials approach to mice. In part due to behavioural differences between rats and mice; but also, because observations from previous studies (Colaccico et al., 2002) saw that mice had higher levels of stress, anxiety, and erratic behaviour. This thesis hypothesise that a smaller number of mice can be used to obtain statistically meaningful finding comparable to that of previous studies that tested recognition memory in mice using standard spontaneous object recognition tasks.

The second objective of this thesis was to further validate and generalise the novel continual trials approach in aging mice and a diseased mouse model. A series of behavioural experiments was carried out to assess the age-related changes in recognition memory (object and object-place) in ageing mice from 7 months of age and 16 months of age (Chapter 4). Following the establishment of age-related changes in recognition memory in normal ageing mice (C57BL/6J), the thesis sought to extend the functionality of the continual trials apparatus to a transgenic mouse model of Alzheimer's disease. The age-related changes of TASTPM (APPxPS1) mice recognition memory (object and object-location) was examined using the continual trials apparatus (Chapter 5).

The third aim of the thesis was to extend the applicability of the continual trials apparatus by applying the continual trials approach investigate the effects of a pharmacological substance on recognition memory in mice. The continual trials task was adapted to incorporate variable retention delays (1-, 4- and 24-hours; Chapter 6) and the newly developed method was used to investigate the role of NMDA receptors on object recognition memory (Chapter 7).

# Chapter 2

## 2.0. General Methods

This chapter details the general protocols employed in experiments in this thesis (chapters 3 – 7). Variants of methods and changes are detailed in the relevant sections.

The procedures of studies in this thesis were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, as well as the EU directive 2010/63/EU.

## 2.1. Apparatus

The apparatus used in this experiment was a rectangular arena (50cm x 42cm x 20cm) comprised of a holding area and an object arena. The two areas were divided by black guillotine doors of which the width of the outer arm doors measured at 10cm and the central arm door measured at 15cm. A schematic diagram and an image of the apparatus can be seen in figure 2.1. The doors were operated by the experimenter during the experiment to allow the animal to shuttle from the object arena to object arena vice versa. During the experiment, the objects were placed at the back-left and back-right corner of the object arena with a distance of approximately 3cm from the walls to allow optimum object exploration. Two food wells, each in holding and object area, were located in the middle of the far end walls of the apparatus in the respective areas (refer to 'black dots' in figure 2.1 left). The apparatus was made out of 10mm opal acrylic and the floors of the apparatus comprised of a grey lego$^{TM}$ surface. The apparatus was covered by a clear Perspex roof measuring at 50cm x 42 cm. An overhead camera was fixed at a height of 1.0 metre above the apparatus to provide a top-down view of the apparatus. The camera was connected to a LG DVDR recorder and a 22 inch screen to allow the experimenter to monitor the animals' activity within the apparatus.

The apparatus was placed in the far-left corner of an experimental room on a table that was 30 inches in height. The room was illuminated by diffused lighting originating from a sole table lamp equipped with a 50w lightbulb. White noise was continuously played in the background during the course of the experiment to mask any extraneous noise.

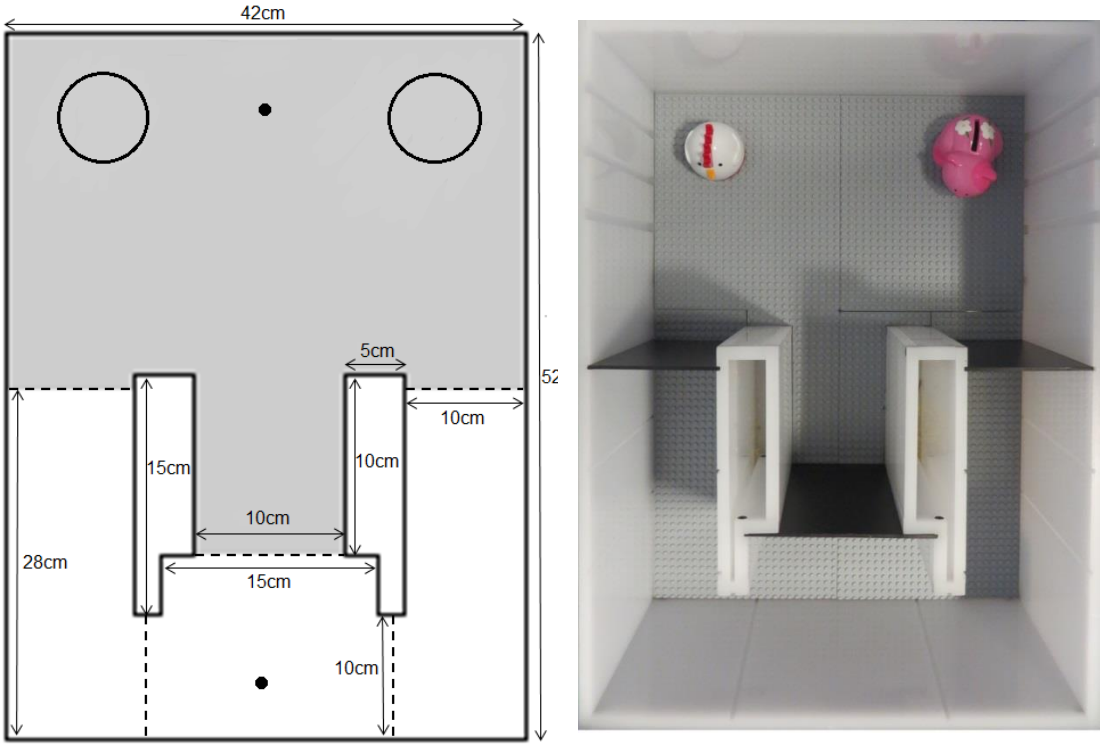



Figure 2.1: *Left* Represents a schematic diagram of the apparatus. The grey area in the apparatus represents the object arena, whereas the white area represents the holding area of the apparatus. The dotted line symbolises the doors that can be opened and closed to allow the animals to shuttle from one compartment to the next. The black dot in the apparatus signifies the 'well' in which the condensed milk solution is placed during the experiment. *Right* depicts an image of the continual trials apparatus. As shown in the image, objects were located in the back-left and back-right corner of the apparatus.

## 2.2. Objects

Various junk objects were used in experiments in this thesis, each of which had different colours, textures, shapes and sizes (examples can be seen in figure 2.2). The objects were made out of different materials, including ceramic, plastic, rubber, glass, metal and combinations of those materials. The minimum dimension of an object used in experiments was 4.5cm in height and 4.0cm in diameter; whereas the maximum dimension of an object was 17.0 x 7.5 cm. Three identical copies of objects were used in the experiment to prevent bias caused by olfactory cues and to ensure that objects were not reused during the experiment. To further reduce potential bias, the objects were wiped down with 70% ethanol wipes in between animals. Copies of objects were used once in a session and were not repeated unless stated otherwise in the method section of the following chapters.

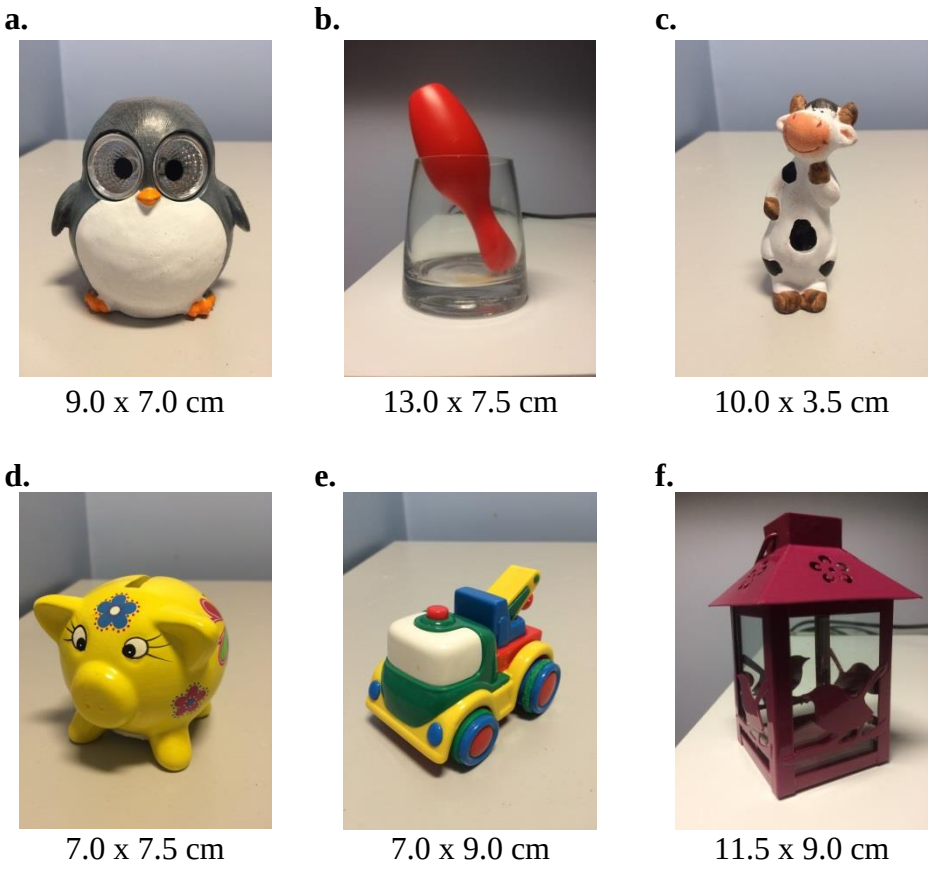



*Figure 2.2* shows examples of objects and their corresponding measurements in height and diameter. The objects were of different sizes, materials and colours. As shown, a) is a bird made out of plastic; b) is an object obtained by a combination of two objects: a plastic bowling pin and a glass cup; c) and d) are made out of ceramic or clay; e) is a truck made out of plastic and rubber and; f) is a lamp made out of metal and glass.

### 2.3. Pre-training and habituation

#### 2.3.1. Spontaneous object recognition and object-location task

After arrival, mice were given 7 days to acclimatise to their new environment prior to receiving five-minute handling sessions from the experimenter over a 5-day period. Following this, the animals, whilst in their home cages, were taken to the experiment room for 10 minutes to acclimatise to the surroundings prior to pre-training.

Pre-training consisted of four stages, with the purpose to habituate the animals to the environment and apparatus.

Stage 1 (Day 1)

Mice, together with their cage groups, were placed in the apparatus in groups to allow free exploration of the maze for 30 minutes. The side arm doors and central door was removed to allow the animals to freely explore the apparatus without any obstruction. Mice were encouraged to explore the apparatus by placement of 0.1ml (50% vol) sweetened condense milk (Nestle); milk was placed at random all over the floors of the apparatus. 1.0ml sweetened condense milk solution was allocated to each mouse. Mice progressed to stage 2 pre-training once 80% of milk solution was consumed.

Stage 2 (Day 2)

Mice were singly placed into the apparatus to freely explore the apparatus for a total of 20 minutes. Identical to stage 1 pre-training, side arm doors and the central door was removed from the apparatus and food was not replenished during this stage of pre-training. 0.1ml droplets of 50% sweetened condensed milk were placed randomly on the floors of the apparatus totalling 1ml for each mouse. Shuttling training only began when animals consumed at least 80% of the milk in the apparatus.

Stage 3 (Day 3-5)

Mice received shuttling training in the apparatus; mice were trained to shuttle between holding and object arena by manipulation of the doors by the experimenter. The animal was initially placed into the holding arena which contained a drop of sweetened condensed milk solution. Once the animal consumed the milk, the experimenter opened the central door to allow the mouse to shuttle through to the object arena. As soon as the animal entered the object arena, the experimenter shuts the central door and replenishes the food well in the holding arena. Immediately after the animal consumed the food in the object arena, the experimenter opened the side arm doors to allow the animal to come through to the holding arena to retrieve food. After the animal returned to the holding arena, the experimenter closed the side arm doors and replenished the food in the object arena. This procedure was repeated until the end of the 10-minute training session. Animals progressed to the next stage when they were able to immediately shuttle between holding and object arena within 10 seconds. Milk droplets were used as motivation to encourage shuttling, placed at areas specified in figure 1 and replenished once consumed by the animals.

The animals were given a time limit of 5 minutes to shuttle from one compartment to the next and animals that took more than 3 minutes were made note of.

Stage 4 (Day 6)

This stage involved exposing mice to objects in the apparatus. The purpose of this stage was to habituate animals to the objects in order to prevent neophobia. Mice were exposed to a pair of identical objects for 3 minutes. During this stage, mice were initially placed in the holding area, the central doors open to allow the animal to shuttle into the testing area where a pair of identical objects was placed at the far corners of the object arena with a distance of 3cm from the walls. After 2 minutes in the object arena, the side

arm doors open so the mice were able to shuttle back into the holding area where the animal will sit for 1 minute while the experimenter switched the objects. This protocol was repeated until mice exposed to all four pairs of objects. Similar to stage 3, 0.1ml droplet of condensed milk were replenished in the holding and object arena each time it was consumed by the mouse. The animals do not re-encounter the objects from stage 4 during test.

## 2.4 Task Protocol

### 2.4.1 Spontaneous object recognition task

For each animal a session constituted 16 trials. A single trial structure was as follows: sample phase, followed by a retention delay, a test phase and an inter-trial interval. A mouse was initially placed in the holding area of the apparatus. During the initial sample phase at the beginning of the session, the central door opened to allow the animal to shuttle through into the object arena of the apparatus which contained a pair of identical objects 'A' (each located at the back-left and back-right corners of the apparatus). The animals were given 2 minutes to explore the objects in the object arena. At the end of the sample phase, the side doors were opened to allow the animal to return to the holding area for 1 minute while the objects were changed to prepare for the test phase. After this 1 minute period, the central door opened once more and the animal shuttled back into the object arena of the apparatus for the test phase. During the test phase of the trial, the animal would be presented with a copy of the familiar object 'A' and a novel object 'B' for 2 minutes. After 2 minutes, the side doors opened and the animal was allowed to return to the holding area for a 1 minute inter-trial interval to wait for the next trial. This procedure was repeated for 16 trials. 0.1mL of 50% sweetened condensed milk solution was replenished in both the holding and object arena each time after it was consumed by the animal and after the animal shuttled to the next compartment (the protocol of this experiment can be seen in figure 2.3 upper).

The location of the novel objects were counterbalanced to prevent any side biases that could occur within the testing session and between animals. This was achieved by having equal numbers of the novel objects presented on the left and right side of the apparatus during test. Furthermore, objects were counterbalanced between animals to minimize possibility of exploratory behaviour driven by object salience. All possible

combinations of the objects (four in total) were worked out, and then assigned to animals in the group, for example, a group of four mice would receive the following: AA:AB; AA:BA; BB:AB; BB:BA.

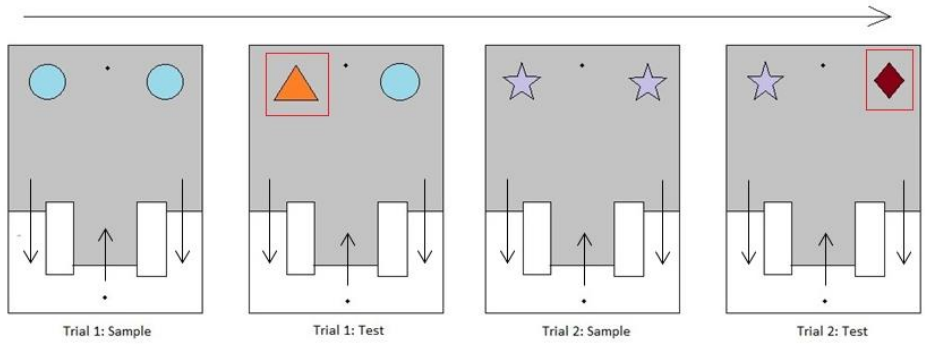
The criteria for ending the testing occurred when the animal failed to shuttle to the next compartment within 3 minutes of the door opening, or at the end of the prescribed 16 trials. If the animal failed to shuttle within the allotted time frame, the testing session would cease and the animal would be excluded from the data analysis of the experiment.

### 2.4.2 Object-location task

The animals in this experiment encountered novel and familiar objects in a 16 trial session. At the start of the session, the central door was opened so the animal could shuttle into the test area which contained novel object 'A' and 'B'. After 2 minutes of exploring the pair of objects, the side door was opened to allow the animal to return to the holding area for a 1 minute retention delay. After 1 minute, the side doors open to allow the animal to shuttle back into the test area which contained a pair of object 'A' (A and A'), in which object A was located in a familiar location and object A' at a novel location. The side doors were opened once more after 2 minutes and the animal shuttled back to the holding area for 1 minute to wait for the next trial. This procedure was repeated for 16 trials (refer to figure 2.3 lower for experimental protocol).

Similar to the spontaneous object recognition task, novel object location were counterbalanced in order to prevent biases that may occur during the session or between animals. The library of objects that were used in the studies was trial unique.
Also, if animals failed to shuttle between compartments within 3 minutes, the animal would then be excluded from the analysis.

Spontaneous object recognition




Object location

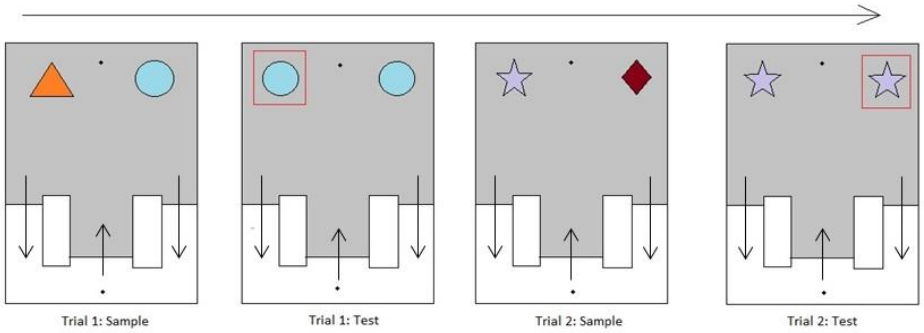



Figure 2.3 *Upper* Represents an example of the continual trials version of the spontaneous object recognition task and the object location task ***(Lower)***. The mouse begins the session in the holding arena (white area) and shuttles to the object arena (grey area) for the start of the sample phase. After two minutes of exploration, the side arm doors open to enable the mouse to return to the holding area. During this time, the experimenter changes the objects to prepare for the test phase. The central door opens to allow the mouse to enter the object arena containing a familiar and novel object. This procedure is repeated for each trial until the mouse has completed the allocated trials within the session. The shape at the back-left and –right corner represents objects from sample to test phase and the novel objects are highlighted with a red square. Both black dots in the apparatus represent food wells.

**2.5 Behavioural analysis**

Animal behaviour throughout the experiment was recorded onto a DVD and manually scored off-line using a stopwatch program (Keypad Scoring, GSK). Object exploration was measured when the nose of the animal was directed towards the object with a distance of less than 1cm or when the paw of the animal was touching the object and their nose was directed within 45° angle from the object. Exploratory behaviour was not measured when the animal was climbing or sitting on the object using it as a platform to rear upwards.

Two measures were used in the studies to determine discrimination between the novel and familiar object: D1 and D2 ratio (Ennaceur and Delaceur, 1988). D1 is obtained from calculating the difference of time spent exploring the novel object minus the familiar object.

D2 ratio was calculated by dividing the difference of the novel and familiar object exploration times with the total exploration time. The resulting D2 scores would fit into a range of value between +1 to -1, with +1 indicating total preference towards the novel object; -1 indicating complete preference towards the familiar object; and 0 showing no preference to either the novel or familiar object. D2 ratio is a discrimination measure in which D1 is corrected by total exploration time (E2) (Sik et al., 2003).

There are two methods to measure D2 over multiple trials, which includes averaged D2 and Updated D2 ratios. The averaged D2 ratio is a 'running average' of trials to the end of the predetermined number of trials. When measured this way, all trials, regardless of exploration times, have equal weighting. The Updated D2 ratio on the other hand, was the calculated D2 score on a given trial derived from the cumulative exploration times up to that trial (Albasser et al., 2010; Ameen-Ali et al., 2012). Ratios derived from this method

results in trials that are weighted differently based on the total exploration times; trials that have higher exploration times will have a higher weighting compared to that with lower exploration times. This method of measurement is more in line with labs that exclude trials with small amounts of exploration times (eg. less than 15 seconds, Frick and Gresack, 2002; Langston and Wood, 2010). However, excluding trials in a continual trials approach would remove potentially important information that may occur during the session. Therefore, the updated D2 score, that weighs trials based on different levels of exploration, is a more suitable measure in a continual trials approach than the exclusion of trials within the session. The formulae for calculating the averaged and updated D2 ratios is shown in figure 2.5.

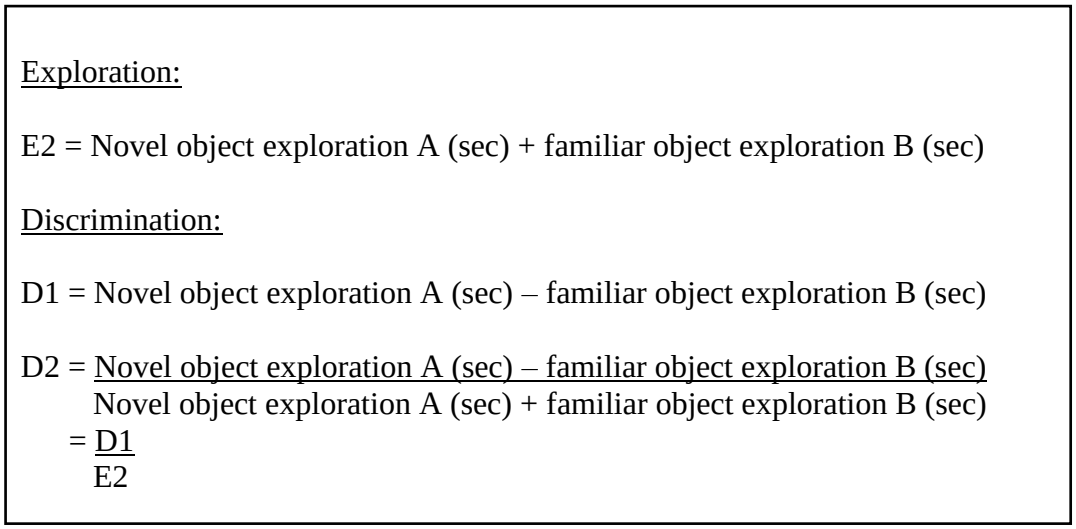



*Figure 2.4*. E2 is the total time spent exploring the familiar object (a) and novel object (b). D1 and D2 are discrimination measures to determine between the novel and familiar objects. D1 is the difference between the novel and familiar object. D2 ratio was obtained by dividing the difference in exploration times (D1) and total exploration times (E2).

Averaged D2 curve

$$T_1 = \left(\frac{D2_{T1}}{T_1}\right)$$
$$T_2 = \left(\frac{D2_{T1} + D2_{T2}}{T_2}\right)$$
$$T_3 = \left(\frac{D2_{T1} + D2_{T2} + D2_{T3}}{T_3}\right)$$
$$T_4 = \left(\frac{D2_{T1} + D2_{T2} + D2_{T3} + D2_{T4}}{T_4}\right)$$
$$T_5 = \left(\frac{D2_{T1} + D2_{T2} + D2_{T3} + D2_{T4} + D2_{T5}}{T_5}\right)$$
$$T_n = \left(\frac{D2_{T1} + D2_{T2} + D2_{T3} + D2_{T4} + D2_{T5} \ldots + D2_{Tn}}{T_n}\right)$$

Updated D2 curve

$$T_1 = \frac{D1_{T1}}{E2_{T1}}$$
$$T_2 = \left(\frac{D1_{T1} + D1_{T2}}{E2_{T1} + E2_{T2}}\right)$$
$$T_3 = \left(\frac{D1_{T1} + D1_{T2} + D1_{T3}}{E2_{T1} + E2_{T2} + E2_{T3}}\right)$$
$$T_4 = \left(\frac{D1_{T1} + D1_{T2} + D1_{T3} + D1_{T4}}{E2_{T1} + E2_{T2} + E2_{T3} + E2_{T4}}\right)$$
$$T_5 = \left(\frac{D1_{T1} + D1_{T2} + D1_{T3} + D1_{T4} + D1_{T5}}{E2_{T1} + E2_{T2} + E2_{T3} + E2_{T4} + E2_{T5}}\right)$$
$$T_n = \left(\frac{D1_{T1} + D1_{T2} + D1_{T3} + D1_{T4} + D1_{T5} \ldots + D1_{Tn}}{E2_{T1} + E1_{T2} + E2_{T3} + E2_{T4} + E2_{T5} \ldots + E2_{Tn}}\right)$$

*Figure 2.5* shows the formulae for calculating the averaged D2 and updated D2 curves within a testing session. $T_1$, $T_2$, $T_3$ represents the trial number (trial 1, trial 2 and trial 3) where Tn denotes the $n^{th}$ trial within the session. D2 represents the discrimination ratio, D1 denotes the difference between novel and familiar object exploration, whereas the E2 represents the total exploration times.

# Chapter 3

## Study 1: Validation of the continual trials apparatus in mice

### 3.1 Introduction

The present chapter of this thesis aimed to validate the mouse version of the continual trials approach to running spontaneous object recognition task and its variants. Methodological issues often associated with spontaneous object recognition tasks were identified (see also introductory chapter 1); and a solution to the shortcomings of the spontaneous object recognition task was proposed in this chapter. The continual trials approach was used to assess spontaneous object recognition and object location memory in mice. Successful adaptation of the continual trials method in mice would then suggest future potential in the field of neuroscience and pharmaceutical research.

The spontaneous object recognition task and its variants have been widely used to investigate different types of recognition memory in rodents, such as memory of an object, object-place, context-place, and a combination of object-place-context (Ennaceur and Delaceur, 1988; Dix and Aggleton, 1998; Eacott and Norman, 2004). These tasks are commonly employed as a measure of memory in the investigations of the effect of lesions (Ennaceur, Neave and Aggleton, 1997; Winters and Bussey, 2005), pharmacological substances (Fan et al., 2010; Zhao et al., 2012) and transgenes (Howlett et al., 2004; Davis et al., 2013) in rodents. Capitalising on an animals' natural preference towards novelty, object recognition memory is demonstrated when an animal preferentially explores a novel object over a familiar object. For example: A standard object recognition task consists of two phases, a sample phase and a test phase. In the sample phase, an animal is exposed to a pair of identical object 'A' (eg. a vase). Following a retention delay, the animal would then be exposed to two objects (test phase), the familiar object 'A' from the sample phase

and a novel object ‘B’ (eg. a vase and a glass bottle). At test, the animal is expected to show preferential exploration towards the novel object ‘B’ (eg. the glass bottle); indicating a form of representation of object ‘A’ (eg. the vase) in memory (Ennaceur, 2010).

Before the widespread use of the spontaneous object recognition task, the delayed non-matched to sample task (DNMS) was used to assess object recognition and to underpin the neurobiological basis of memory in animals. This task was initially used in monkeys to investigate the effects of lesions on memory (Mishkin, 1978; Mishkin and Delacour, 1975, Eacott et al., 1994) and further adapted to test rodent memory (Aggleton, 1985; Mumby et al., 1990; Mumby & Pinel, 1994; Kesner et al., 1993). However, the DNMS studies in rats have been shown to be unreliable, with some studies reporting impairments (Mumby et al., 1992; 1996), while other studies reported intact performance (Aggleton, 1985, Mumby et al., 1996; Clark et al., 2001) in the task after lesions to the hippocampus.

It should be noted that, there have been several issues surrounding the use of DNMS task in rats that may have resulted in conflicting findings. One of which requires an animal to undergo extensive training involving a high number of trials to ensure an animal learn the matching or non-matching rules (Dix and Aggleton, 1999). Also, the findings from the task would be difficult to interpret especially over long delays. This difficulty arises from having to discern if performance was a result of delay-dependent memory impairment, from the animal forgetting the non-matching rule required of the task, or the changes in motivation resulting from the presence of food reward (Clark et al., 2001). It is therefore essential to utilise a task assessing memory without the need to undergo lengthy training sessions and food reward.

The spontaneous object recognition task however, is relatively easy to administer, and unlike the DNMS task, does not require food reward and animals do not need to undergo lengthy training sessions. Also, performance levels in the spontaneous object recognition task has been consistent across species (Clark and Martin, 2005; van Goethem et al., 2012). Despite the advantages of the spontaneous object recognition task, data collection could be time consuming due to the one trial a day nature of these tasks. Furthermore, there are substantial procedural differences in which the standard object recognition has been conducted across different labs, such as, lighting conditions, food deprivation or lack thereof and the presence of white noise.

External stress inducing factors could also lead to impairments in spontaneous recognition tasks (Yuan et al., 2009; Baker and Kim, 2002). Hurst and West (2010) demonstrated that particular types of handling induced aversion and anxiety in rodents, subsequently affecting performance in behavioural experiments. For example, when an animal is rapidly and repeatedly taken in and out of the arena, the animals could suffer from stress, which in turn might drive the animals' behaviour towards the familiar object and away from the novel object, masking the animals' recognition capabilities. Baker and Kim (2002) provided evidence that when an animal was exposed to uncontrollable stress, after a 3-hour delay, recognition memory in the animals were severely impaired.

Therefore, to address the limitations of the spontaneous object recognition task, Albasser and colleagues (2010) introduced the bow-tie maze which combined features of the DNMS task (multiple trials per session) and the spontaneous object recognition task (spontaneous preference for novelty). The bow-tie maze consisted of two compartments which were divided by a guillotine door. Both compartments acted as object arenas. In brief, the rat was placed in one of the compartments which contained object 'A'. After the

rat explored the object, the door was opened, and the rat shuttled to the other compartment containing a copy of the familiar object ‘A’ paired with a novel object ‘B’. Soon after, the door was opened to allow the animal to shuttle back into the initial compartment, now containing a copy of object ‘B’ and a novel object ‘C’. This procedure was repeated for the whole session allowing 30 trials. In this study, instead of presenting a new set of objects at the beginning of each trial, the novel objects in one trial will serve as a familiar object in the following trial. To encourage active exploration of objects, rats were trained to displace objects to obtain a food reward placed in a well concealed by each of the objects. The protocol of the bow-tie maze was further modified (Albasser et al., 2010), and the one-well procedure was introduced, where food was placed in between the test objects instead of under the objects. They reasoned that the modification was examined because it would exclude exploration time that were drawn from the attempt to displace objects and the protocol would be suitable for small rodents (such as mice) that are unable to displace objects. The one-well protocol was then tested in mice. In this experiment, (Albasser et al., 2010, experiment 4) instead of food pellet, 0.1 mL of condensed milk solution was placed at the far walls of both the compartment in between the pair of objects. The one-well concept used in the experiment was especially suitable for the testing of smaller rodents such as mice because less food would be consumed and thus a smaller chance that exploration would be driven by changes in motivation as a result of food consumption. Although the bow-tie maze presented a solution to several shortcomings in the spontaneous object recognition task such as, data collection that is time consuming and considerable behavioural variance; the inherent structure of the bow-tie maze, in which the compartments are essentially a mirror reflection of each other makes the bow-tie maze paradigm inappropriate to test spatial memory because of the difficulty in discriminating allocentric and egocentric approaches which is essential in performing spatial memory

tasks (Ameen-Ali et al., 2012). Due to the difficulty in running spatial tasks, the bow-tie maze is not directly comparable to other studies of spontaneous tasks.

Ameen-Ali et al., recently (2012) introduced a new paradigm with a similar concept to the bow-tie maze by combining features of the DNMS task and spontaneous object recognition task but which allowed for the testing of not only the spontaneous object recognition, but also the variants of the task (eg. object-location task and object-in-context task). In contrast to the bow-tie maze, the continual trial apparatus consisted of a holding area where the animal was placed before the start of the session and in between trial and an object area to hold objects during the sample and test phases of each trial (see figure 1.2 and 1.3 for comparison between the bow-tie and continual trials apparatus). The two compartments in the continual trials apparatus was divided by a central door and two side doors. Several experiments were run in this study, but the general protocol was: Initially, the animal was placed in the holding area, soon after, the central door opened, and the animal shuttled into the object area containing a pair of identical objects 'A' (sample phase). After the animal explored the objects, the side doors were opened to allow the animal to return to the holding area while the experimenter changed the objects in the object area. The central door opened once more, and the animal shuttled back into the object area which now contained a copy of the familiar object 'A' from the sample phase and a novel object 'B' (test phase). The side doors were opened, and the animal returned to the holding area to await the start of the next trial. This protocol was repeated for the number of trials specified within the session. The advantage of the continual trial apparatus in comparison with the bow-tie maze was that instead of having two compartments that acted as object areas, the continual trial apparatus had only one designated object area which more closely resembles the approach used in typical spontaneous object recognition studies. This enabled successful investigation of object recognition memory and spatial

memory in the apparatus. Furthermore, performance of rats in the study was comparable to other studies of spontaneous object recognition and complex variants of the task (Ameen-Ali et al., 2013; Ameen-Ali et al., 2015), maintaining statistical power whilst using approximately 50% less animals. Despite successful investigations into different types of memory in rats, the apparatus has not been validated in mice.

Recently, there has been an increasing demand in the use of mice in scientific procedures in the UK, this is partially due to the technological advancement in mouse transgenic models. This is especially true with transgenic models of neurodegenerative diseases, whereby the spontaneous object recognition task and its variants are commonly used to tease out memory impairments of a transgenic line or to test drug efficacy (Howlett et al., 2004; Davis et al., 2013; van der Staay et al., 2011). Based on Home Office statistics (Home Office, 2014), from 2009 until 2011, the use of mice in scientific procedures were level at about 2.6 million however there was a rapid increase in the year of 2012, when the number of mice used in scientific procedures increased to 3 million, this figure was maintained in 2013. The increase was largely attributed to advances in transgenic animal models. For example, when a search was conducted on the "Science Direct" database from the year of 2014-2017 (Search terms: 'spontaneous object recognition' OR 'novel object recognition' OR 'object recognition' AND 'mouse' or 'mice'), returned 2474 journal articles. Within the first 25 of these journal articles there were a total of 860 mice being used for research involving spontaneous object recognition tasks. This means that, an upwards of 17,025 mice used for studies using spontaneous object recognition task each year. Furthermore, these statistics do not include unreported studies and studies performed in pharmaceutical companies (Ameen-Ali et al., 2012).

The current study aimed to replicate the Ameen-Ali et al., 2012 study of spontaneous object recognition task and object-location task in mice rather than rats; achieved by adapting the 'one-well concept' (Albasser et al., 2010) to Ameen-Ali and colleagues' continual trials apparatus. This paradigm would allow the testing of multiple trials within a session while measuring preferential exploration through spontaneous novelty preference in mice. Similar to Ameen-Ali et al., (2012), this continual trial apparatus consists of two compartments, namely the holding area (where the mouse held in between trials) and an object area.

The purpose of this study was to examine whether the animal reduction found in Ameen-Ali et al., (2012) study was replicable in the present study of the continual trial apparatus in investigations of recognition memory in mice. This was achieved through replicating two experiments in Ameen-Ali et al., (2012) study. Experiment 1 of this study was a multiple trials version of the standard object recognition task, whereas experiment 2 was a more complex variant of spontaneous object recognition: the object-location task (what-where) (Eacott and Norman, 2004; Langston and Wood, 2010; Davis et al., 2013).

## 3.2 Material and Methods

### 3.2.1 Apparatus

The animals in this experiment were tested in the continual trials apparatus detailed in Chapter 2, section 2.1**.** During the experiment, the objects were placed at the back-left and back-right corner of the object arena with a distance of approximately 3cm from the walls to allow optimum object exploration. The floor of the apparatus was lined with a grey lego$^{TM}$ surface. See figure 2.1 for the schematic diagram of the continual trials apparatus.

### 3.2.2 Objects

Various junk objects were used in this experiment, each of which had different colour, texture, shape and size. Multiple copies were used in the experiment to prevent potential bias resulting from olfactory cues. Animals did not re-encounter objects during a session in the experiment. (Refer to Chapter 2, section 2.2, figure 2.2 for examples of junk objects).

### 3.2.3 Pre-training

All animals in this experiment received handling and pre-training sessions detailed in Chapter 2, Section 2.3. Habituation and pre-training of animals in this experiment lasted a total of 16 days (5 day handling session; 11 day pre-training).

### 3.2.4 Behavioural analysis

Animal behaviour was recorded onto a DVD and manually scored using a stopwatch program. Object exploration was measured when the nose of the animal was directed towards the object with a distance of less than 1cm or when the paw of the animal

was touching the object and their nose was directed within 45° angle from the object. Exploratory behaviour was not measured when the animal was climbing or sitting on the object using it as a platform to rear upwards. D1 and D2 ratio were used as measures of discrimination (Ennaceur and Delaceur, 1988). Further details of the behavioural analysis were described in Chapter 2, Section 2.5.

## 3.3 Experiment 1: Novel object recognition

### 3.3.1 Subjects

Eight experimentally naive female C57bl/6j mice (Charles River, UK) were used as subjects in this experiment. The animals were housed in groups of 4 under diurnal conditions (12-hour light-dark cycle; 0700 – 1900 hours). Sawdust bedding and nesting material were provided as a source of enrichment. Behavioural testing occurred during the light phases of the day. The animals were food deprived to 90-95% of their free feeding weight and thus maintained throughout the study. Water was available ad-libitum. The animals were 11 weeks old at the start of the experiment and weighed between 17.5 and 21.2 grams.

### 3.3.2 Protocol

The protocol of the spontaneous object recognition task was detailed in Chapter 2, Section 2.4.1, figure 2.3 *Upper*. Briefly, a mouse was placed into the holding area of the apparatus for 1 minute, after which, the experimenter opened the central door, and the animal shuttled into the object area. The object area contained a pair of identical objects. At the end of 2 minutes, the side arm doors opened, and the animal returned to the holding area for 1 minute. During which, the experimenter changed the objects to a new pair of objects (a copy of the familiar object and a novel object). The central arms door opened once more, so the animal could shuttle into the object arena to explore the pair of objects. Each animal in this experiment received a testing session which consisted of 16 trials in the continual trials apparatus.

### 3.3.3 Results

Performance of novel object discrimination was determined by comparing the mean D1 measure of the group against zero (one sample two-tailed t-test), findings show that mice spent significantly more time exploring the novel object over the familiar object, mean D1 = 13.62; $t(7) = 7.52$, $p < 0.001$. Further analysis of the averaged and updated D2 ratio against zero with a one-sample (two-tailed) t-test, found that the animals performed above chance in discriminating the novel from the familiar object (mean averaged D2 = 0.46, $t(7) = 17.59$, $p < 0.001$; mean updated D2 = 0.50; $t(7) = 18.58$, $p < 0.001$). See figure 3.1.

To investigate the probability of performance level changes within the session, the trials within the session were separated into 4 blocks of 4 trials. This was achieved by obtaining the mean averaged D2 ratio for the 4 trials within each block for each animal. By conducting a repeated measures ANOVA, no block effects were found, $F(3,21) = 0.738$, $p = 0.738$, indicating no changes in the levels of performance within the session (figure 3.2).

Possible proactive interference within the session was assessed by comparing the D2 scores of trials with the lowest likely interference (trials 1 and 2) against trials with the highest likely proactive interference (trials 15 and 16) (Albasser et al., 2010). The analysis was conducted with a paired samples t-test and there was no evidence of proactive interference within the session (low proactive interference mean D2 = 0.37; high proactive interference mean D2 = 0.53; $t(7) = -1.74$, $p = 0.126$). See figure 3.3.

A post-hoc power calculation was conducted with the G*power 3.1 program to obtain the statistical power of the current experiment. Comparisons were then made to a previous study that employed the spontaneous object recognition task in mice (Sanderson et al., 2011). The effect size of the current experiment was 6.21 with a calculated power of 1.0 from a sample size of 8 subjects whilst Sanderson et al., 2011 had an effect size of 1.87 with a

calculated power of 0.99 (sample size of 11 subjects). Based on these findings, the statistical power of the current experiment (with smaller number of animals) was comparable to that of a previous study. A summary of studies and its corresponding effect sizes is found in table 3.2.

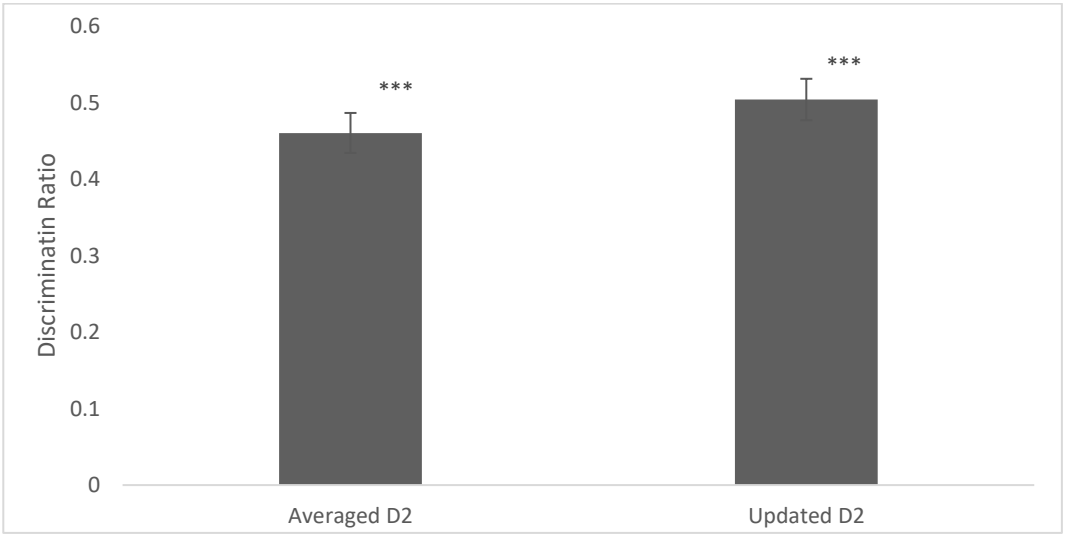



*Figure 3.1* represents performance of mice (averaged and updated D2 ratio) in the continual trials version of the spontaneous object recognition task. Analysis found that animals were successful in discriminating the novel from the familiar object. Vertical bars represent the mean and the standard error of the mean.

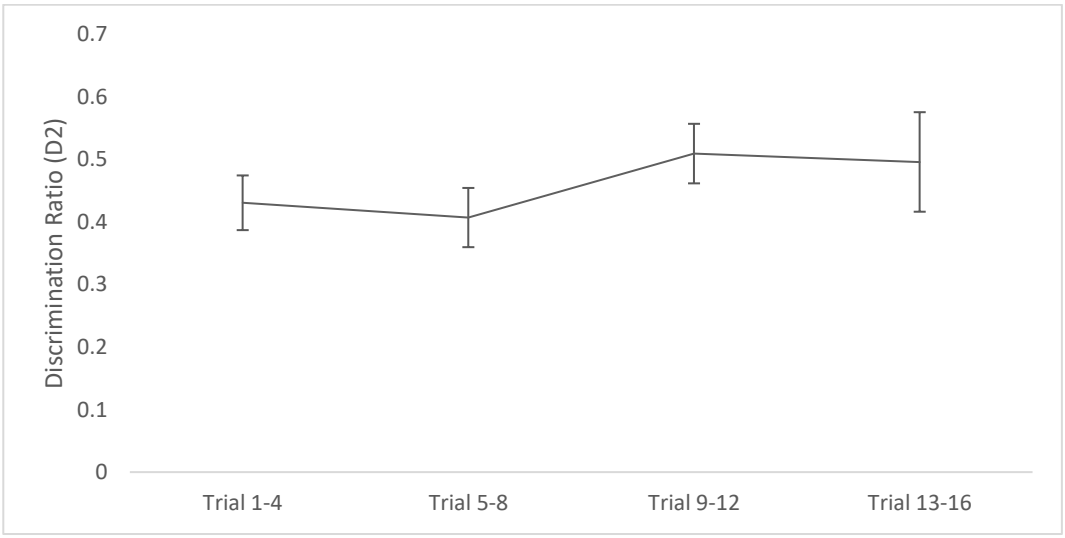



*Figure 3.2* represents performance changes across the testing session. D2 ratio averaged over 16 trials creating 4 blocks. Block effects were not found, indicating that performance levels did not change during the session. Error bars indicate the standard error of the mean.

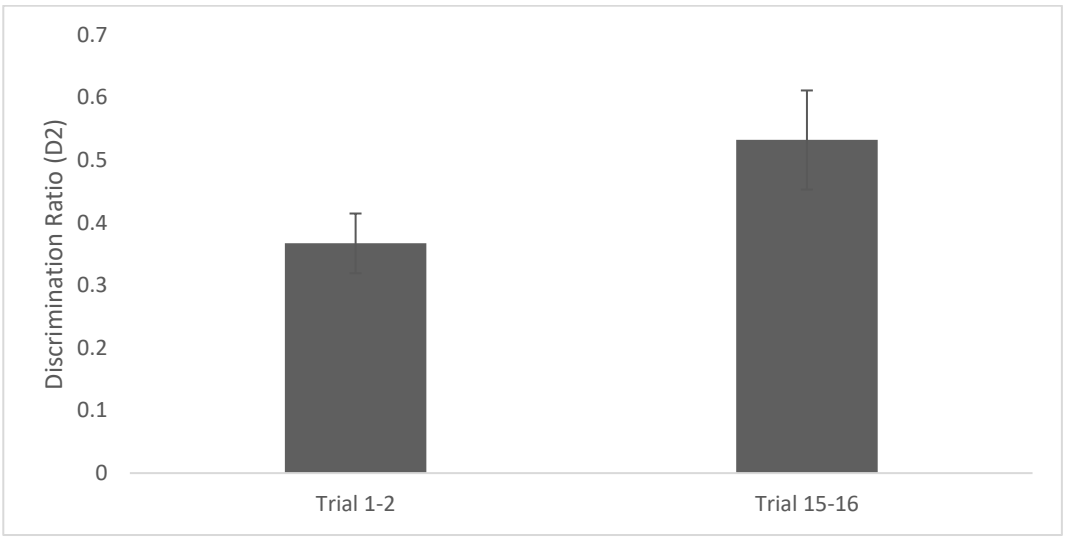



*Figure 3.3* shows no evidence of proactive interference between trials with the least likely interference (trials 1 and 2) and highest likely interference (trials 15 and 16). Bar graphs represent mean discrimination ratio (D2) between trials and error bars indicate standard error of the mean.

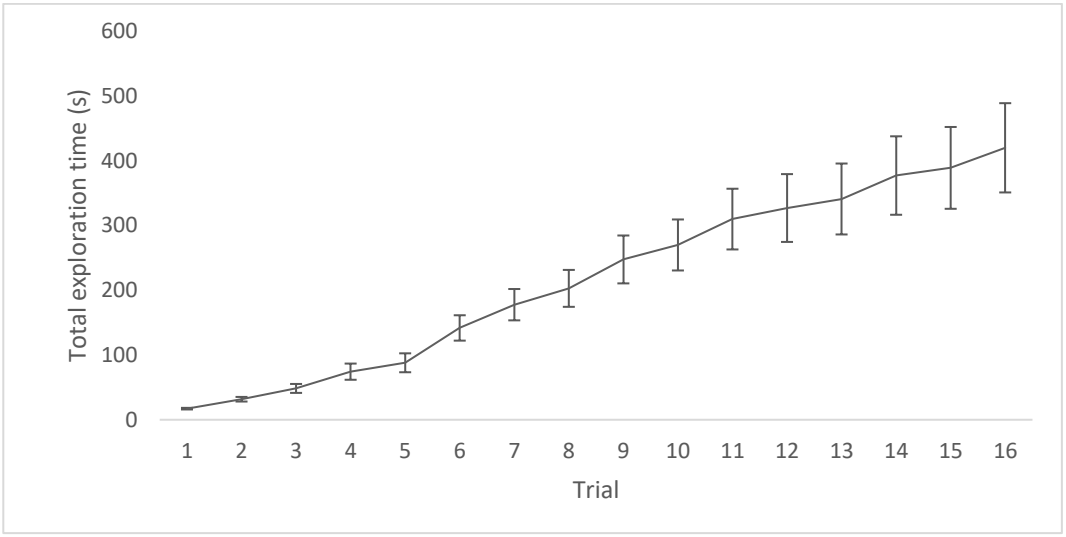



*Figure 3.4* shows the cumulative of total exploration times of test phases within the session. The graph shows a linear increase in total exploration time, indicative of continuous exploration until the end of the session. The cumulative exploration time at trial 16 was 419.76 seconds, which show that on average, animals spent 26.24 seconds exploring both novel and familiar objects at each trial. Error bars indicate the standard error of the mean.

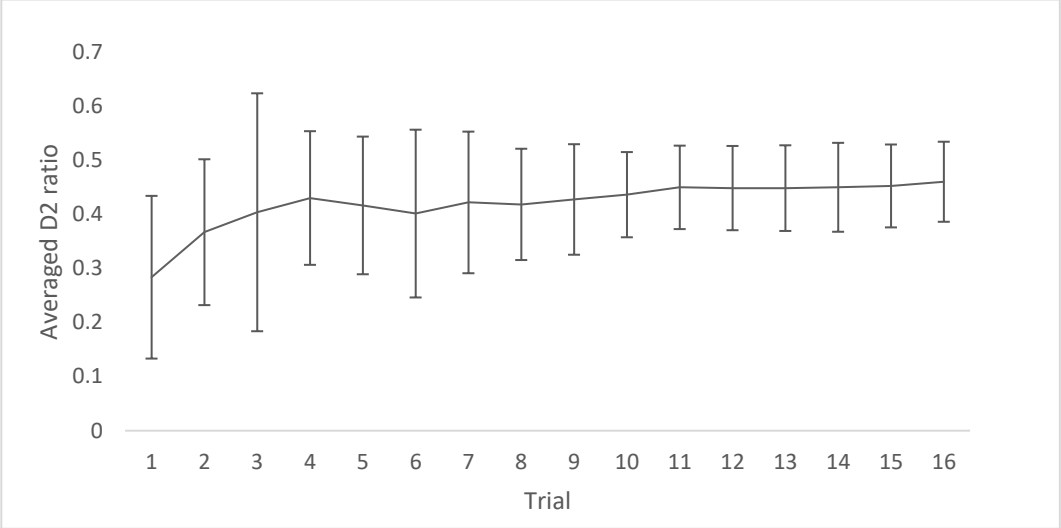



*Figure 3.5* represents averaged D2 ratio across 16 trial testing session. Performance level gradually increased and was stable until the end of the session. The averaged D2 ratios were obtained by calculating the 'running average' for each trial within the session. Mean averaged D2 at trial 16 = 0.46. Error bars indicate the standard error of the mean.

### 3.3.4 Discussion

Experiment 1 was a replication of Ameen-Ali et al., (2012; Experiment 2) study in mice. This experiment was designed to be a continual trials version of the spontaneous object recognition memory in mice. Performance of mice in the multiple trials version of the spontaneous object recognition task was comparable to previous studies of spontaneous object recognition (Ameen-Ali et al., 2012; Albasser et al., 2010; Dix and Aggleton, 1999; Sik et al., 2003; Eacott and Norman, 2004; Sanderson et al., 2011). Also, based on the power analysis (section 3.3.3), the present experiment has shown a potential reduction of 25% in the number of mice used in the continual trials approach, whilst maintaining statistical power.

Similar to Ameen-Ali et al., (2012), the performance level of the animals was consistent throughout the 16 trials session. The animals maintained satisfactory levels of

discrimination between the novel and familiar object throughout the session and demonstrated continuous exploratory behaviour throughout the testing session. There was no evidence of proactive interference build-up in this experiment. The current experiment used two different measures of discrimination: the averaged D2 and the updated D2 ratios. Based on the findings of the experiment (see figure 3.1), performance of mice using both discrimination ratios were similar, suggesting that both the averaged and updated D2 scores may be used to describe performance in the continual trials apparatus.

The following experiment (experiment 2) examined the continual trials version of a more complex variant of the spontaneous object recognition task investigating spatial memory: the object-location (What-Where) task.

## 3.4 Experiment 2: Object-location task

### 3.4.1 Subjects

Four female C57bl/6j mice used in experiment 1 served as subjects in this experiment. Housing conditions of the animals were identical to that of experiment 1. The animals were 15 weeks old at time of testing and weighed between 18.3 and 22.4 grams.

### 3.4.2 Protocol

The animals in this experiment encountered novel and familiar object location in a 16 trial testing session. At the start of the session, the central door was opened so the animal could shuttle into the object area which contained novel object 'A' and 'B'. After 2 minutes of exploring the pair of objects, the side door was opened to allow the animal to return to the holding area for a 1 minute retention delay. After 1 minute, the side doors opened to allow the animal to shuttle back into the object area which contained a pair of objects 'A' (A and A'), in which object A was located in a familiar location and object A' at a novel location. The side doors were opened once more after 2 minutes and the animal shuttled back to the holding area for 1 minute to wait for the next trial. This procedure was repeated until the number of designated trials were fulfilled. Details of the experimental protocol could be seen in Chapter 2, Section 2.4.2, figure 2.3 *lower*.

### 3.4.3 Results

To determine if the animals performed above chance in discriminating the object in the novel location over the familiar location, a one-sample (two-tailed) t-test was used to compare the group D1 scores, the updated D2 and averaged D2 ratio of the group against zero. It was found that the animals performed above chance by showing preference for objects in a novel location over objects at a familiar location (mean D1 = 2.83, $t(3) = 28.20$,

$p < 0.001$; Averaged $d2 = 0.12$, $t(3) = 5.302$, $p < 0.05$; Updated $D2 = 0.13$, $t(3) = 10.97$, $p < 0.005$). Refer to figure 3.6.

As in experiment 1, performance across the session was measured by comparing blocks of 4 trials. Blocks were obtained by calculating the mean averaged D2 ratios of four consecutive trials across all animals. A repeated measures ANOVA found that there were no performance changes during the session $F(3, 9) = 0.668$, $p > 0.05$ (figure 3.7).

As in the previous experiment, a paired samples t-test was used to analyse the presence of proactive interference during the session. Proactive interference was measured by the comparison of the first two trials (lowest proactive interference) and the final two trials of the session (highest proactive interference). There was also no evidence of proactive interference within the session, $t(3) = -0.46$, $p > 0.05$; low proactive interference mean $d2 = 0.15$; high proactive interference mean $d2 = 0.22$ (figure 3.8).

As in experiment 1, a post-hoc power calculation was conducted by using the G*power 3 program to obtain the statistical power of the object-location task in the current experiment. Comparisons were then made to previous studies employing the object location task (Davis et al., 2013). The effect size of the current experiment was 2.65 with a calculated power of 0.99 from a sample size of 4 subjects whilst Davis et al., 2013 had an effect size of 1.20 with a calculated power of 0.96 (sample size of 10 subjects). Based on these findings, the statistical power of the current experiment (with smaller number of animals) was comparable to that of a previous study. A summary of studies and its corresponding effect sizes is found in table 3.2.

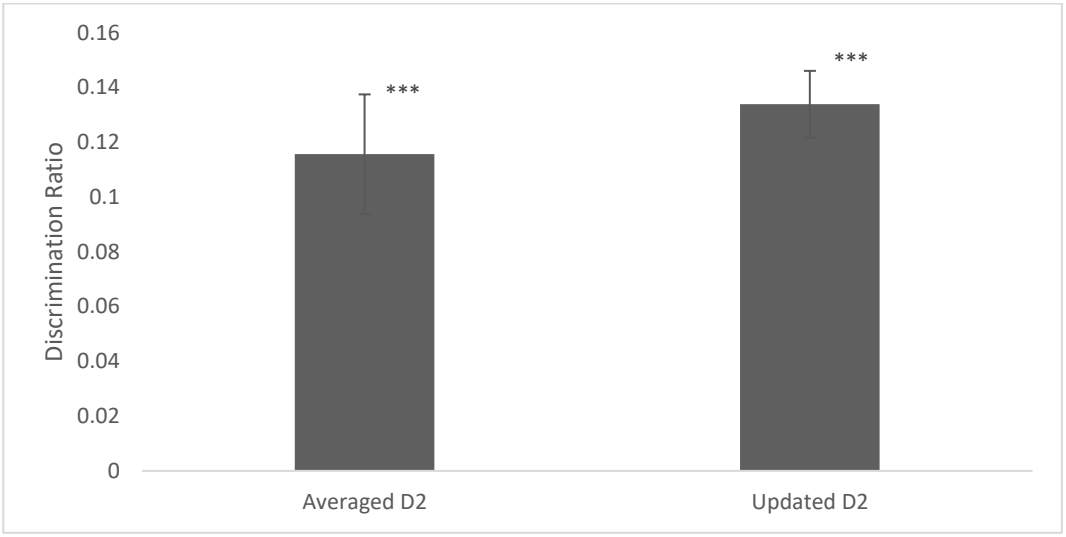



*Figure 3.6* represents performance of mice (averaged and updated D2 ratio) in the continual trials version of the object location task. Analysis found that animals were successful in discriminating the novel location from the familiar location of the object. Error bars indicate the standard error of the mean.


*Figure 3.7* represents performance level changes across the testing session. Block effects were not found, indicating that performance levels did not change during the session. D2 ratio averaged over 16 trials creating 4 blocks. Error bars indicate the standard error of the mean.

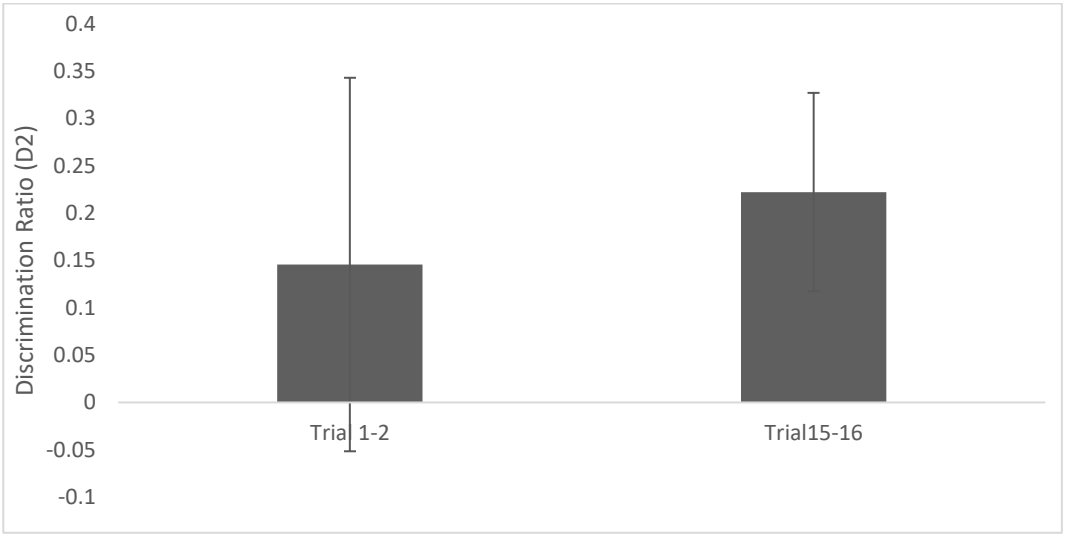



*Figure 3.8* shows no evidence of proactive interference between trials with the least likely interference (trials 1 and 2) and highest likely interference (trials 15 and 16). Bar graphs represent mean discrimination ratio (D2) between trials and error bars indicate the standard error of the mean.

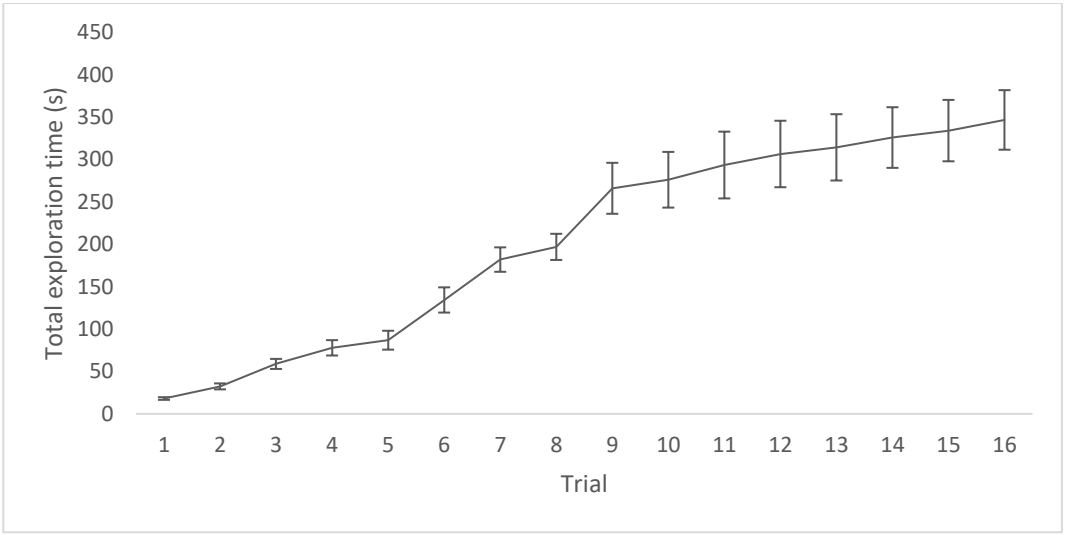



*Figure 3.9* represents the cumulative of total exploration times of test phases across the session. The graph shows a linear increase in total exploration time, indicative of continuous exploration until the end of the session. The cumulative exploration time at trial 16 was 346.69 seconds, which show that on average, animals spent 21.67 seconds exploring both novel and familiar object locations at each trial. Error bars indicate the standard error of the mean.

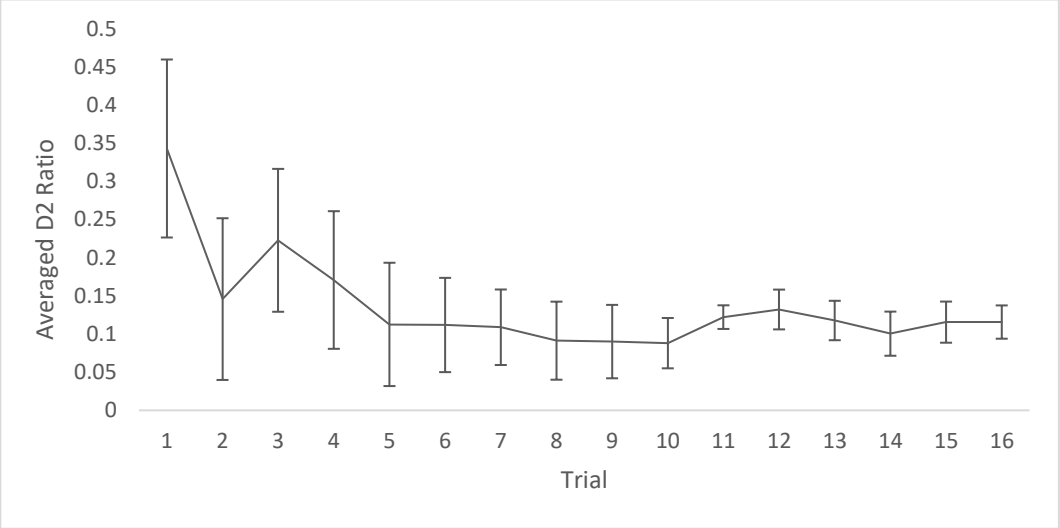



*Figure 3.10* Averaged D2 ratio across 16 trial testing session. There was a decrease in performance and performance stabilised from trial 5 until the end of the session. The averaged D2 ratios were obtained by calculating the 'running average' for each trial within the session. Mean averaged D2 at trial 16 = 0.46. Error bars indicate the standard error of the mean.

### 3.4.4 Discussion

Experiment 2 aimed to replicate Experiment 3 of Ameen-Ali et al., (2012) study. The object-location task was designed to test location memory in animals. Even with a small number of animals used in this experiment, performance was comparable to previous studies of the object-location task, with the potential to save approximately 50% of mice whilst maintaining statistical power (Ameen-Ali et al., 2012; Dix and Aggleton, 1999; Eacott and Norman, 2004; Davis et al., 2013).

There was no evidence of change in the levels of performance in experiment 2. Although the object-location task in experiment 2 was more complex, whereby the mice are required to form associations between the objects and their respective location by discriminating the familiar and novel location of the object, discrimination levels of mice remained constant throughout the session. There was also no evidence of proactive interference resulting from the presentation of multiple objects during the session. As in the previous experiment, two types of discrimination ratios were used to indicate object location memory, and performance of mice in the object-location task resulted averaged D2 and the updated D2 scores (with a mean of 0.12 and 0.13 respectively). This suggests that both discrimination ratios are suitable measures of memory in the continual trials approach to running spontaneous tasks.

| | Trial number | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Task** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
| **SOR** | 0.28 | 0.45 | 0.48 | 0.51 | 0.36 | 0.33 | 0.55 | 0.39 | 0.50 | 0.52 | 0.59 | 0.43 | 0.45 | 0.47 | 0.49 | 0.58 |
| **OL** | 0.34 | 0.15 | 0.22 | 0.17 | 0.11 | 0.11 | 0.11 | 0.09 | 0.09 | 0.09 | 0.12 | 0.13 | 0.12 | 0.10 | 0.12 | 0.12 |

*Table 3.1* details the performance level (Averaged D2 ratio) of mice in the spontaneous object recognition (SOR) and object location (OL) task across the session.

| **Spontaneous object recognition** | **Effect Size** | **Power** |
|---|---|---|
| Vogel-Cierni et al., 2013 | 1.95 | 1.00 |
| McNulty et al., 2012 | 4.89 | 1.00 |
| Balderas et al., 2008 | 9.6 | 1.00 |
| Walf et al., 2009 | 4.75 | 1.00 |
| Heyward et al., 2012 | 10.67 | 1.00 |
| Wimmer et al., 2012 | 4.00 | 1.00 |
| Fan et al., 2012 | 5.29 | 1.00 |
| Palchykova et al., 2006 | 1.68 | 0.99 |
| Han et al., 2013 | 3.2 | 0.99 |
| Wang et al., 2008 | 16 | 1.00 |

| **Object location** | **Effect size** | **Power** |
|---|---|---|
| Murai et al., 2007 | 3.2 | 1.00 |
| Assini et al., 2009 | 4.00 | 1.00 |
| Vogel-Ciernia et al., 2013 | 9.00 | 1.00 |
| McNulty et al., 2012 | 3.30 | 1.00 |
| Heyward et al., 2012 | 3.33 | 1.00 |
| Wimmer et al., 2012 | 2.5 | 0.99 |
| Fan et al., 2012 | 7 | 1.00 |
| Wang et al., 2008 | 17.5 | 1.00 |

*Table 3.2* shows a summary of studies using the spontaneous object recognition and object location task, and its corresponding effect size and statistical power. The post-hoc power calculation was conducted using the G*power 3 program.

### 3.5 General Discussion

The results of the present chapter provided evidence that performance of mice in the continual trial apparatus was comparable to previous studies (Ameen-Ali et al., 2012, Albasser et al., 2010; Dix and Aggleton, 1999; Langston and Wood, 2010) of the spontaneous object recognition and object-location task.

The main aim of the study was to validate Ameen-Ali et al., (2012) continual trials approach in mice, subsequently reducing the number of mice typically used in spontaneous object recognition type studies. The increased data collection within a single testing session could decrease the potential day-to-day behavioural noise which may affect performance and increase reliability of the task. Furthermore, the amount of stress suffered by the animals was also reduced by minimizing the amount of handling the animal was subjected to during the study (Hurst and West, 2010).

There was no indication of proactive interference build-up within this study. In multiple trials experiments (16 in this experiment), with the increasing number of objects being presented, there would be a build-up of proactive interference. Proactive interference occurs as a result of multiple presentations of objects taxing the memory load of an animal which in turn causes a reduced discrimination ratio towards the end of the experiments (Albasser et al., 2010). There was no change in the level of performance in this study. In fact, the discrimination between the novel and familiar objects was constant. Moreover, there was no difference in the performance of the animals in trials with the lowest and highest proactive interference suggesting that the testing of multiple trials within a session would not have any negative or potentially detrimental effects.

All mice in this study continuously shuttled until the end of the testing session. This was unlike what Ameen-Ali and colleagues found in their 2012 continual trials study in rats.

In their study, they found that 16% of rats ceased shuttling and were unable to complete the testing session. Mice in the present study received 11 days of pre-training to the procedures of the continual trials apparatus; whilst the rats in Ameen-Ali et al., (2012) study received 5 days of pre-training. The increased length of habituation and pre-training that mice received in this study may have reduced the chances of mice ceasing to shuttle between the compartments.

A concern relating to running multiple trial tasks in mice was whether the animals would continuously consume food throughout the testing session or performance in the task would be affected by irregular food consumption. It was observed that although all mice in this study ceased food consumption halfway (approximately during trial 8) through the testing session (and resumed in later trials), the cessation of food consumption did not in any way affect the movement of animals in between the holding and object area. Furthermore, a preliminary analysis (data not shown) has shown that task performance was constant regardless of whether mice consumed food or not. The baiting protocol used in this study was based on the one-well concept introduced by Albasser et al., (2010). Instead of individually baiting the objects with food pellets, (Ameen-Ali et al., 2012), mice in this study were baited by condensed milk solution between the objects close to the far end walls of the compartments with the aim of motivating the animal to shuttle in between areas. Albasser et al., (2010; experiment 3) compared the difference of the animal performance when using the one-well (the bait was placed in between the objects at the far wall of the compartment) concept and two-well concept (where the objects were individually baited) protocol and found that there was no difference in performance between the animals were assigned to 'one-well' or 'two-well' protocol. This showed that the animals still spontaneously explored the object even if the objects were not baited. Similar to Albasser et al., (2010), the one-well protocol was successfully applied to the experiments; the mice in this study had a strong preference for novelty even though the objects were not individually baited. The one-well

protocol was designed to be suitable for running multiple trials for smaller rodents such as mice, and potentially transgenic mice models.

A potential of the continual trial apparatus was demonstrated in Albasser et al., (2010) study which involved manipulating retention delays of the trials within the session. This consisted of investigating the effects of various retention delays on rodents' memory. By implementing delays into the continual trial apparatus, there is potential for the continual trial apparatus to be used to investigate the effects of drugs at different time points.

In conclusion, this study presented a continual trial apparatus adapted from Ameen-Ali et al., (2012) that was successfully developed for mice. This study examined various tasks of recognition memory typically utilised in rodent literature. It should be noted that the number of animals tested in the continual trial apparatus was vastly reduced compared to other literature and the animals continually explored the objects even after exposed to multiple trials. These findings suggest that the continual trial apparatus was successfully adapted to mice in a range of recognition tasks and the apparatus has potential to reduce animal numbers used in spontaneous type tasks and speed up data collection.

Following the successful validation of the continual trials approach in object recognition and object location memory in mice, the next chapter of this thesis aimed to examine performance of aging mice in the spontaneous object recognition and object location task using the continual trials approach.

# Chapter 4

## Study 2: Effects of ageing on recognition memory in the continual trials apparatus.

### 4.1 Introduction

Ageing is associated with cognitive decline, particularly in the ability to exhibit rich contextual representations of a memory (Johnson et al., 2017; Cansino, 2009). This has been found to be also true in animals, where monkeys, rats and mice show a gradual decline in the ability to perform tasks involving episodic like memory and spatial memory (Cavoy & Delaceur, 1993; Robitsek et al., 2008; Hernandez et al., 2013; Aggleton et al., 1989). Performance impairments were found in the delayed non-matching to sample (DNMS; described in chapter 1) task in both aged monkeys (Rapp and Amaral, 1991; Shamy et al., 2006) and rats (Aggleton et al., 1989). Aged rats were also found to be impaired in a spatial variant of the DNMS task, delayed non-match to place (DNMP) when compared to young rats (Dunnett et al., 1988; Aggleton et al., 1989). When tested in the standard object recognition task, aged rats were found to perform no differently from young rats (Cavoy and Delaceur, 1993). However, at delays of more than 15 minutes, aged rats were found to be impaired in the task (Bartolini et al., 1996; Burke et al., 2010). Aged rats were also found to be impaired in various spatial tasks such as the Morris water maze task (Aitken & Meaney, 1989; Gage et al., 1989; Ando & Ohashi, 1991; Joyal et al., 2000).

A vast majority of animal studies investigating the effects of age on cognitive performance utilised a cross-sectional design; in which the performance of a group of young animals is compared to an aged group. Furthermore, animals in both the young and aged group are often matched in their experience levels. This calls into question the validity of the paradigm and transferability of those findings to human studies, which usually comprised of a cross-sectional design comparing older adults and young adults which differ in

education, socio-economic differences, and cultural factors (Hofer & Sliwinski, 2001; Salthouse & Nesselroade, 2002; Hedden & Gabrieli, 2004).

The impairments found in longitudinal studies are often less pronounced compared to findings in cross-sectional studies (Caprioli et al., 1991). By incorporating a longitudinal approach, several studies were able to tease out behavioural performance changes within a group of animals throughout different time points of their life (Markowska & Savonenko, 2002; Joyal et al., 2000) and track the effects of prior experience on behavioural performance (Dellu et al., 1997). Therefore, utilizing a longitudinal design to assess cognitive decline in animals will allow the study to more closely resemble the levels of experience often found in the adult ageing population.

Recognition and spatial memory in this study were assessed by measuring animals' performance in the multiple trials version of the spontaneous object recognition and object-location task. The spontaneous object recognition task (Ennaceur and Delaceur, 1988; Aggleton, 1985; Ameen-Ali et al., 2012) is a two-trial test of recognition memory consisting of a sample and a test phase. During the sample phase, an animal is presented with a pair of identical objects, and after an inter-trial interval, the animal is then presented with the familiar object from the sample phase and a novel object. This task capitalises on a rodents' natural propensity to explore novelty. The object-place (Save et al., 1992; Davis et al., 2013; Langston and Wood, 2010; Eacott & Norman, 2004; Ameen-Ali et al., 2012) task on the other hand, a spatial variant of the object recognition task, is a task to measure the location memory of an object.

The experiments in the present chapter used the continual trials approach to running spontaneous tasks which have previously been validated in study 1 (see chapter 3). The continual trials paradigm allows for the testing of multiple trials of the spontaneous task within a testing session, thus resulting in a more reliable task. The advantages of running multiple trials within the session is particularly relevant for ageing studies, since many data

points are consecutively collected from a single animal, a smaller number of mice may be used to obtain meaningful results. Furthermore, reducing the handling that an animal receives in the continual trials would in turn reduce the effects of anxiety that may have an effect on task performance. Since the impairment of object recognition memory was not age-dependent (Cavoy and Delaceur, 1993) unless a delay was incorporated into the task (Bartolini et al., 1996; Burke et al., 2010), suggesting that interference may be an issue. The continual trials approach and the possible proactive interference may occur as a result of running multiple trials within a session, may serve to be as an advantage to tease out deficits in ageing mice.

Proactive interference is said to occur when prior memory conflicts with the retrieval of subsequent memory (Baddeley, 1974; May et al., 1995; Underwood, 1957) and is evident across different species (Hasher et al., 2002; Kane & Engle, 2000; Grant, 1975; Edhouse & White, 1988). Furthermore, studies also found that proactive interference disproportionately affects the older population in both monkeys and rats (Moss, Rosene & Peters, 1988; Bartus & Dean, 1979; Dunnett, Martel & Iverson, 1990). To illustrate, Moss and colleagues (1988) found that, unlike young adult monkeys, which demonstrated improved accuracy from the middle of the testing session in the 10 trial DNMS task; older monkeys failed to show similar patterns of performance, indicating a greater susceptibility to proactive interference. Animals in the current study faced two potential forms of interference. The first being having the sets of objects that were presented in the spontaneous object recognition task reused in the object location task. The second being proactive interference that may occur as a result of running multiple trials within a single session. The continual trials approach, with its increased possible proactive interference may be more sensitive to the effects of ageing because of the memory load demands.

In this study, we sought to establish recognition and spatial memory in normal ageing mice in the continual trials apparatus with a longitudinal approach. Also, this study aimed to

investigate the effects of interference on memory, both across task, when sets of objects were reused; and within the testing session, to measure proactive interference. In addition to that, we investigated the effects of prior experience on performance in both spontaneous object recognition and object-place tasks. The experiments in this study were conducted to further extend the continual trials approach to the effects of ageing and to provide a more reliable approach to investigating recognition memory in aged mice.

## 4.2 Materials and Methods

### 4.2.1 Subjects

Sixteen naïve male (n = 8) and female (n =8) C57BL/6J mice (Charles River, UK) were used as subjects in this experiment. Mice were housed singly or in groups of up to four in individually ventilated cages (IVC) under 12-hour light-dark cycle (0700-1900hours). Sawdust bedding, a cardboard tunnel, plastic igloo, and hammock were provided as forms of enrichment. Prior to habituation, all mice were food deprived to 85-90% of their free feeding weight and thus maintained all throughout behavioural testing. Water was available ad-libitum throughout the duration of the study. Animals were behaviourally tested at 7, 10, 14 and 16 months old. Mice weighed between 26.4 – 39.6 grams at the start of the experiment.

Subjects in this experiment were divided into two groups: an experienced and a naïve group. The experienced group (group 1) consisted of 12 mice (6 males and 6 females) and were tested at all ages and the naïve group (group 2) was 4 naïve mice (2 males and 2 females) that were first tested at 10 months of age. The group of naïve mice was used to assess the effects of previous experience on recognition memory performance. The naïve group was allocated a small sample size due to logistical issues.

According to the NIH Strain Survival Information (https://www.nia.nih.gov/research/dab/aged-rodent-colonies-handbook/strain-survival-information) and Turturro et al., (1999), C57BL/6J mice should show a 90% survival rate at 19 months old for males and 18 months old for females. Therefore, at 16 months of age (which is the point where animals are the oldest in this study), the mortality rate of mice should be below 10%. It should be noted however that these rates were derived from a large population and the survival rates in smaller groups will be variable. Details of exclusions and deaths in this study are listed in Table 4.1 below.

| | **Death** | **Failure to Complete Shuttling** | **Side bias** |
|---|---|---|---|
| **Experienced (N = 12)** | 1 | 1 | 1 |
| **Naïve (N = 4)** | 2 | 0 | 0 |

*Table 4.1* details the exclusions and deaths of mice at the end of the study (when the animals are 16 months old). The survival rate of the animals in this study stood at 81.25%, which was lower than the value detailed in Turturro et al., 1999.

### 4.2.2 Apparatus

As in the previous study, animals in the current study were tested in the continual trials apparatus detailed in Chapter 2, section 2.1 (refer to figure 2.1). During the study, the objects were placed at the back-left and back-right corner of the object arena with a distance of approximately 3cm from the walls to allow for optimum object exploration. The floor of the apparatus was lined with a grey lego$^{TM}$ surface.

### 4.2.3 Objects

Various junk objects were used in this study, each of which had different colours, textures, shapes and sizes. Multiple copies of 3 were used in the experiment to prevent bias

caused by olfactory cues. Animals did not re-encounter objects during a session in the experiment. (Refer to Chapter 2, section 2.2, figure 2.2 for examples of junk objects).

### 4.2.4 Behavioural analysis

Object exploration was scored when the animals' nose was directed towards the object at <1cm or when the animals' paw was touching the object with their nose directed within 45° of the object. Behaviours including sitting, climbing on and using the object as a platform were not counted as exploration. Mice behaviour throughout the experiment was recorded and scored offline using a stopwatch program. Two primary measures, namely D1 and D2 ratios were used to determine levels of discrimination between novelty and familiarity (Ennaceur and Delaceur, 1988; refer to chapter 2, section 2.5).

### 4.2.5 Pre-training and habituation

All animals in this experiment received handling and pre-training sessions detailed in Chapter 2, Section 2.3.1. Habituation and pre-training of animals in group 1 in this experiment lasted a total of 15 days (5-day handling session; 10-day pre-training); whereas naïve animals (group 2) session lasted for 7 days.

Prior to being tested at 10, 14 and 16 months, because animals were previously habituated to the apparatus, mice received a single session of shuttling training.

### 4.2.6 Testing protocol

#### 4.2.6.1 Object recognition and Object location at 7, 10 and 16 months of age

##### 4.2.6.1.1 Spontaneous object recognition task

All mice received 16-trial testing sessions at 7 and 10 months, and a 12-trial testing session at 16 months old. Briefly, a single trial structure consists of a sample and test phase with an inter-trial-interval in between phases. The central door was opened to allow the animal to shuttle from the holding area to the object area which contained a pair of identical objects (e.g. a pair of circle). After 2 minutes, the side arm doors were opened, and the animal shuttled back into the holding area for 1 minute. During this time, the experimenter would change the objects around in the object area to prepare for the test phase. The central arm door opened once more, and the animal shuttled into the object area where the animal was presented with a copy of the object from the sample phase (e.g. a circle) and a novel object (e.g. a triangle). At the end of the test phase, the side arms doors opened, and the animal returned to the holding area. This procedure was repeated until the end of the testing session. Diluted sweetened condense milk (Nestle, 0.1mL, 50% concentration) was replenished in the holding and object area after consumption by the animal (refer to figure 2; chapter 2, section 2.4.1 for further details including counterbalancing details).

##### 4.2.6.1.2 Object-location task

As in the spontaneous object recognition task, all mice received 16-trial testing sessions at 7 and 10 months and a 12-trial test at 16 months of age. Initially, the central door was opened, and the animal shuttled from the holding area to the object area. Once in the object area, the animal was given 2 minutes to explore two different novel objects (e.g. a circle and triangles). At the end of the sample phase, the side arms door opened to allow the animal to return to the holding area for 1 minute whilst the experimenter changed the objects

to prepare for the test phase. The central door opened once more, and the animal shuttled into the object area. The animal was presented with copies of a pair of identical objects (e.g. a pair of triangle) that the animal encountered during the sample phase. After exploring the objects for 2 minutes, the side arms door was opened, and the animal returned to the holding area once more. This procedure was then repeated until the end of the test session. Animals were tested in the object location task 7 days after completion of the spontaneous object recognition task. Diluted sweetened condense milk (Nestle, 0.1mL, 50% concentration) was replenished in the holding and object area after consumption by the animal (refer to figure 2; chapter 2, section 2.4.2 for further details such as counterbalancing).

The library of objects that were used in the spontaneous object recognition task was reused in the object location task, but in reverse order. For example, objects used in the SOR task were as follows: AA then AB at test; in the object-location task, the following reverse order was therefore used: BA at sample and BB at test.

#### 4.2.6.2 Investigating interference effects of reused object sets at 14 months

This experiment was designed explicitly to explore whether the reuse of objects between the spontaneous object recognition task and object location task caused interference which impacted recognition memory. The present experiment predicted that there will be some evidence of interference caused by the reuse of objects from the spontaneous object recognition task. The testing procedure of this experiment was identical to that of the previous experiments (7, 10 and 16), with the exception that all tasks ran with 12 trials in a session. The animals ran through four tasks in this experiment in the following order: (1) Object-location task with objects set A; (2) Spontaneous object recognition task with objects set B; (3) Object-location task with objects set B, but in reversed order; and (4) object-

location task with objects set C. All tasks were tested 7 days apart from each other. Details of the testing protocol of the spontaneous object recognition and object-location task can be found in chapter 2, section 2.4.1 and 2.4.2 respectively.

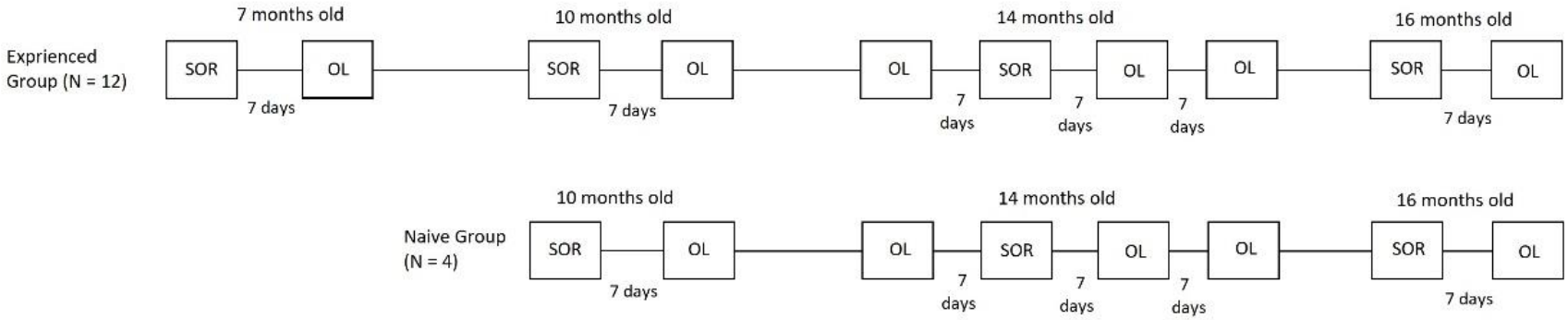



*Figure 4.1* represents the experimental timeline of the present study. Mice were separated into two groups, the experienced group (N = 12) and the naïve group (N = 4). At each age, animals were tested in the spontaneous object recognition (SOR) and object-location (OL) task. Tasks were tested 7 days apart from each other.

## 4.3 Results

### 4.3.1 Effect of age (7, 10, 14 and 16 months) on task performance (SOR and OL).

Only nine of 12 experienced mice were included in the analysis in the current section. Mice that failed to complete shuttling (N = 1), had a significant side bias (N = 1) and died (N = 1) were not included in the analysis. Due to the small remaining sample size, sex differences will not be analysed in the current study.

An Age*Task ANOVA was used in order to determine the effects of ages on task performance of the animals. An analysis of D1 measure (figure 4.2) show that there was no effect of age (: $F(3, 824) = 0.42$, $p > 0.05$), but found an effect of task: $F(1, 8) = 21.09$, $p = 0.002$. This show that animal performance did not change across ages but performance in the spontaneous object recognition task was better than that of the object location task. No age*task interaction was found ($F(3, 24) = 0.35$, $p > 0.05$).

An analysis (ANOVA) of the averaged D2 scores (figure 4.3) found an effect of age ($F(3, 24) = 5.96$, $p = 0.03$), and an effect of task: Task: $F(1, 8) = 85.84$, $p < 0.001$. A post-hoc Bonferroni pairwise comparison revealed that performance of mice at 14 months of age was impaired compared to performance at 7 months ($p = 0.022$). These findings indicate that there was an age-related change in performance of mice and performance in the spontaneous object recognition task was better than that of the object location task. However, no age*task interaction was found ($F(3, 24) = 0.29$, $p > 0.05$).

A further ANOVA was used to determine if exploration times (figure 4.4) differed across ages and task types found an effect of age-related changes to total exploration times of both objects ($F(3, 24) = 4.52$, $p = 0.012$) and also found that animals explored objects significantly less in the object location task compared to the spontaneous object recognition task $F(1, 8) = 54.55$, $p < 0.001$. Results also show that there was no age* task interaction: $F(3, 24) = 0.86$, $p = 0.474$. A post-hoc pairwise comparison of the exploration times between

all ages found that 7 month old mice explored the pair of objects significantly less than 16 month old mice ($p = 0.045$).

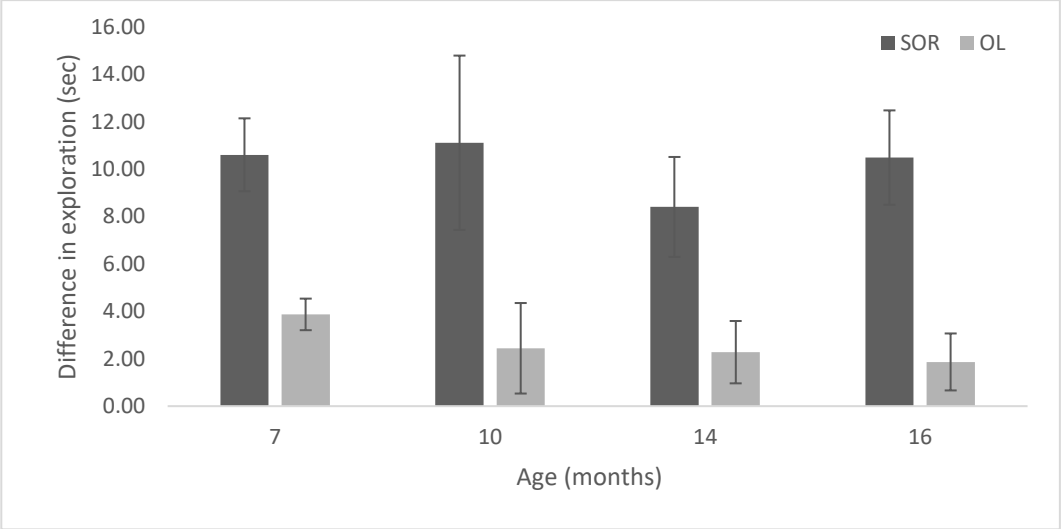



*Figure 4.2* represents mean difference in exploration (D1 scores) of mice at 7-, 10-, 14- and 16-month of age in the spontaneous object recognition and object location task based on the difference of time spent between the novel and familiar object and object-locations. No age-related decline in object recognition and object location memory was found, but performance levels of animals in the object location task was significantly worse compared to the object recognition task. The bars represent the mean and standard error of the mean.

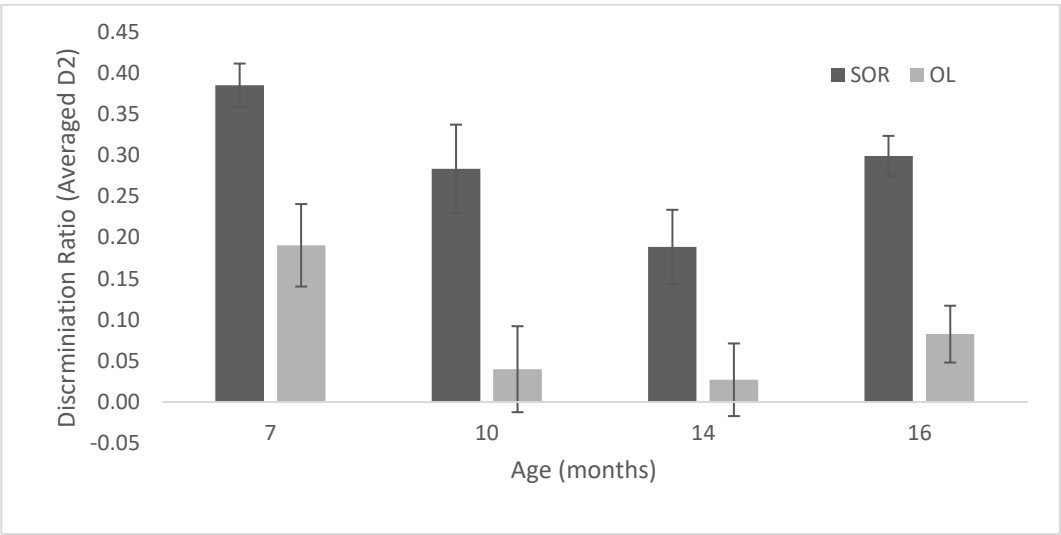



*Figure 4.3* represents performance of mice at 7-, 10-, 14- and 16-month of age in the spontaneous object recognition and object location task based on the averaged D2 ratios. There was an age-related change in object recognition and object location memory was found, with a decline in performance of mice at 14 months compared to performance at 7 months old. The findings also indicated that performance of mice was worse in the object location task compared to the object recognition task. The bars represent the mean and standard error of the mean.

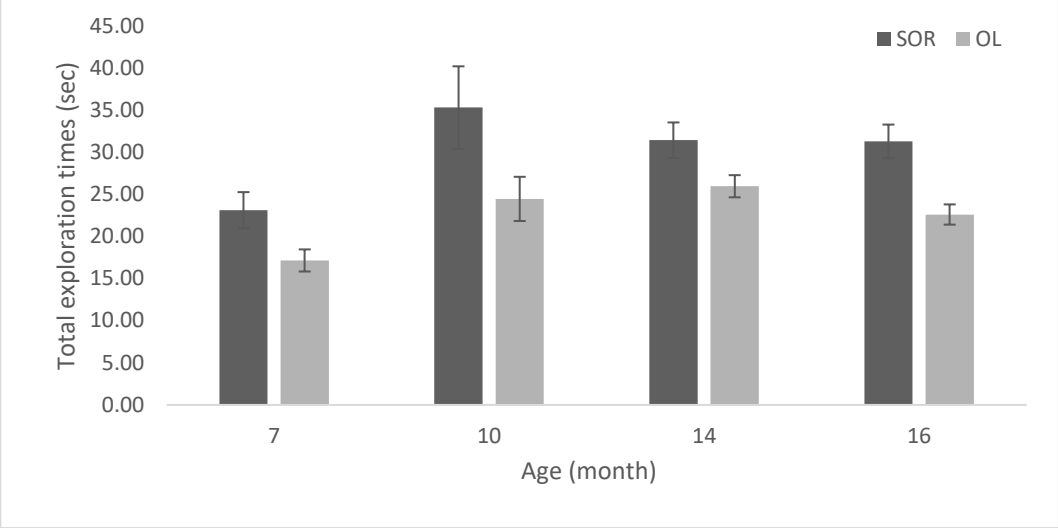



*Figure 4.4* represents the mean and the standard error of the mean of the total exploration times of mice at 7-, 10-, 14- and 16-months of age in the spontaneous object recognition and object location task. The total exploration times of mice at 7 months were lower compared to exploration times at 16 months of age. Total exploration times of mice in the spontaneous object recognition task was significantly higher than the object location task. The bars represent the mean and standard error of the mean.

### 4.3.2 Interference affected by repeated object use between tasks at 14 months old mice

Mice that completed all testing at 14 months of age were included in the analysis in this current experiment (N = 8). As in the previous section, mice that failed to complete shuttling or died during the experiment were excluded from the analysis.

In order to see whether the reuse of object sets affected animal performance in the object location task, Repeated Measures ANOVA was conducted on D1 measure (figure 4.5) and averaged D2 (figure 4.6) of the animals in the group. The results found that the reuse of objects between the tasks did not affect performance when analysis was conducted with the D1 scores: $F(2, 14) = 0.24$, $p > 0.05$ and averaged D2 scores: $F(2, 14) = 1.09$, $p = 0.362$. Further analysis using a repeated measures ANOVA on the total exploration times (figure 4.7) spent exploring the objects in the test phases of the experiment also found that exploration levels did not differ across experiments ($F(2, 14) = 2.35$, $p = 0.132$).

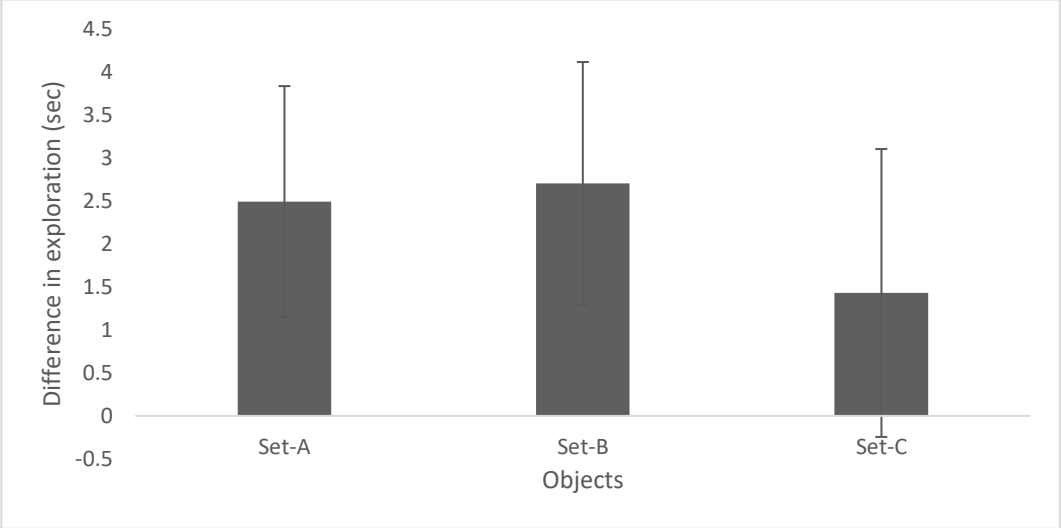



*Figure 4.5* represents the performance of 14-month-old mice when objects were reused in between tasks based on the difference in exploration between the novel and familiar location. Object Set-B was initially used in the spontaneous object recognition task then reused in the object location task of the current experiment. Performance levels between tasks did not differ from each other suggesting no evidence of interference occurring from the reuse of objects impacting the performance levels of mice. The bars represent the means and standard error of the mean.

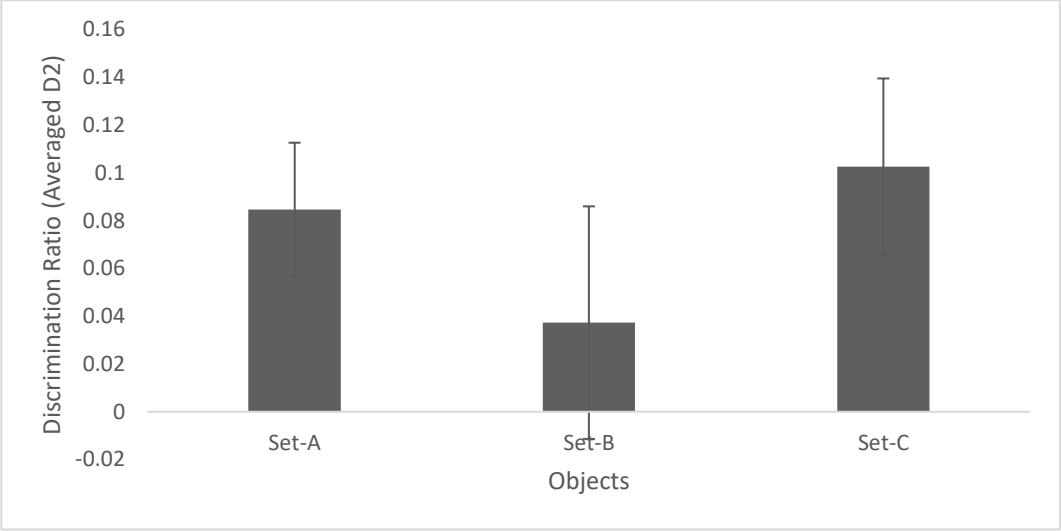



*Figure 4.6* represents the performance of 14-month-old mice when objects were reused in between tasks based on the averaged D2 ratios. Object Set-B was initially used in the spontaneous object recognition task then reused in the object location task of the current experiment. Performance levels between tasks did not differ from each other suggesting no evidence of interference occurring from the reuse of objects impacting the performance levels of mice. The bars represent the means and standard error of the mean.

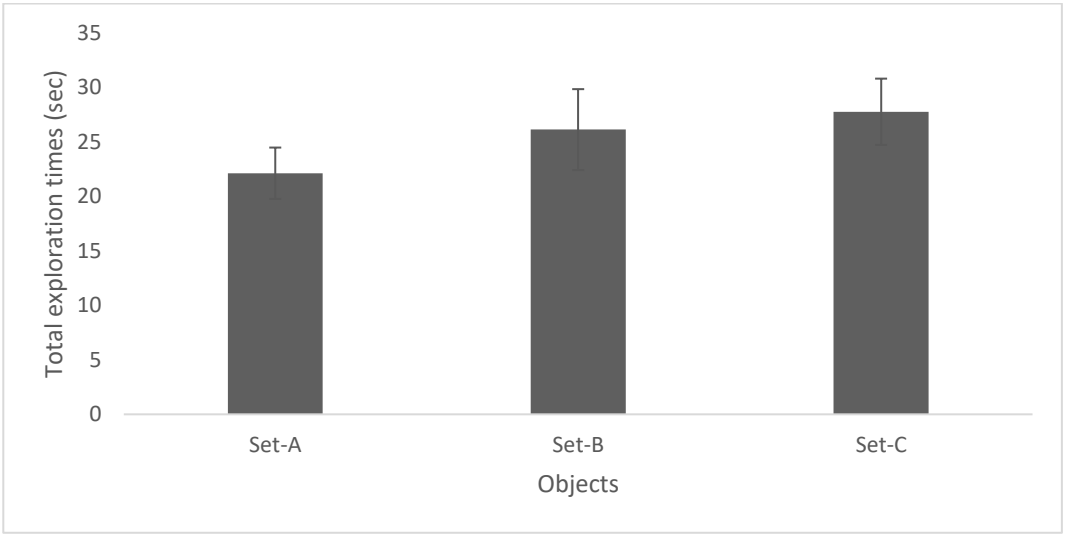



*Figure 4.7* represents the total exploration times of 14-month-old mice. The exploration levels of the group of mice did not differ between tasks. The bars represent the means and standard error of the mean.

### 4.3.3 Experience levels and performance in the spontaneous object recognition and object location task

The analysis in this section was conducted by comparing performance of the experienced (N = 12) and the naïve (N = 4) group at 10 months of age.

To investigate if experience levels affected performance of mice in the object recognition and object location task, performance of the naïve and experienced group (mean D1 scores and averaged D2 ratios) were compared using a 2x2 (task*group) ANOVA in both the spontaneous object recognition and object location task. The results found that the experience levels of mice had no effect on object recognition memory and object location memory in both the D1 scores ($F(1,14) = 1.32$, $p = 0.27$) and averaged D2 ratio ($F(1,14) = 0.15$, $p = 0.707$) (figure 4.8 and 4.9 respectively).

A further task*group ANOVA was used to analyse the exploration levels of the experienced and naïve group in the spontaneous object recognition and object location task. There was an effect of task, $F(1, 14) = 22.43$, $p < 0.001$; with mice exploring the objects more in the spontaneous object recognition task compared to the object location task. There

was no task*group interaction, F(1, 14) = 0.30, p = 0.866; suggesting that experience levels did not have an effect on performance in the spontaneous object recognition and object location task (figure 4.10).

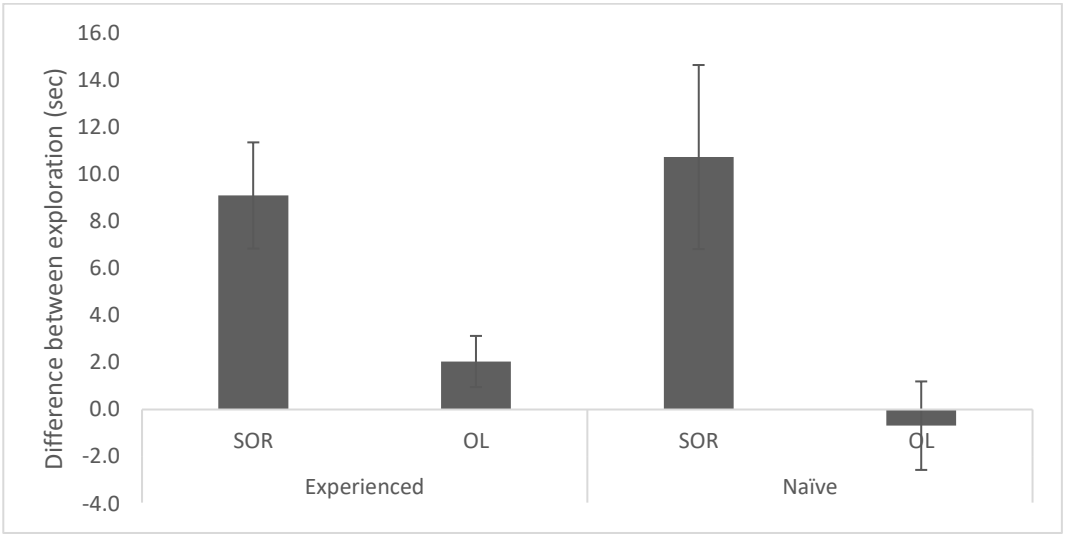



*Figure 4.8* depicts the performance levels (difference between the novel and familiar object/location) of experienced and naïve mice in the spontaneous object recognition and object location task. Experience levels of mice at 10 months of age had no effect on performance in the spontaneous object recognition and object location task. The bars represent mean and the standard error of the mean.

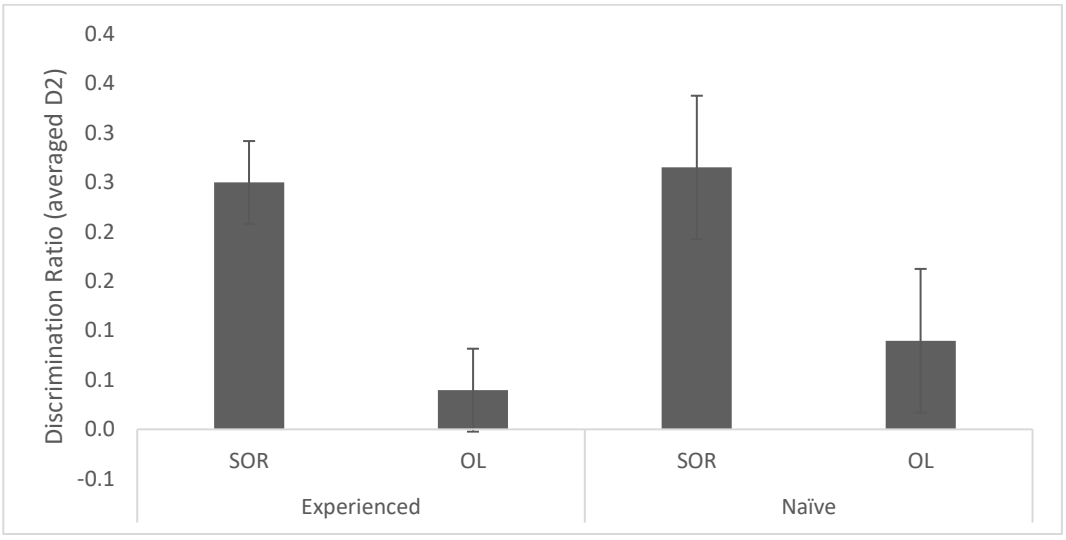



*Figure 4.9* shows the performance levels of experienced and naïve mice in the spontaneous object recognition and object location task. Experience levels had no effect on object recognition and object location memory. The bars represent the mean and the standard error of the mean.

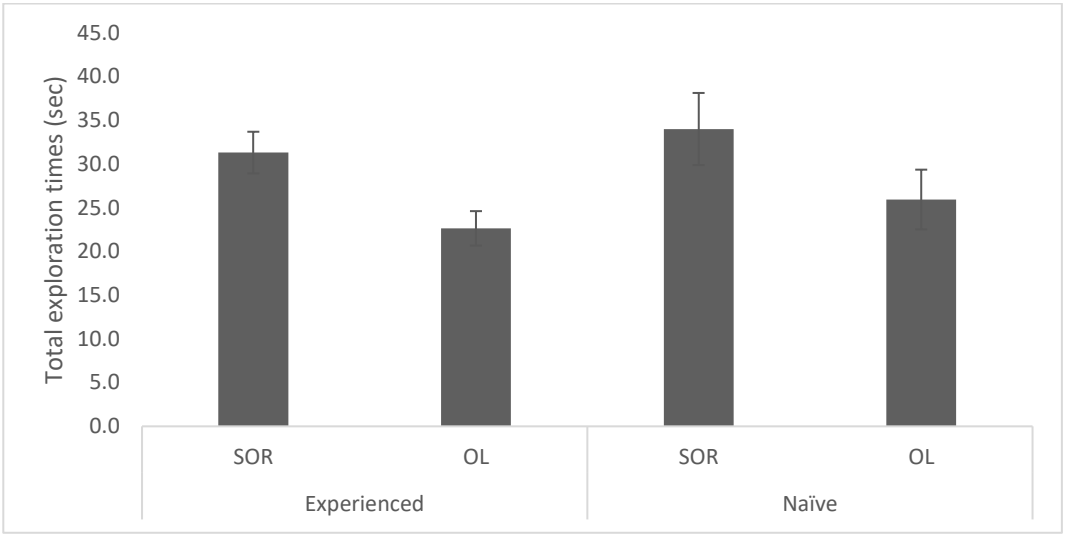



*Figure 4.10* represents the total exploration times of the experienced and naïve group in the spontaneous object recognition and object location task. Both naïve and experienced mice spent less time exploring the objects in the object location task. The bars represent the mean and the standard error of the mean.

### 4.3.4 Performance of mice at 7, 10, 14 and 16 months old in the spontaneous object recognition and object location task.

At 14 and 16 months of age, mice received 12 trials testing session instead of 16 trial test at 7 and 10 months. This change was made due to animal welfare concerns related to being placed in the apparatus for prolonged periods of time. Due to this, data from 7 and 10 months were analysed by using 16 trials and at a cut-off point of 12 trials.

#### 4.3.4.1 Spontaneous object recognition task at 7 months old

One sample (two-tailed) t-tests were used to determine if performance of mice in the spontaneous object recognition task was above chance by comparing means of D1 scores and averaged D2 ratio against zero when testing session consisted of 16 trials and at a cut-off point of 12 trials. Results show that animals were able to discriminate between the novel and familiar object at 7 months of age when the testing session was 16 trials long. D1 score: $t(11) = 7.985$, $p < 0.001$; Averaged D2: $t(11) = 14.06$, $p < 0.001$. At the 12-trial cut-off point, findings show that mice at 7 months also demonstrated object recognition memory. D1 score: $t(11) = 8.41$, $p < 0.001$; Averaged D2: $t(11) = 18.14$, $p < 0.001$ (Figure 4.11 and 4.12).

In order to investigate if animal performance changed over the session, the averaged D2 scores of all animals were separated into four blocks of 4 trials. Blocks of trials for individual animals were obtained by calculating a mean of the averaged D2 scores within that block. A test of repeated measures ANOVA found no evidence of performance changes within the testing session ($F(3, 33) = 1.532$, $p = 0.225$, refer to figure 4.13 *left*).

#### 4.3.4.2 Object location task at 7 months of age

One sample (two-tailed) t-tests were used to determine if mice performance of the object location task was above chance by comparing means of D1 scores and averaged D2 ratio against zero when testing session consisted of 16 trials and at a cut-off point of 12 trials. Results show that animals were able to discriminate between the novel and familiar locations at 7 months of age when the testing session was 16 trials long. D1 score: $t(11) = 7.18$, $p < 0.001$; Averaged D2: $t(11) = 4.32$, $p = 0.001$. At the 12-trial cut-off point, findings show that mice at 7 months also demonstrated object location memory. D1 score: $t(11) = 7.83$, $p < 0.001$; Averaged D2: $t(11) = 5.01$, $p < 0.001$ (Figure 4.11 and 4.12).

In order to investigate if animal performance changed over the session, the averaged D2 scores of all animals were separated into four blocks of 4 trials. Blocks of trials for individual animals were obtained by calculating a mean of the averaged D2 scores within that block. A test of repeated measures ANOVA found no evidence of performance changes within the testing session, $F(3, 33) = 0.497$, $p = 0.687$, see figure 4.13 *right*.

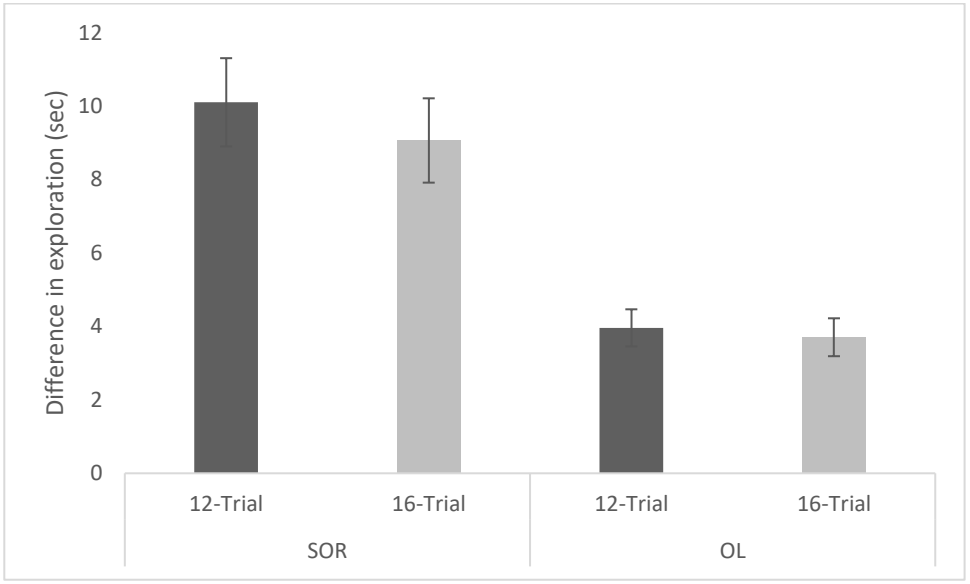



*Figure 4.11* represents the performance of 7-month-old mice in the spontaneous object recognition and object location task. The dark grey bars represent performance levels at a 12-trial cut off point, whereas the light grey bars represent performance of mice in the 16-trial testing session. The bars represent the mean difference of exploration between the novel and familiar object/object-location and the standard error of the mean.

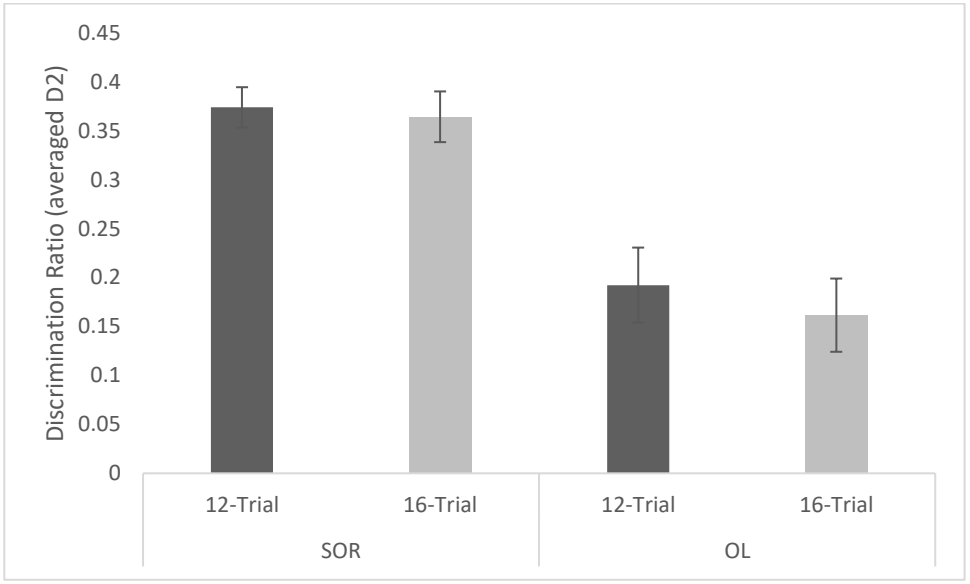



*Figure 4.12* represents the performance of 7-month-old mice in the spontaneous object recognition and object location task. The dark grey bars represent performance levels at a 12-trial cut off point, whereas the light grey bars represent performance of mice in the 16-trial testing session. The bars represent the mean averaged D2 ratio and the standard error of the mean.

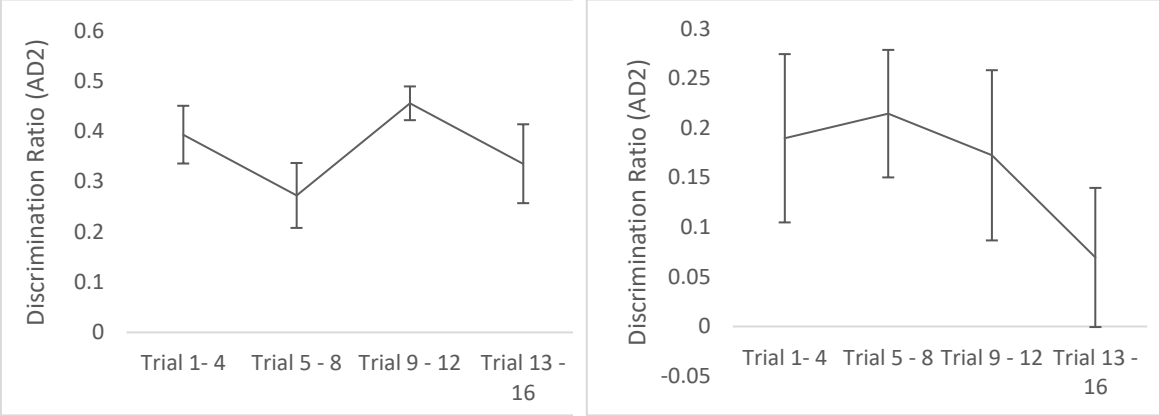



*Figure 4.13* depicts the changes in performance levels of 7 months old mice in the object recognition task (*left)* and object-location task (*right*). The blocks were calculated by obtaining the mean of the first four trials (trials 1 – 4) and each consecutive blocks of four trials thereafter until the end of the session. Performance levels of mice showed no change throughout the testing session in both the object recognition and object location task, suggesting little amounts of interference within the session. The vertical bars represent the standard error of the mean.

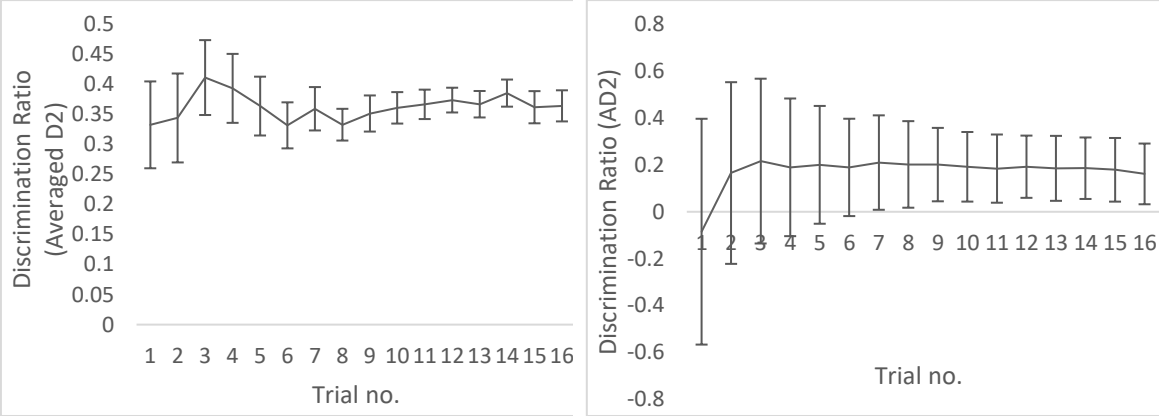



*Figure 4.14* represents the averaged D2 curves for both the spontaneous object recognition and object location task. The averaged D2 scores of each trial were obtained by calculating the 'running average' of each trial within the session. The vertical bars represent the standard error of the mean.

### 4.3.4.3 Spontaneous object recognition task at 10 months of age.

Data from the experienced and naïve group were collated since analysis in section 4.3.3 found that experience levels of mice had no effect on performance in the spontaneous object recognition and object location task.

As seen in the previous experiment, one sample (two-tailed) t-tests were used to determine if mice performance of the spontaneous object recognition task was above chance

by comparing means of D1 scores and averaged D2 ratio against zero when testing session consisted of 16 trials and at a cut-off point of 12 trials. Results show that animals were able to discriminate between the novel and familiar object at 10 months of age when the testing session was 16 trials long. D1 score: $t(15) = 5.01$, $p < 0.001$; Averaged D2: $t(15) = 7.23$, $p < 0.001$. At the 12-trial cut-off point, findings show that mice at 7 months also demonstrated object recognition memory. D1 score: $t(15) = 4.49$, $p < 0.001$; Averaged D2: $t(15) = 7.29$, $p < 0.001$ (refer to figure 4.15 and 4.16).

As in the previous experiment, to investigate if animal performance changed over the session (figure 4.17 *left*), the averaged D2 scores of all animals were separated into four blocks of 4 trials. Blocks of trials for individual animals were obtained by calculating a mean of the averaged D2 scores within that block. A test of repeated measures ANOVA found no evidence of performance changes within the testing session ($F(3, 45) = 1.277$, $p = 0.294$).

#### 4.3.4.4 Object location task at 10 months of age

One sample (two-tailed) t-tests were used to determine if performance of mice on the object location task was above chance by comparing means of D1 scores and averaged D2 scores against zero when testing session consisted of 16 trials and at a cut-off point of 12 trials. Results show that animals failed to discriminate between the novel and familiar locations at 10 months of age when the testing session was 16 trials long. D1 score: $t(15) = 1.42$, $p = 0,176$; Averaged D2: $t(15) = 1.78$, $p = 0.095$. At the 12-trial cut-off point, findings show that mice at 10 months also did not demonstrate object location memory. D1 score: $t(15) = 1.26$, $p = 0.226$; Averaged D2: $t(15) = 0.91$, $p = 0.347$ (see figure 4.15 and 4.16).

In order to investigate if animal performance changed over the session, the averaged D2 scores of all animals were separated into four blocks of 4 trials. Blocks of trials for individual animals were obtained by calculating a mean of the averaged D2 scores within

that block. A test of repeated measures ANOVA found no evidence of performance changes within the testing session ($F(3, 45) = 0.153$, $p = 0.927$); refer to figure 4.17 *right*.

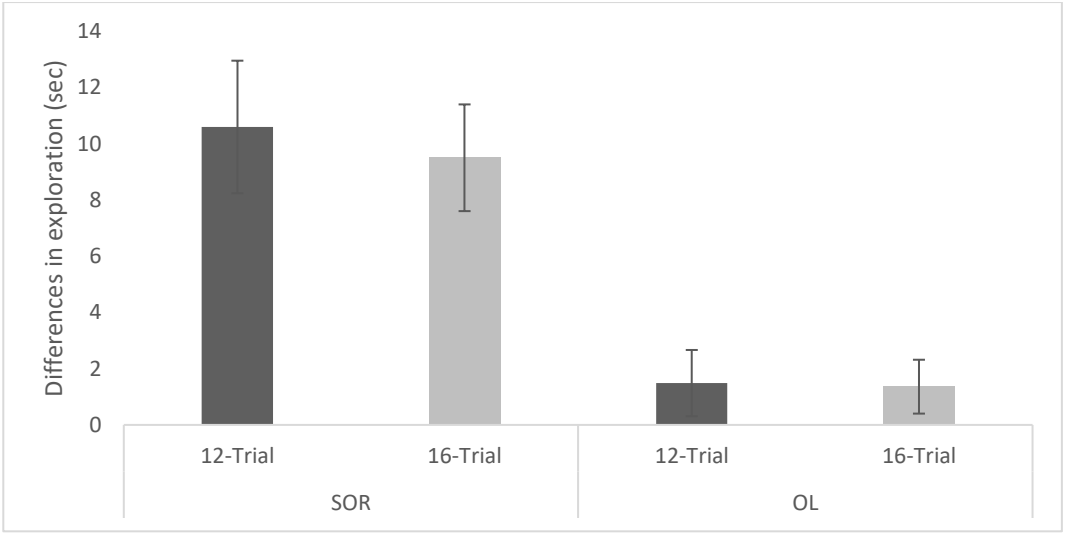



*Figure 4.15* represents the mean differences in exploration (D1) performance of 10-month-old mice in the spontaneous object recognition and object location task. The dark grey bars represent performance levels at a 12-trial cut off point, whereas the light grey bars represent performance of mice in the 16-trial testing session. The bars represent the mean difference of exploration between the novel and familiar object/object-location and the standard error of the mean.

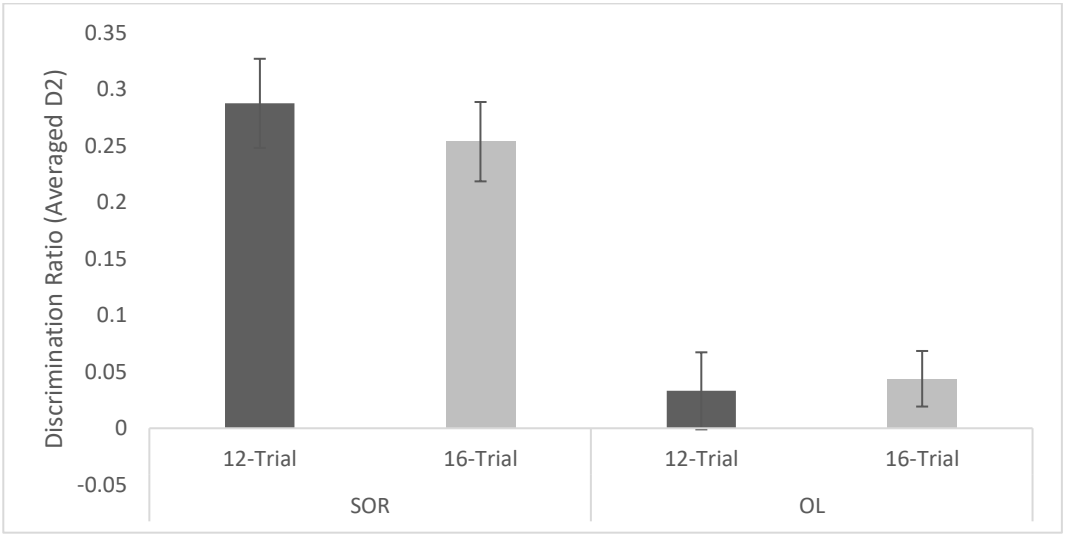



*Figure 4.16* represents the performance of 10-month-old mice in the spontaneous object recognition and object location task. The dark grey bars represent performance levels at a 12-trial cut off point, whereas the light grey bars represent performance of mice in the 16-trial testing session. The bars represent the mean averaged D2 ratio and the standard error of the mean.

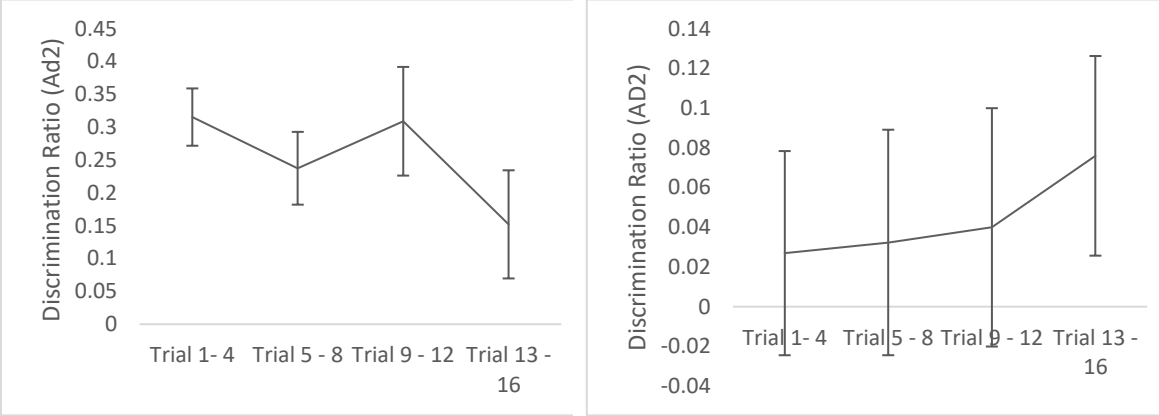



*Figure 4.17* depicts the changes in performance levels of 10 months old mice in the object recognition task (*left)* and object-location task (*right*). The blocks were calculated by obtaining the mean of the first four trials (trials 1 – 4) and each consecutive blocks of four trials thereafter until the end of the session. Performance levels of mice showed no change throughout the testing session in both the object recognition and object location task, suggesting little amounts of interference within the session. The vertical bars represent the standard error of the mean.

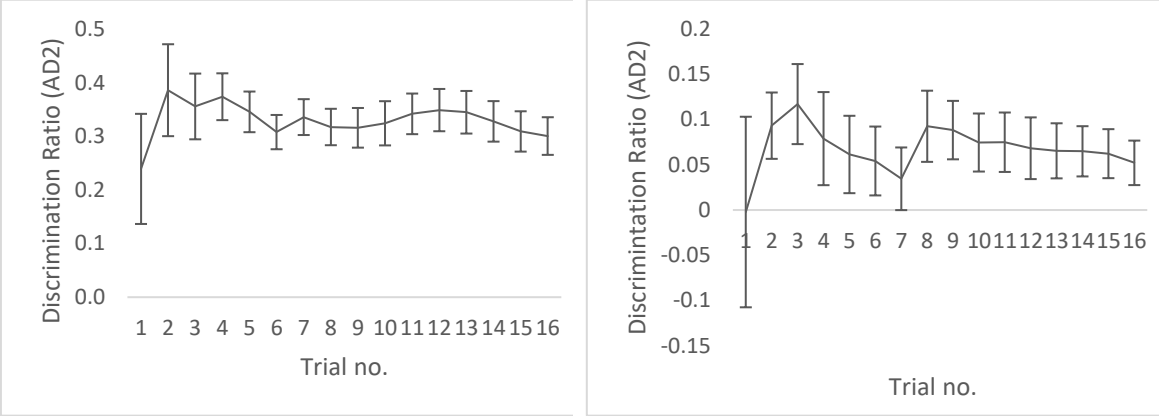



*Figure 4.18* represents the averaged D2 curves for both the spontaneous object recognition and object location task. The averaged D2 scores of each trial were obtained by calculating the 'running average' of each trial within the session. The vertical bars represent the standard error of the mean.

#### 4.3.4.5 Object location task (Set A) at 14 months

One sample (two-tailed) t-tests were used to determine if performance of mice in the object location task (Set A) was above chance by comparing means of D1 scores and averaged D2 ratio against zero (refer to figure 4.5 and 4.6 respectively). The analysis found that, at 14 months of age, animals were able to discriminate the objects in the novel location from objects in a familiar location (D1 scores: $t(12) = 3.01$, $p = 0.01$; averaged D2: $t(12) =$

3.83, p = 0.002).

In order to investigate if animal performance changed over the session, the averaged D2 scores of all animals were separated into three blocks of 4 trials. Blocks of trials for individual animals were obtained by calculating a mean of the averaged D2 scores within that block. A test of repeated measures ANOVA found no evidence of performance changes within the testing session, $F(2, 24) = 1.358$, $p = 0.276$ (see figure 4.19).

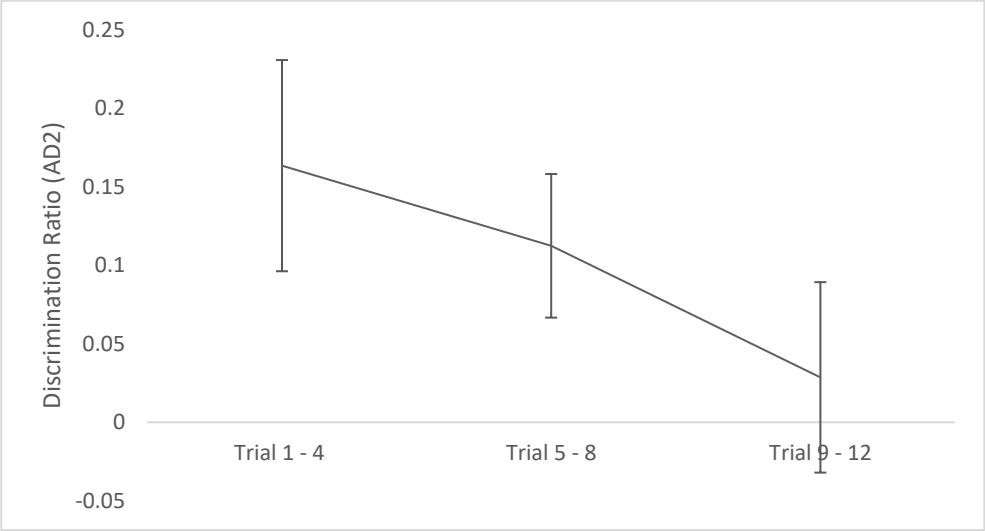



*Figure 4.19* depicts the changes in performance levels of 14 months old mice in object-location task (Set A). The blocks were calculated by obtaining the mean of the first four trials (trials 1 – 4) and each consecutive block of four trials thereafter until the end of the session. Performance levels of mice showed no change throughout the testing session in both the object recognition and object location task, suggesting little amounts of interference within the session. The vertical bars represent the standard error of the mean.

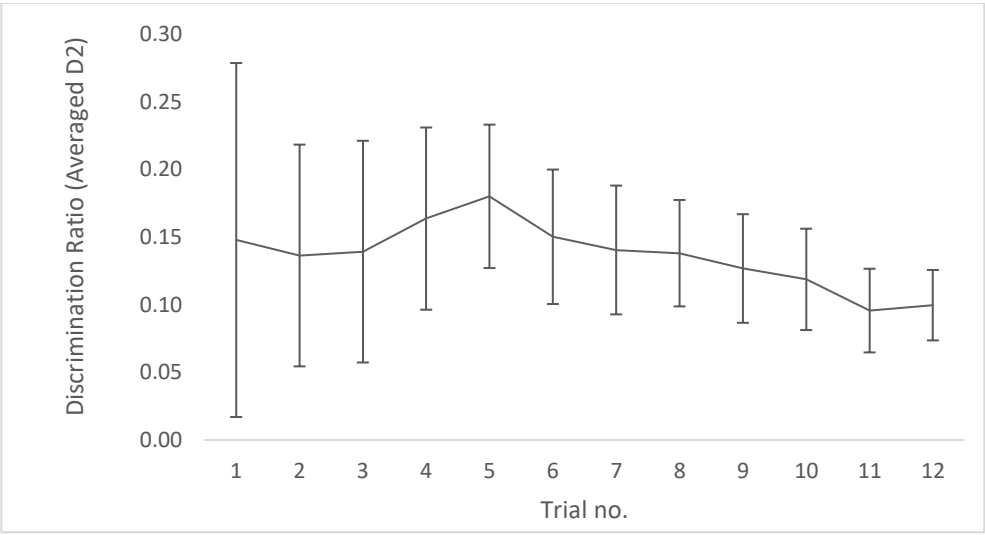



*Figure 4.20* represents the averaged D2 curve for the object location task (set A). The averaged D2 scores of each trial were obtained by calculating the 'running average' of each trial within the session. The vertical bars represent the standard error of the mean.

#### 4.3.4.6 Spontaneous object recognition task (Set b) at 14 months

One sample (two-tailed) t-tests were used to determine if performance of mice in the spontaneous object recognition task was above chance by comparing means of D1 scores and averaged D2 ratio against zero. Results show that animals show ability to discriminate between the novel and familiar object at 14 months of age when the testing session consisted of 12 trials: D1 score: $t(13) = 5.31$, $p < 0.001$; Averaged D2: $t(13) = 6.45$, $p < 0.001$ (figure 4.21 and 4.22).

In order to investigate if animal performance changed over the session, the averaged D2 scores of all animals were separated into three blocks of 4 trials. Blocks of trials for individual animals were obtained by calculating a mean of the averaged D2 scores within that block. A test of repeated measures ANOVA found no evidence of performance changes within the testing session, $F(2, 26) = 0.99$, $p > 0.05$ (figure 4.23 *left*).

#### 4.3.4.7 Object location task (Set B) at 14 months

As in the previous section, a one-sample (two-tailed) t-tests were used to determine if performance of mice in the object location task was above chance by comparing means of D1 scores and averaged D2 scores against zero. The findings show that 14-month-old mice demonstrated object-location memory (refer to figure 4.21 and 4.22) when discrimination was analysed with the D1 scores ($t(14) = 2.47$, $p = 0.027$); but failed to discriminate the objects in the novel location from the objects in the familiar location when analysis was conducted on the averaged D2 ratio ($t(14) = 1.78$, $p = 0.097$).

In order to investigate if animal performance changed over the session, the averaged D2 scores of all animals were separated into three blocks of 4 trials. Blocks of trials for individual animals were obtained by calculating a mean of the averaged D2 scores within that block. A test of repeated measures ANOVA found no evidence of performance changes within the testing session, $F(2, 28) = 2.18$, $p = 0.132$ (see figure 4.23 *right*).

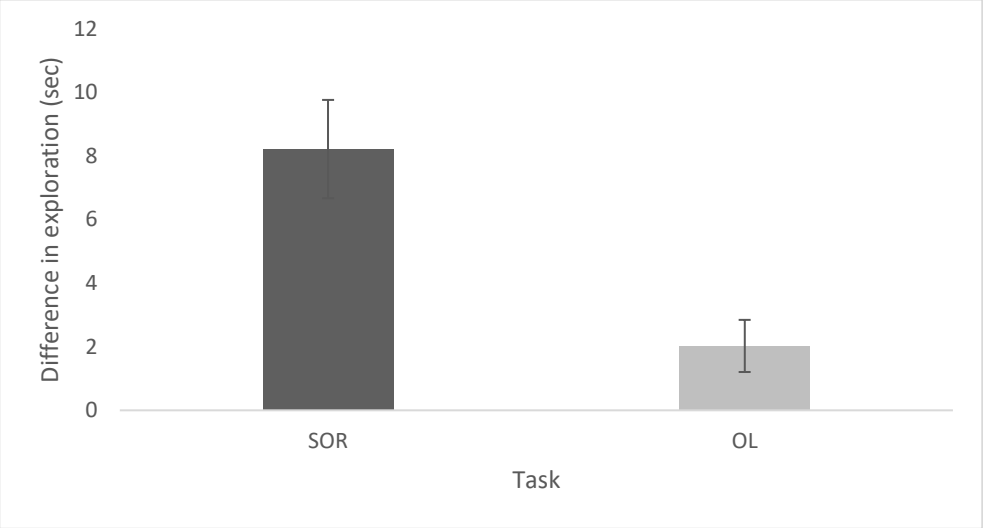



*Figure 4.21* represents the performance of 14-month-old mice in the spontaneous object recognition and object location task (Set B). The dark grey bar represents the mean difference in exploration (D1 scores) in the spontaneous object recognition task, whereas the light grey bar represent performance in the object location task. The bars represent the mean and the standard error of the mean.

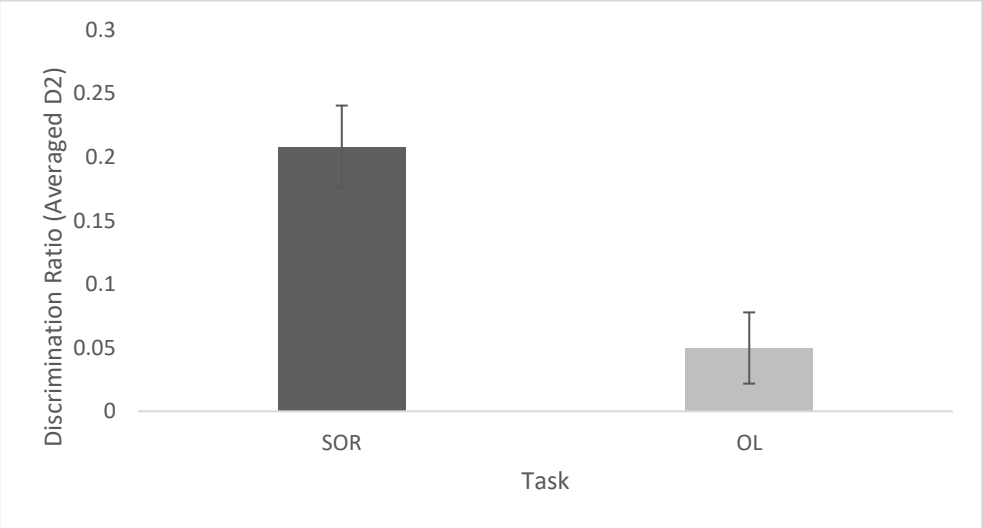



*Figure 4.22* represents the performance of 14-month-old mice in the spontaneous object recognition and object location task (Set B). The dark grey bar represents the mean discrimination ratio (averaged D2) in the spontaneous object recognition task, whereas the light grey bar represent performance in the object location task. The bars represent the mean and the standard error of the mean.

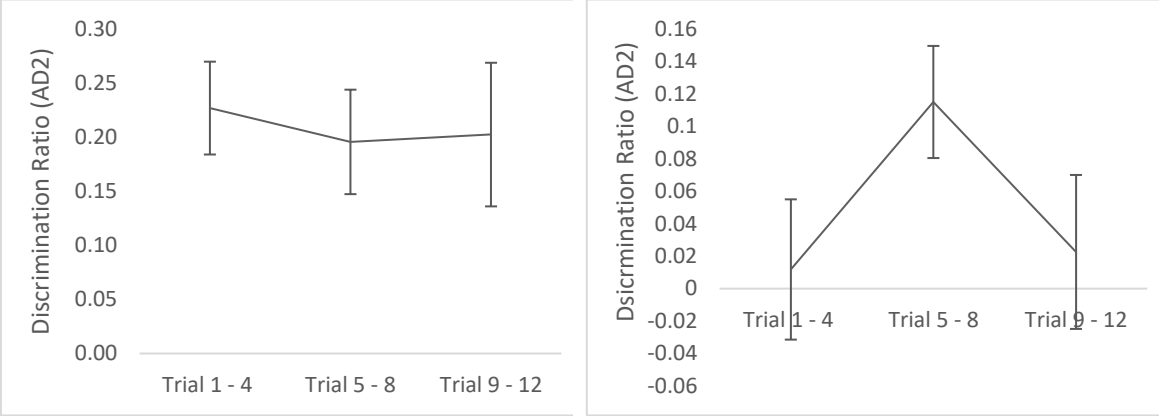



*Figure 4.23* depicts the changes in performance levels of 14 months old mice in the object recognition task (*left)* and object-location task (*right*) using object set B. The blocks were calculated by obtaining the mean of the first four trials (trials 1 – 4) and each consecutive block of four trials thereafter until the end of the session. Performance levels of mice showed no change throughout the testing session in both the object recognition and object location task, suggesting little amounts of interference within the session. The vertical bars represent the standard error of the mean.

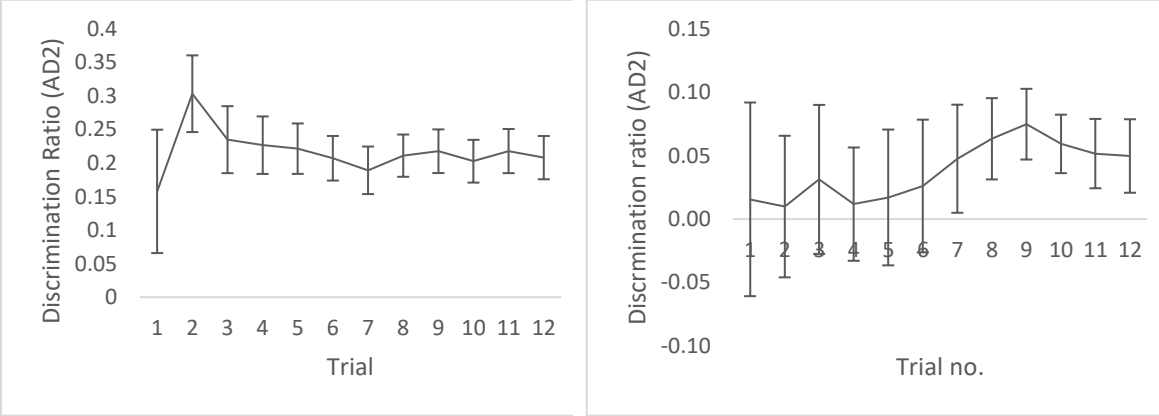



*Figure 4.24* represents the averaged D2 curves for both the spontaneous object recognition (*left*) and object location (*right*) task using object set B. The averaged D2 scores of each trial were obtained by calculating the 'running average' of each trial within the session. The vertical bars represent the standard error of the mean.

#### 4.3.4.8 Object location task (Set C) at 14 months

As in the previous experiment, one-sample (two-tailed) t-tests were used to determine if performance of mice in the object location task (Set A) was above chance by comparing means of D1 scores and averaged D2 ratios against zero. Results show when analysis was conducted on the Averaged D2 scores, mice performance was above chance; $t(13) = 3.45$, $p = 0.004$. But failed to discriminate between the novel and familiar location at

14 months of age in D1 score: $t(13) = 2.12$, $p = 0.054$ (refer to figure 4.5 and 4.6).

In order to investigate if animal performance changed over the session (figure 4.25), the averaged D2 scores of all animals were separated into three blocks of 4 trials. Blocks of trials for individual animals were obtained by calculating a mean of the averaged D2 scores within that block. A test of repeated measures ANOVA found no evidence of performance changes within the testing session ($F(2, 26) = 2.751$, $p = 0.082$).


*Figure 4.25* depicts the changes in performance levels of 14 months old mice in object-location task (Set C). The blocks were calculated by obtaining the mean of the first four trials (trials 1 – 4) and each consecutive blocks of four trials thereafter until the end of the session. Performance levels of mice showed no change throughout the testing session in both the object recognition and object location task, suggesting little amounts of interference within the session. The vertical bars represent the standard error of the mean.

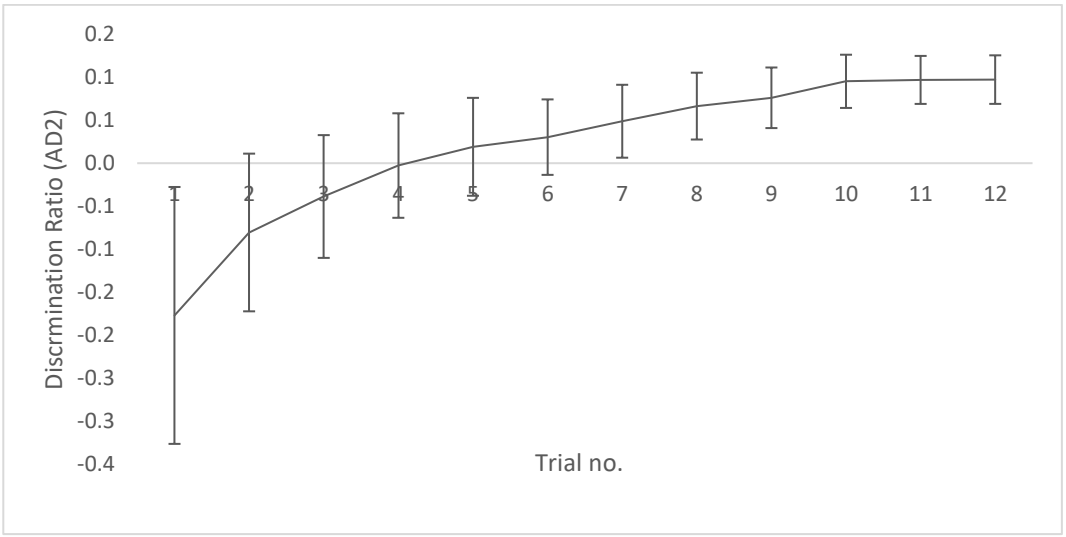



*Figure 4.26* represents the averaged D2 curve for the object location task (set C). The averaged D2 scores of each trial were obtained by calculating the 'running average' of each trial within the session. The vertical bars represent the standard error of the mean.

#### 4.3.4.9 Spontaneous object recognition task at 16 months of age

As in the previous experiment, one-sample (two-tailed) t-tests were used to determine if performance of mice in the spontaneous object recognition task was above chance by comparing means of D1 scores and averaged D2 ratio against zero. Results have shown that animals demonstrate the ability to discriminate between the novel and familiar object at 16 months of age when the testing session consisted of 12 trials, D1 score: $t(10) = 5.66$, $p < 0.001$; Averaged D2: $t(10) = 14.32$, $p < 0.001$ (see figure 4.27 and 4.28).

In order to investigate if animal performance changed over the session (figure 4.29 *left*), the averaged D2 scores of all animals were separated into three blocks of 4 trials. Blocks of trials for individual animals were obtained by calculating a mean of the averaged D2 scores within that block. A test of repeated measures ANOVA found no evidence of performance changes within the testing session ($F(2, 20) = 0.977$, $p = 0.394$).

#### 4.3.4.10 Object location task at 16 months of age

As in previous experiments, one-sample (two-tailed) t-tests were used to determine if performance of mice in the object location task was above chance by comparing means of D1 scores and averaged D2 ratio against zero. Results show that 16-month-old mice failed to distinguish the novel and familiar object-locations; D1 scores: $t(9) = 1.14$, $p = 0.284$; Averaged D2 score $t(9) = 1.79$, $p = 0.107$ (figure 4.27 and 4.28).

In order to investigate if animal performance changed over the session (figure 4.29 *right*), the averaged D2 scores of all animals were separated into three blocks of 4 trials. Blocks of trials for individual animals were obtained by calculating a mean of the averaged D2 scores within that block. A test of repeated measures ANOVA found no evidence of performance changes within the testing session ($F(2, 18) = 0.532$, $p = 0.597$).

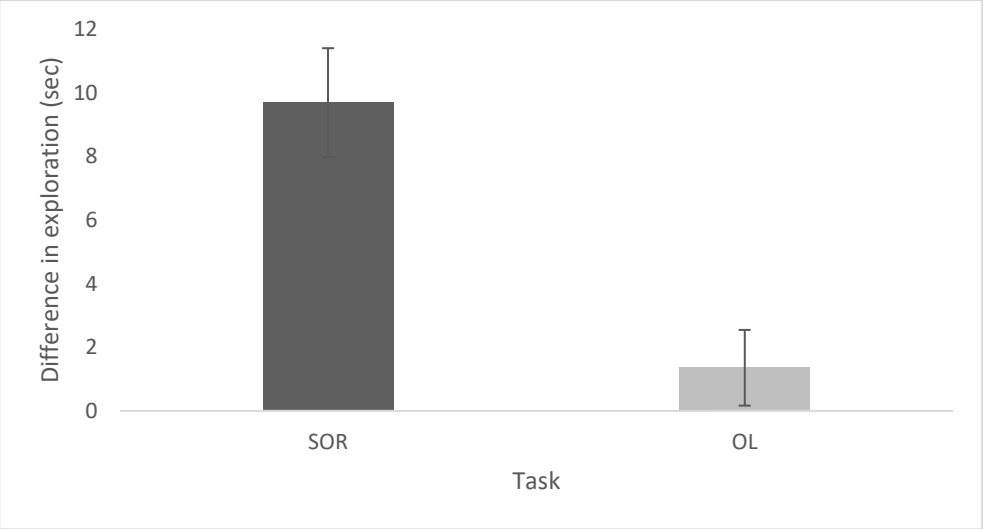



*Figure 4.27* represents the performance of 16-month-old mice in the spontaneous object recognition and object location task. The dark grey bar represents the mean difference in exploration (D1 scores) in the spontaneous object recognition task, whereas the light grey bar represent performance in the object location task. The bars represent the mean and the standard error of the mean.

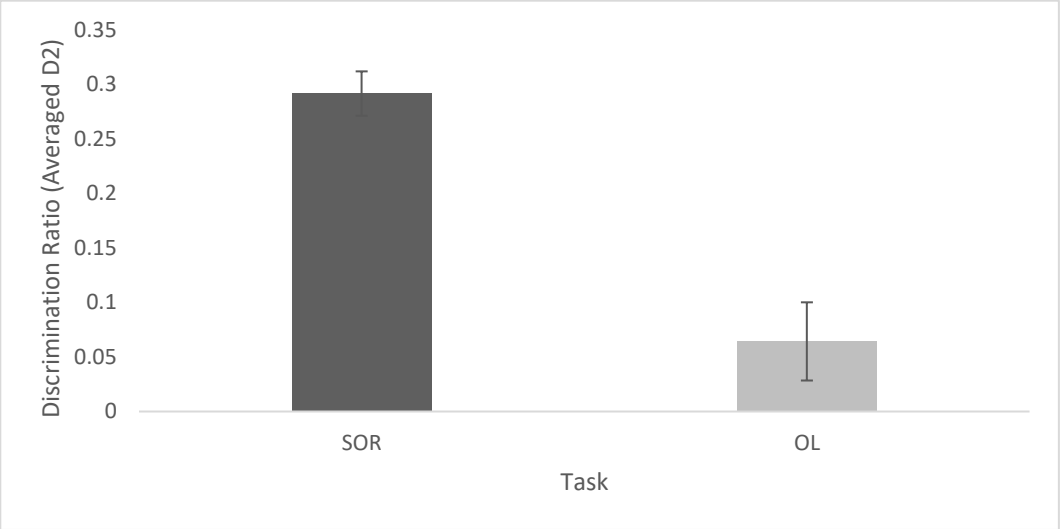



*Figure 4.28* represents the performance of 16-month-old mice in the spontaneous object recognition and object location task. The dark grey bar represents the mean discrimination ratio (averaged D2) in the spontaneous object recognition task, whereas the light grey bar represent performance in the object location task. The bars represent the mean and the standard error of the mean.

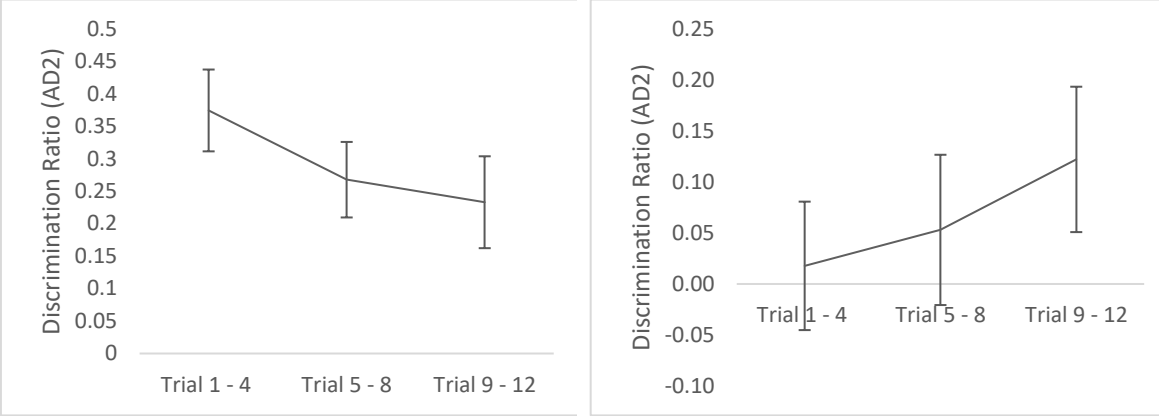



*Figure 4.29* depicts the changes in performance levels of 16 months old mice in the object recognition task (*left)* and object-location task (*right*). The blocks were calculated by obtaining the mean of the first four trials (trials 1 – 4) and each consecutive block of four trials thereafter until the end of the session. Performance levels of mice showed no change throughout the testing session in both the object recognition and object location task, suggesting little amounts of interference within the session. The vertical bars represent the standard error of the mean.

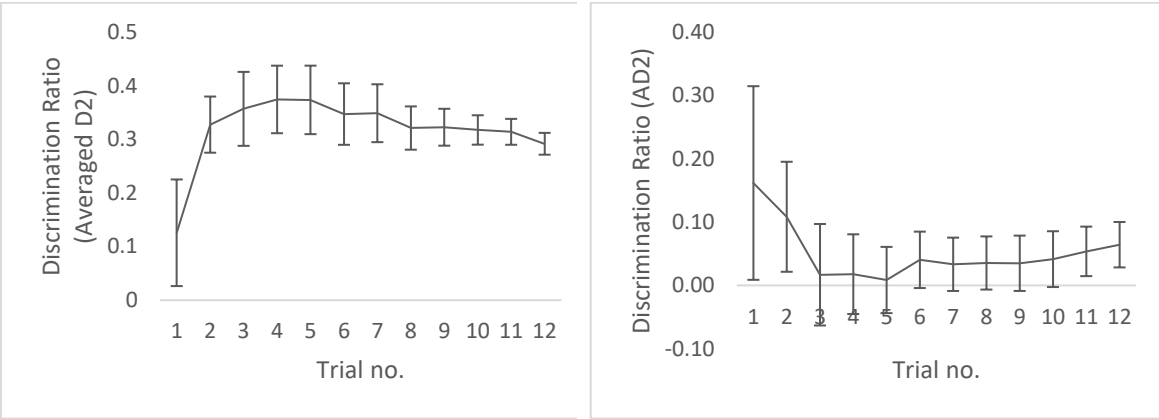



*Figure 4.30* represents the averaged D2 curves for both the spontaneous object recognition (*left*) and object location (*right*) task. The averaged D2 scores of each trial were obtained by calculating the 'running average' of each trial within the session. The vertical bars represent the standard error of the mean.

| | | Trial number | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age** | **Task** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
| **7** | **SOR** | 0.33 | 0.34 | 0.41 | 0.39 | 0.36 | 0.33 | 0.36 | 0.33 | 0.35 | 0.36 | 0.37 | 0.37 | 0.37 | 0.39 | 0.36 | 0.36 |
| | **OL** | -0.09 | 0.42 | 0.32 | 0.11 | 0.24 | 0.13 | 0.33 | 0.15 | 0.20 | 0.11 | 0.11 | 0.28 | 0.10 | 0.19 | 0.08 | -0.10 |
| **10** | **SOR** | 0.24 | 0.53 | 0.30 | 0.43 | 0.23 | 0.12 | 0.50 | 0.19 | 0.30 | 0.40 | 0.52 | 0.43 | 0.30 | 0.11 | 0.05 | 0.17 |
| | **OL** | -0.002 | 0.19 | 0.17 | -0.04 | -0.01 | 0.02 | -0.08 | 0.50 | 0.06 | -0.05 | 0.08 | -0.005 | 0.03 | 0.06 | 0.03 | -0.10 |
| **14** | **OL** | 0.15 | 0.13 | 0.15 | 0.24 | 0.25 | 0.001 | 0.08 | 0.12 | 0.04 | 0.05 | -0.13 | 0.14 | | | | |
| | **SOR** | 0.16 | 0.45 | 0.10 | 0.20 | 0.20 | 0.14 | 0.08 | 0.36 | 0.27 | 0.07 | 0.37 | 0.10 | | | | |
| | **OL** | 0.02 | 0.004 | 0.07 | -0.05 | 0.04 | 0.07 | 0.18 | 0.17 | 0.17 | -0.08 | -0.02 | 0.03 | | | | |
| | **OL** | -0.18 | 0.02 | 0.05 | 0.11 | 0.11 | 0.09 | 0.16 | 0.19 | 0.15 | 0.27 | 0.11 | 0.10 | | | | |
| **16** | **SOR** | 0.13 | 0.53 | 0.42 | 0.43 | 0.37 | 0.22 | 0.36 | 0.13 | 0.34 | 0.27 | 0.28 | 0.05 | | | | |
| | **OL** | 0.16 | 0.06 | -0.17 | 0.02 | -0.03 | 0.20 | -0.01 | 0.05 | 0.03 | 0.10 | 0.18 | 0.18 | | | | |

*Table 4.2* details the performance levels (averaged D2 ratio) of mice within the testing session in the spontaneous object recognition (SOR) and object location (OL) task at 7-, 10-, 14- and 16 months of age.

## 4.4 Discussion

The present study aimed to investigate changes in recognition and spatial memory in C57 mice over time, the levels of proactive interference in a multiple trials task and reusing objects between tasks, and the effects of prior experience on task performance. The results of this study found that from 7 to 16 months, mice did not show marked decline in performance in recognition and location memory, although performance in the spatial task was significantly worse than that of the recognition task. Findings from the study also found no evidence of proactive interference within a testing session in both the spontaneous object recognition and object location task at 7, 10, 14 and 16 months of age. Finally, this study found that prior experience did not affect task performance.

The present study found no age-related impairment in both recognition and spatial memory in mice. In a study conducted by Cavoy and Delaceur (1993), they found that, recognition memory was not age-dependent when delays were 5 minutes. But other studies however, have shown evidence of age-related impairments in recognition memory with delays of over 15 minutes (Burke et al., 2012, Burke & Barnes, 2010). Thus, the presence of an age-dependent effect of recognition memory would be unlikely with a 1-minute delay used in the present study.

Spatial memory of ageing mice in the current study did not demonstrate age-related decline; but performance in the object-location task were below chance levels from 10 months old. Previous evidence in cross-sectional studies have indicated that the onset of age-related decline of spatial reference memory occurs at around 18 months (Markowska, 1999), but others have argued that such decline could only be observed at around 20-24 months of age (Wimmer et al., 2012). The oldest age of which mice in this study were tested was 16 months, which is considered early old age (Markowska & Savonenko, 2002), whereas a rodent would be considered aged at around 22 – 24 months of age (Shukitt-Hale et al., 2001; Maasberg et al., 2012).

Therefore, by testing mice at a small window of 7 to 16 months, the current study predicted that there will be some evidence of an age-related decline in performance in the object-location task. This is because mice were middle aged (16 months) when testing ended, which was before the age (22 months old) where rapid memory decline caused by ageing would occur. Longitudinal studies investigating memory in ageing rodents typically test animals at three time points: young age, middle aged and old age. To illustrate this, Joyal and colleagues (2000) tested a group of CD-1 mice at 3, 17 and 22 months of age; the same could also be said in other papers investigating age-related changes in memory (Dellu et al., 1997; Markowska & Savonenko, 2002; Ando & Ohashi, 1991). Theories pertaining human ageing have shown that rapid development occurs from early age until about 20 then stabilises until about 60 before going through a sharp decline (Craik & Bailystok, 2006).

Furthermore, the current study uses a longitudinal design to examine age-related changes to recognition memory in mice but did not find an age-dependent decline in object recognition and object location memory. Studies utilising a longitudinal design often find that cognitive impairments are often less pronounced compared to studies with a cross-sectional design (Joyal et al., 2000; Markowska & Savonenko, 2002; Caprioli et al., 1991). Whilst cross-sectional studies are advantageous for evaluating cognitive differences between groups of animals (young vs. old animals), the experience levels of animals in these studies often do not mirror experiences in the human population. Longitudinally designed studies however, provides a solution to the shortcomings of cross-sectional studies by testing the same cohort of animals over several time points and this design serves to more closely resemble ageing studies in the human population in terms of experience levels. However, because experience levels of mice in this study would increase, in part due to being tested multiple times throughout different ages, the present chapter investigated the effects of experience may have on object recognition and object location memory by comparing mice

that had prior experience in both the spontaneous object recognition and object location task at 7 months, with a naïve group.

The present study found that prior experience in the tasks of object recognition and object location memory did not improve nor impair performance in those tasks. The effects of experience levels in this study was examined by comparing experienced mice (that were initially tested at 7 months) and a naïve group at 10 months old in the spontaneous object recognition and object location task in the continual trials apparatus. Previous longitudinal studies (Markowska and Savonenko, 2002) have found that prior training experience in the task resulted in protective effect which preserved performance in both reference memory and working memory tasks. Furthermore, a study conducted by Bierley and colleagues (1985) have found that once rats received training in radial arms maze at a young age, the skills obtained at a young age do not deteriorate when rats ages, creating a protective effect. Also, longitudinal studies examining memory in animals have found that the practise effects of being retested causes performance of the task to be constant (Ando & Ohashi, 1991; Dellu et al., 1997) or even improve (Caprioli et al., 1991). Therefore, although the present study found that experience levels had no effect on performance in the spontaneous object recognition and object location task, previous experience in the task may have resulted in protective effects that contributed to constant performance levels up till 16 months of age.

The current study found little evidence of proactive interference occurring within the testing session, whereby performance levels of mice did not significantly change during the testing session of the spontaneous object recognition and object location task across all ages (7, 10, 14 and 16 months old). The continual trials approach used in the present study allowed the investigation of increased interference and its effects on aging mice. Instead of running one trial a day, the present study was able to run multiple trials (12 and 16 trials) within a single testing session. Despite the use of trial unique objects, the animals were tested within a single context, and in rapid succession (1 min inter-trial interval), thus increasing the

interference within the task. The present study also found that 14 month old mice showed little interference caused by the reuse of objects between the task. There may be increased interference within the object location task due to the reuse of objects that were previously seen in the spontaneous object recognition task and previous memory of the objects from the spontaneous object recognition task may interfere with the acquisition or retrieval of object location memory. Proactive interference has been found to disproportionately affect the older population (Moss, Rosene & Peters, 1988; Bartus & Dean, 1979); but this cannot be concluded in the present study, because cross-sectional comparisons between older mice and younger mice were not made.

The present study tested the object recognition and object location memory in a group of mice from 7 to 16 months of age in a continual trials apparatus and found no age-related impairments in both tasks. Object-location memory was impaired compared to object recognition memory across all ages. The current study also found that prior experience (7 months old) in both the spontaneous object recognition and object location task had no effect on performance levels at a later age (10 months old). Finally, there was little evidence of proactive interference which occurred within the testing session; and the reuse of previously encountered objects (from the spontaneous object recognition task) did not adversely affect performance in the object location task.

This study utilises a longitudinal design, instead of a cross-sectional design, which have not been widely utilized in ageing studies involving animals. Although the use of longitudinal design has the advantage of assessing how memory changes within a cohort over time, the effects are often diminished compared to the findings in a cross-sectional study (Hedden and Gabrieli, 2004). To resolve this, future possible work may aim to combine the cross-sectional and longitudinal design to investigate age-related decline in recognition memory in mice using the continual trials apparatus. The comparison between the cross-sectional and longitudinal study would allow for a more reliable conclusion with

regards to the age-related changes in memory. The current study has shed light on normal mouse behaviour; and this is an important prerequisite before quantifying and understanding behavioural performances of aged population with severe cognitive decline such as Alzheimer's and dementia.

In the following chapter (Study 3), this thesis further validates the continual trials apparatus and examine the paradigms' potential in the investigation of memory in a diseased mouse model. Age-related changes of the object recognition and object location memory of a transgenic mouse model of Alzheimer's Disease was examined using the continual trials apparatus.

# Chapter 5

## Study 3: Evaluating object recognition and location memory of the TASTPM (APP/PS1) mouse model for Alzheimer's Disease

### 5.1 Introduction

The previous chapter in this thesis validated the continual trials approach to evaluating recognition memory in ageing mice and found that ageing mice were able to successfully exhibit object recognition and object location memory. Following that, the present chapter will validate the continual trials apparatus in a transgenic model of Alzheimer's Disease with known age-related recognition memory impairments. The current study aimed to replicate the findings of previous literature (Howlett et al., 2004) by examining the age-related decline of recognition memory in TASTPM (APP/PS1) mice using the continual trials apparatus.

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common cause of dementia in the elderly population (Bilkei-Gorzo, 2014). The prevalence of AD is age-dependent, increasing as the population ages. A recent meta-analysis by Niu et al., (2017) found that, the prevalence of AD in Europe was 0.97% for patients between ages 65-74 and 22.53% for patients older than 85 years old; and with the increasing ageing population, the prevalence of AD is bound to rise. AD patients typically exhibit progressive decline of cognitive function including short- and long-term memory loss, episodic memory loss, language difficulties and executive dysfunction (Balducci and Forloni, 2011). Histopathological symptoms and diagnosis include the presence of extracellular deposits of amyloid beta (Aβ) plaques and intracellular neurofibrillary tangles / hyperphosphorylated tau and in later stages of the disease, extensive neuronal loss within the hippocampus and cortex (Czech and Grueninger, 2013). There are two forms of the disease: Familial and sporadic AD. Familial AD (FAD) affects around 5% of the total cases of AD, whereas 95%

of AD patients have sporadic AD (Pardon and Rattray, 2008). FAD is typically early onset and often progresses more rapidly compared to the sporadic form of AD. The molecular study of FAD has led to the discovery of three known mutations that cause familial AD: amyloid precursor protein (APP), presenilin-1 (PS1) and presenilin-2 (PS2); and these mutation results in the deposition and aggregation of the 42-amino acid of amyloid-beta (Aβ42).

The use of transgenic mouse models in therapeutic research of AD have been centred on the amyloid cascade hypothesis, which postulates that Aβ peptide deposition in the brain is the main cause of AD and that neurofibrillary tangles (tau), neuronal loss and dementia is a result of Aβ deposition (Hardy and Higgins, 1992; Karran et al., 2011). Thus, most therapeutics have been aimed at reducing the deposition of amyloid-β. To do this, transgenic mouse models overexpressing mutations of the amyloid precursor protein (APP) and/or presenilin (PS) – proteins that are linked to familial forms of AD – have been used to understand pathological developments of the disease. Examples of transgenic mouse models of AD include Tg2576, APP23, APP/PS1, 3xTgAD and 5xFAD. Details of these AD mouse models are summarised in Table 5.1.

This experiment used the TASTPM mice model of AD, which is a transgenic mouse model overexpressing the Swedish double (K670N and M671L) and presenilin-1 (M164V) familial mutation (Howlett et al., 2004). Like human AD patients, TASTPM mice exhibit progressive amyloid plaque deposition that is detectable from 3 – 6 months (Howlett et al., 2004). The development of Aβ load is age-related; present at low levels at 3 months but increasing in load and concentration by 7 and 12 months (Howlett et al., 2008; Grillo et al., 2013). Howlett and colleagues (2008) further report neuronal loss, particularly in the hippocampus of TASTPM mice. Investigations into the plasma of TASPM mice found that Aβ levels were detectable from 1 -13 months and Aβ levels within the plasma for older animals were less than younger mice (Hallé et al., 2015). Howlett et al., (2008) reported

observations of hyperphosphorylated tau in TASTPM mice from 4 months and this phosphor-tau labelling increased from 6 – 8 months although no further age-related changes were seen.

Apart from pathological developments of amyloid plaques, TASTPM mice has been found to exhibit age-related memory decline similar to human AD patients. Work by Howlett and colleagues (2004) has shown that recognition memory of TASTPM mice was impaired from the age of 6 months compared to wildtype littermates and this cognitive impairment coincided with the presence of matured Aβ plaques that disrupts neural activity. A longitudinal study investigating TASTPM and wildtype performance in the object recognition task from 4 – 8 months found a lack of age-related decline (Scullion et al., 2011).

TASTPM mice also exhibited age-related impairment contextual memory task from 5.5 months old; other studies report impairments at 8 and 11 months of age compared to wildtype mice (Pardon et al., 2009; Perren et al., 2003, Pugh et al., 2007). Rattray and colleagues however, found that TASTPM mice demonstrated deficit in extinction of the contextual fear conditioning between 3 – 4 months, in a weaker conditioning procedure, prior to onset of reported memory impairment. This suggest that mice had impaired cognitive flexibility during the early development of amyloid pathology (Pardon et al., 2009, Rattray et al., 2009). Furthermore, work done by Scullion et al., (2011) investigating spatial memory found that TASTPM mice showed an age-related decline in performance on the spontaneous alternation task in the T-maze compared to controls. Also, TASTPM mice performance in the Morris Water Maze at 4 months of age was comparable to wildtype mice; but escape latencies were significantly longer in 8-month-old TASTPM compared to wildtype controls. TASPM mice also show decreased motor activity in the locomotor activity test, increased feeding over a 24-hour period and lower body weight compared to wildtype mice (Pugh et al., 2007).

In the previous study (Chapter 4), this thesis examined longitudinal changes of object recognition and location memory of C57 mice from 7 – 16 months of age using the multiple trials approach. Using the same continual trials approach, this study sought to examine the longitudinal changes of recognition and object location memory in TASTPM mice. The multiple trials approach to running the spontaneous object recognition task and its variants has been shown to decrease potential stress caused by repeated handling, and in turn lowers variance, increases task sensitivity and statistical power (see chapter 3, study 1). The reduction of stress is especially important in studies investigating the cognitive abilities of transgenic animals. Pre-clinical studies have reported links between stress and Alzheimer's disease (Pardon and Rattray, 2008). In fact, Dong et al., (2004; see also Kang et al., 2007) have reported that repeated exposure to stressors have been shown to elevate amyloid beta plaque levels and deteriorate memory in APP mice (see Pardon, 2008 for review).

The present study had two aims: 1) to validate the continual trials approach to running recognition tasks in diseased mice to demonstrate whether they can complete the task and; 2) to provide a behavioural paradigm that helps clarify conflicting findings in the literature. This study sought to investigate whether TASTPM mice exhibit age-related decline in object recognition and location memory. To do this, animals received multiple trials version of the spontaneous object recognition and object location task at 7 and 10 months of age. Naïve TASTPM mice were introduced at 10 months to examine the effects of experience on the spontaneous object recognition and object location task performance.

| Mouse Model | Transgene | Promoter | APP mutation | PS1 and tau mutation | Amyloid Pathology | Age of onset | Behavioural impairments | Age of onset | References |
|---|---|---|---|---|---|---|---|---|---|
| **Tg2576** | Human APP | Hamster PrP | KM670/671NL (Swedish) | None | High plaque concentration in the cortex, subiculum and pre-subiculum | 11 - 13 months | Spatial learning; Episodic-like memory | 10 months | Hsiao et al., 1996; Good et al., 2007; Taglialatela et al., 2009 |
| **APP23** | Human APP | Thy-1 | KM670/671NL (Swedish) | None | High plaque in the neocortex and hippocampus; neuronal loss | 6 months | Spatial learning | 3 months | Struchler-Pierrat et al., 1997; Calhoun et al., 1998; Kelly et al., 2003 |
| **APPswe/ PS1dE9** | Human APP/PS1 | Mouse PrP | KM670/671NL (Swedish) | deltaE9 | Aβ plaque present at 6 months; high concentration in hippocampus and neocortex at 9 months | 6 months | Contextual memory; Spatial learning | 6 months | Jankowsky et al., 2004; Volianskis et al., 2010 |
| **TASTPM** | Human APP/PS1 | Thy-1 | KM670/671NL (Swedish) | M146V | Aβ plaque deposits; high concentration in hippocampus and neocortex; neuronal loss at 10 months | 6 months | Object recognition; Contextual memory | 6 months | Howlett et al., 2004; Pardon et al., 2009; Scullion et al., 2011 |
| **3xTgAD** | Human APP, PS1 and tau | Thy-1 | KM670/671NL (Swedish) | M146V and tau.P301L | Extracellular amyloid deposits in the frontal cortex; tau pathology (tangles) from 12 months | 6 months | Spatial memory; Contextual memory; Episodic-like memory | 6.5 months | Oddo et al., 2003; Stover et al., 2015; Davis et al., 2013 |
| **5xFAD** | Human APP/PS1 | Thy-1 | KM670/671NL (Swedish), I716v (Florida), V7171 (London) | M146L, L286V | Amyloid deposition; neuronal loss | 2 months | Spatial memory; Contextual memory | 4 - 5 months | Oakley et al., 2006; Kimura and Ohno, 2009; Ohno, 2009 |

*Table 5.1* Summary of mouse models that are commonly used in AD research, a comprehensive overview of transgenic mouse models can be found on the Alzheimers Research Forum website: Abbreviations: http://www.alzforum.org/research-models/alzheimers-disease. APP, amyloid precursor protein; PS1, presenilin-1; Prp, prion protein; Aβ, amyloid beta.

## 5.2 Materials and Methods

### 5.2.1 Subjects

The current study was performed using 16 (8 = male; 8 = female) naïve TASTPM mice overexpressing the hAPP695swe mutation (TAS10) and the Presenilin-1 M146V mutation were backcrossed with C57Bl/6J mice (Howlett et al., 2004) and sourced from GlaxoSmithKline, UK. Animals were housed in groups of up to 4 in individually ventilated cages (IVC) under controlled diurnal conditions (0700 – 1900hours). All animals received sawdust bedding, cardboard tube and a hammock in the cages as enrichment. All experiments occurred during the light phase. Water was available ad libitum throughout the study. The animals were food deprived to 90-95% of their free feeding body weight and maintained as thus throughout the duration of the study. Dependent on groups (see below), animals were 7 or 10 months old at the start of behavioural testing and weighed between 20.8 – 30.8 grams. Four of 16 mice died before behavioural testing began.

Subjects in this experiment were divided between two groups. The experienced group consisted of 8 mice which were tested at 7 and 10 months old; whereas the naïve group was composed of 4 naïve mice, of which the purpose was to investigate if experience influenced performance. Mice in the naïve group were tested when they were 10 months old.

### 5.2.2 Apparatus

As in chapter 4, this experiment was conducted in an apparatus detailed in Chapter 2, section 2.1, figure 2.1. Objects were placed in the back-left and –right corner of the apparatus 3cm from the walls to allow animals to circle the objects during exploration. The floors of the apparatus were lined with a grey Lego$^{TM}$ surface and the walls of the apparatus were white.

### 5.2.3 Objects

Junk objects of various colours, shapes and sizes were used in this experiment. Three copies of objects were used as to prevent animals from re-encountering objects; this was to ensure that biases resulting from olfactory cues did not occur. The objects used in this experiment were trial-unique. For examples of objects, see Chapter 2, section 2.2, figure 2.2.

### 5.2.4 Habituation and training protocol

Mice in this experiment received handling and habituation training as described in chapter 2, section 2.3.1. The experienced group received pre-training at 7 months and the naïve group received pre-training at 10 months old. Prior to test, at 10 months, animals in the experienced group were subjected to a single session of shuttling training, due to having been trained when they were 7 months old. Habituation and pre-training lasted 9 days and 7 days for the experienced and naïve groups respectively.

### 5.2.5 Testing protocol

All animals in this experiment received either one or two 16 trial testing sessions of both the spontaneous object recognition and object location task, depending on group allocation. Group 1 received a 16-trial testing session at 7 and 10 months, whereas Group 2 received a 16-trial testing session at 10 months old. Detailed description of the object recognition and the object location task can be found in Chapter 2, section 2.4.1 and 2.4.2 respectively. Task protocols for the object recognition and object location task are described below.

#### 5.2.5.1 Spontaneous object recognition task

Initially, an animal was placed into the holding area of the continual trials apparatus. After, the central door open and the animal shuttled into the object area. During this phase (sample phase) the animal was presented with a pair of identical objects (a pair of objects A), located at the back-left and right corner of the object areas. After 2 minutes of exploration, the side arm doors opened, and the mouse returned to the holding area for 1 minute. During this time, the experimenter swapped the objects around to prepare for the test phase. The central door opened once more and the mouse shuttled into the object area, this time presented with a copy of the familiar object from the sample phase and a novel object (objects A and B). The mouse was given 2 minutes to explore the pair of objects before returning to the holding area via the central door. The mouse waited in the holding area, whilst the experimenter changes the objects to prepare for the next trial. This procedure was repeated until the end of the testing session. 0.1mL of 50% condensed milk solution (Nestle, UK) were replenished each time after it was consumed by the animal.

#### 5.2.5.2 Object location task

As in the spontaneous object recognition task, a trial of the object location task consisted of a sample and test phase. The trial structure is identical with the exception that animals were presented initially with a novel pair of objects (objects A and B) and an identical pair of familiar objects (objects A and A') of which one of the objects would be in a novel location. As in previous chapters, the library of objects that were used in the object recognition task was reused for the object location task, but in reverse order. For example, objects used in the SOR task were as follows: Objects AA then AB at test; in the object-location task, the following reverse order was therefore used: Objects BA at sample and BB at test. Refer to Chapter 2, section 2.4 for details regarding counterbalancing and exclusion criteria.

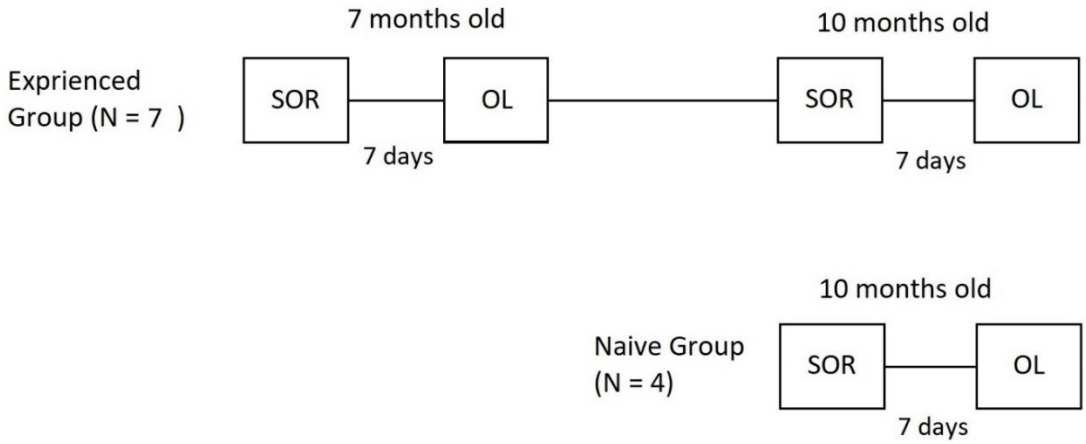



*Figure 5.1* represents the experimental timeline of the current study. The experienced group was tested at 7 and 10 months of age in the spontaneous object recognition task; whereas the naïve group were tested at 10 months of age. Animals were subject to object location memory tests 7 days after the completion of the spontaneous object recognition task. SOR = spontaneous object recognition task; OL = object location task.

### 5.2.6 Behavioural analysis

As in previous experiments, all animal behaviour during the experiment was recorded and scored offline using a stopwatch program. When an animals' nose was directed towards the object and was within 1cm or when the animals' nose was directed towards the object within a 45° angle and their paw was touching the object counts as exploratory behaviour. However, behaviours such as sitting, climbing and using the objects as leverage to rear upwards were not counted as exploratory behaviour. Two primary discriminatory measures (D1 and averaged D2) were used to determine discrimination levels between novelty and familiarity in this experiment (Ennaceur & Delaceur, 1988). See Chapter 2, Section 2.5 for further details.

| | 7 months | 10 months | |
|---|---|---|---|
| | | Experienced | Naïve |
| **Spontaneous object recognition** | 7 | 6 | 4 |
| **Object location** | 6 | 6 | 4 |

*Table 5.2* shows the number of mice that were tested in the object recognition and object location task at 7 and 10 months old (experienced and naïve group).

### 5.3 Results

Animals that have completed testing in both the spontaneous object recognition and object location task a 7 and 10 months of age were included in the analysis (see table 5.2). The analysis was conducted using a 2x2 ANOVA comparing the effects of age on task performance.

An analysis of D1 measure showed that there were no effect of age ($F(1,4) = 2.16$, $p = 0.22$), and task ($F(1, 4) = 6.89$, $p = 0.059$). This indicated that TASTPM mice demonstrated no age-related decline, and performance between the spontaneous object recognition and object location task were at similar levels (see figure 5.2). We also found no interaction between age and task, ($F(1, 4) = 0.26$, $p > 0.05$), indicating that TASTPM mice did not demonstrate age-related decline in object recognition and location memory.

An analysis (2x2 ANOVA) of the averaged D2 scores (figure 5.3) found no effect of age $F(1, 4) = 3.11$, $p = 0.15$, but found an effect of task: $F(1, 4) = 27.05$, $p < 0.01$ on performance of TASTPM mice. No age*task interaction was found, $F(1, 4) = 0.28$, $p > 0.05$. TASTPM showed no age-related decline in relation to task performance; but performance was significantly worse in task requiring location memory in comparison to the recognition memory task.

A further 2x2 ANOVA of the total exploration times of animals at 7 and 10 months in the SOR and OL task found that there were no effect of age, $F(1, 4) = 0.44$, $p > 0.05$ and task, $F(1, 4) = 5.16$, $p = 0.086$. This indicated that total exploration times of animals at 7 and 10 months across both the SOR and OL task were similar (figure 5.4).

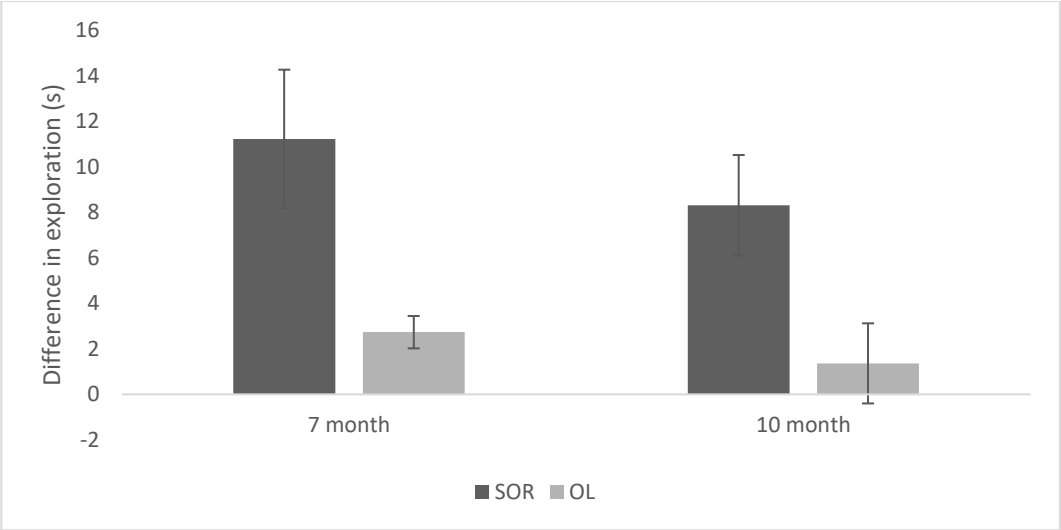



*Figure 5.2* represent the performance (differences in exploration, D1) of TASTPM mice at 7 and 10 months in a recognition and location memory task. TASTPM mice did not show age-related decline in performance on both the spontaneous object recognition and object location task. Vertical bars represent standard error of the mean.

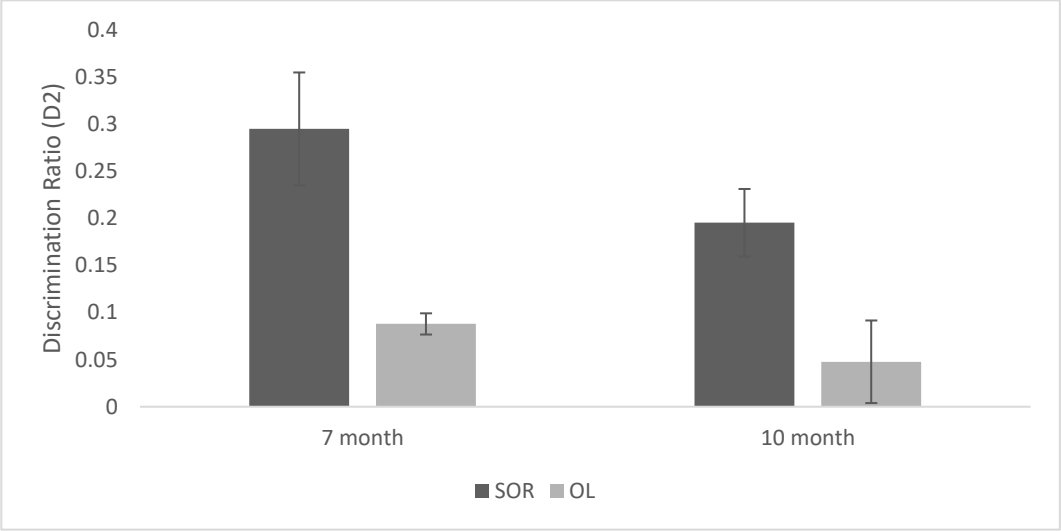



*Figure 5.3* represents performance of TASTPM mice at 7 and 10 months in the spontaneous object recognition and object location task. Analysis found an effect of task, which indicate that performance of TASTPM mice were worse in the object location task compared to the spontaneous object recognition task. Animals did not show an age-related decline in performance. Vertical bars represent standard error of the mean.

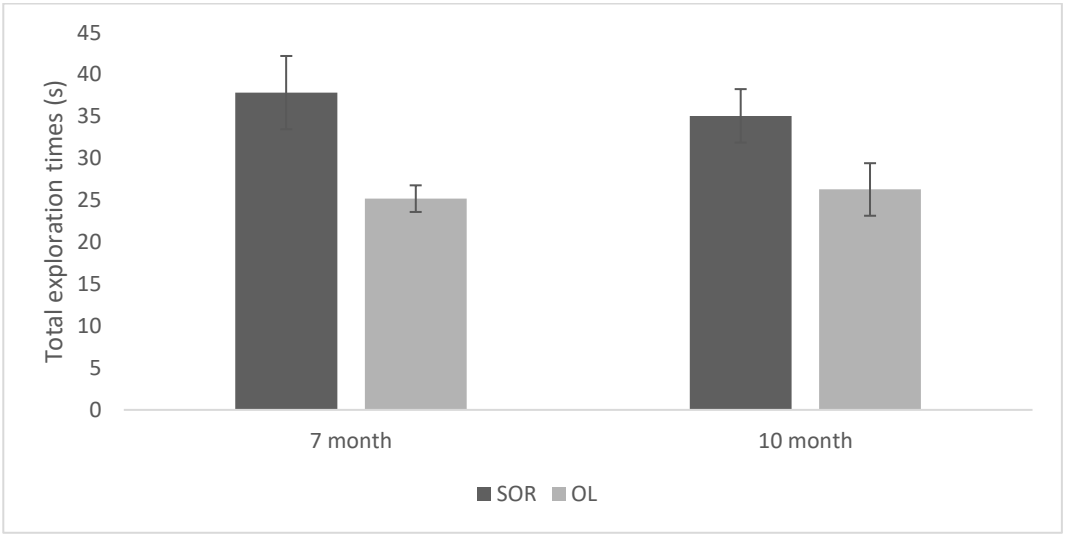



*Figure 5.4* represents the total exploration times of TASTPM mice at 7 and 10 months in the spontaneous object recognition and object location task. Animals show similar exploration times across ages and task. Vertical bars represent the standard error of the mean.

In order to evaluate the effects of experience (experienced vs naïve) on task performance (spontaneous object recognition and object location), a 2x2 ANOVA was conducted to compare performance of experienced and naïve mice using D1 scores and averaged D2 ratios.

The findings show that when D1 scores were analysed (figure 5.5), there was no effect of task, F(1, 8) 0.42, p = 0.742, suggesting that performance levels between the spontaneous object recognition and object location task were similar. The analysis also found that the experienced and naïve group performance levels were similar, F(1, 8) = 0.155, p = 0.704. Task*experience interaction was not significant, F(1, 8) = 0.38, p = 0.554, indicating that prior experience in both task did not impact performance levels at 10 months of age. The analysis of averaged D2 scores (figure 5.6) revealed similar findings, with no effect of task, F(1, 8) = 4.22, p = 0.074; and performance levels between the naïve and experienced group were similar, F(1, 8) = 1.302, p = 0.287. Finally a task*experience analysis showed that prior task experience had no effect on performance levels at 10 months, F(1, 8) = 1.213, p = 0.303.

A further 2x2 ANOVA analysing the total exploration times (figure 5.7) of the experienced and naïve group in both the spontaneous object recognition and object location task found that exploration times in between tasks were similar, $F(1, 8) = 4.957$, $p = 0.057$; and the experienced and naïve group had similar levels of exploration, $F(1, 8) = 0.249$, $p = 0.632$. A task*exploration analysis on the total exploration times found that previous experience in both the spontaneous object recognition and object location task had no effect on exploration times in both tasks, $F(1, 8) = 0.454$, $p = 0.519$.

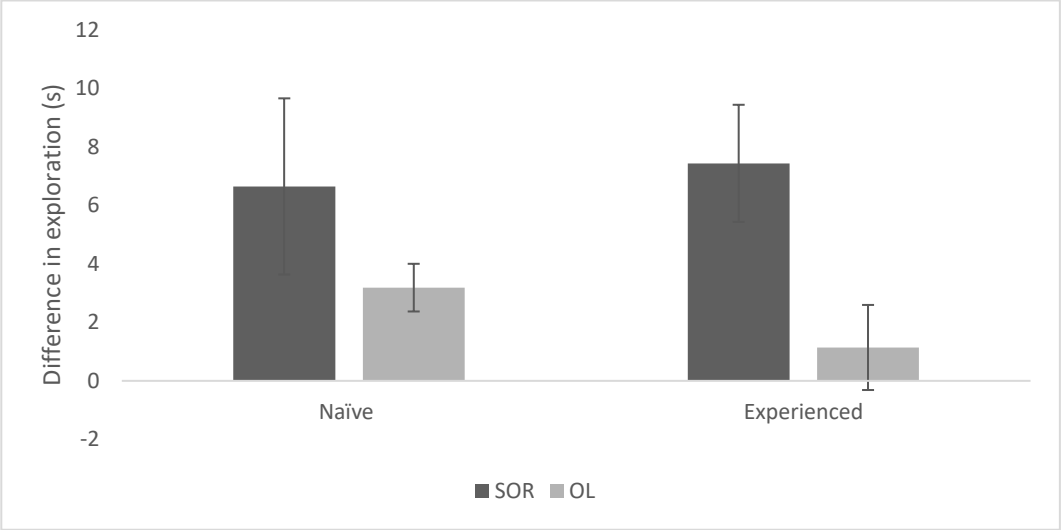



*Figure 5.5* represents the effects of experience on performance (difference between exploration) of mice in the SOR and OL task at 10 months. Experience had no effect on performance in both the spontaneous object recognition and object location task. Vertical bars represent the standard error of the mean.

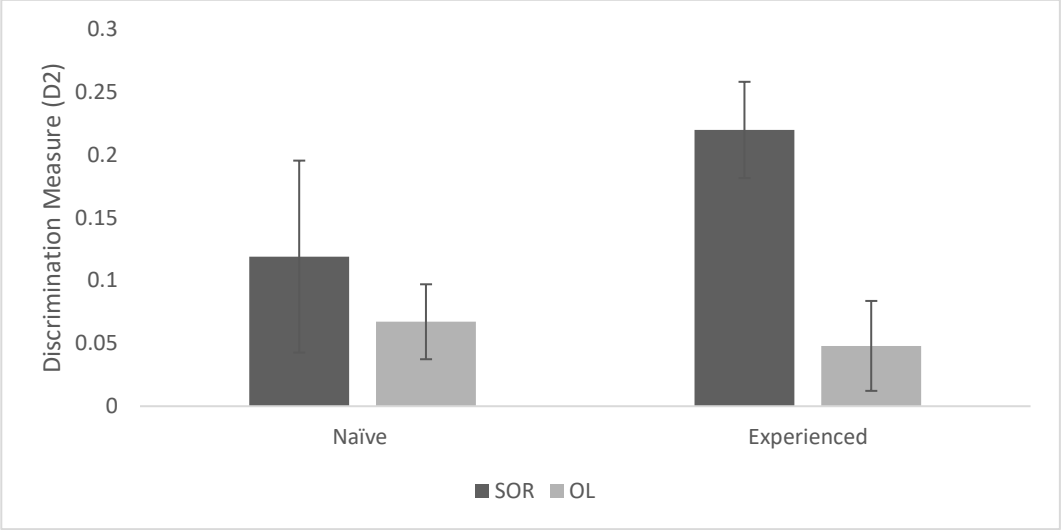



*Figure 5.6* represents the effects of experience on performance level (D2 measure) of 10 month old mice in tasks of recognition and location memory. Analysis found that experience did not affect performance of TASTPM mice in the SOR and OL task at 10 months old. Vertical bars represent standard error of the mean.

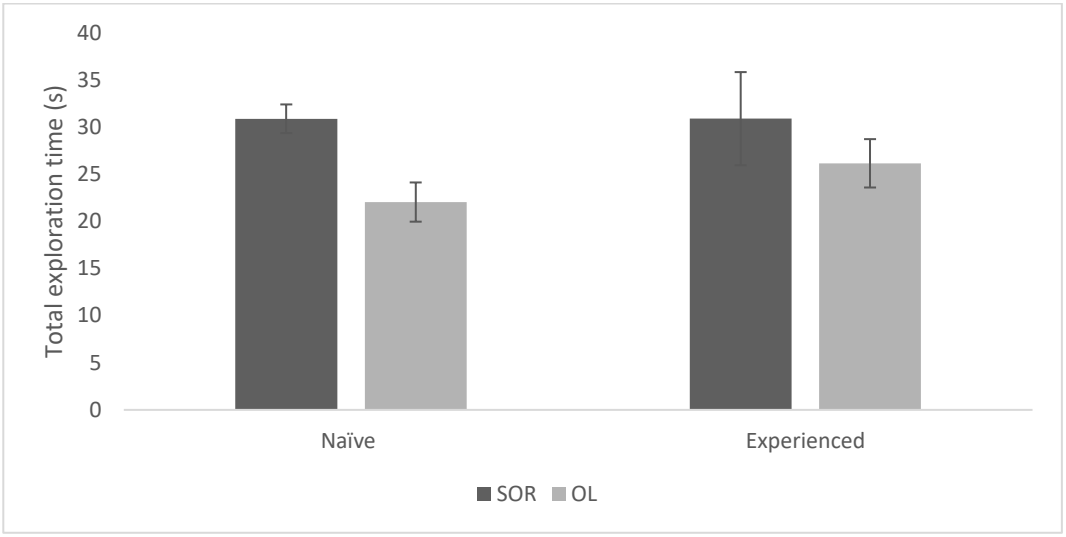



*Figure 5.7* shows the total exploration time of naïve and experienced TASTPM mice. Total exploration times did not differ across experience levels and tasks. Vertical bars represent standard error of the mean.

### 5.3.1 Performance of animals at 7 months

#### 5.3.1.1 Spontaneous object recognition

As in previous experiments, performance levels of seven-month-old TASTPM mice in the spontaneous object recognition task was determined by comparing the mean D1 measure and averaged D2 of the group against zero (One-sample t-test; two-tailed). Findings showed that analysis using D1 and averaged D2 scores showed that, at 7 months of age, TASTPM mice successfully discriminated the novel from the familiar object; mean D1 (±SEM) = 10.22 (±2.23), $t(6) = 4.58$, $p < 0.005$; mean Averaged D2 (±SEM) = 0.29 (±0.04), $t(6) = 7.13$, $p < 0.001$. See graph 5.8 for details.

As in previous experiments, to determine whether performance levels of 7 month old TASTPM mice changed within the session, trials (total trial number: 16) within the session were divided into 4 blocks of 4 trials. Blocks were calculated by obtaining the means of averaged D2 scores for the first 4 consecutive trials of each animal and each consecutive group of 4 trials. The blocks obtained were then analysed using a repeated measures ANOVA, no block effect was found, $F(3, 18) = 1.57$, $p = 0.23$, indicating that performance levels of animals did not change within the session. Refer to figure 5.11.

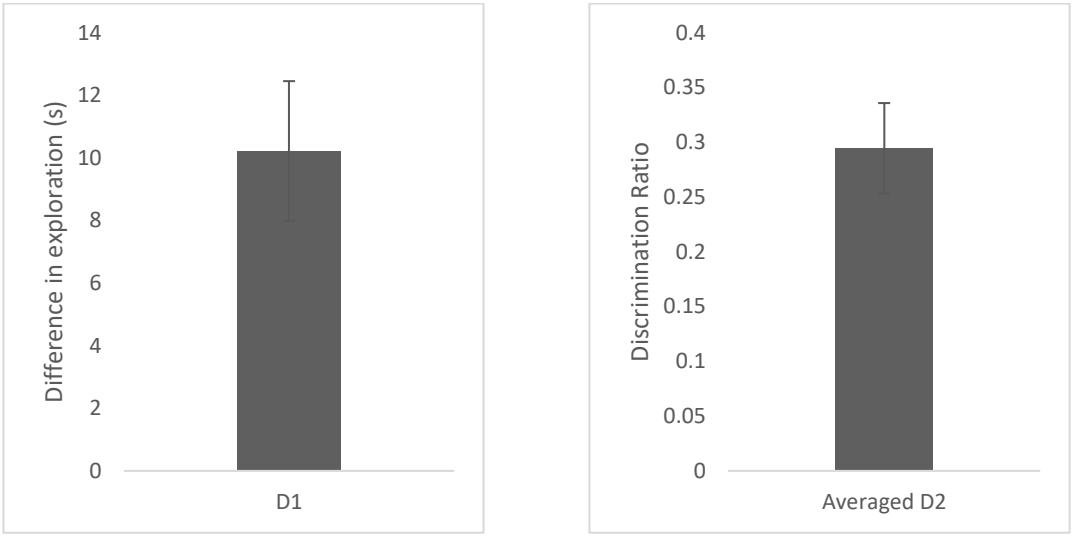



*Figure 5.8* depicts performance of TASTPM mice in the SOR task at 7 months based on D1 measures (*left)* and the averaged D2 ratio (*right)*. Both analyses were based on all animals that were tested in the SOR at 7 months of age (n = 7). TASTPM mice showed above chance performance in the SOR task for both D1 and averaged D2 ratio. Vertical bars represent the standard error of mean.

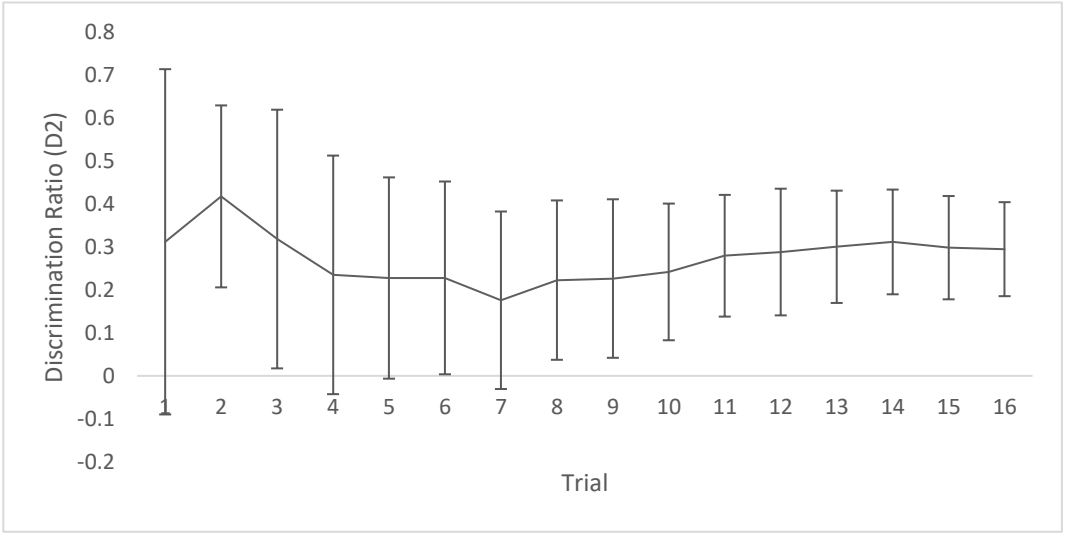



*Figure 5.9* represents the averaged D2 curve of the spontaneous object recognition task at 7 months. Performance of TASTPM mice were stable throughout the session. Averaged D2 ratios were calculated by obtaining the 'running average' of each trial within the session. Verical bars represent the standard error of the mean.

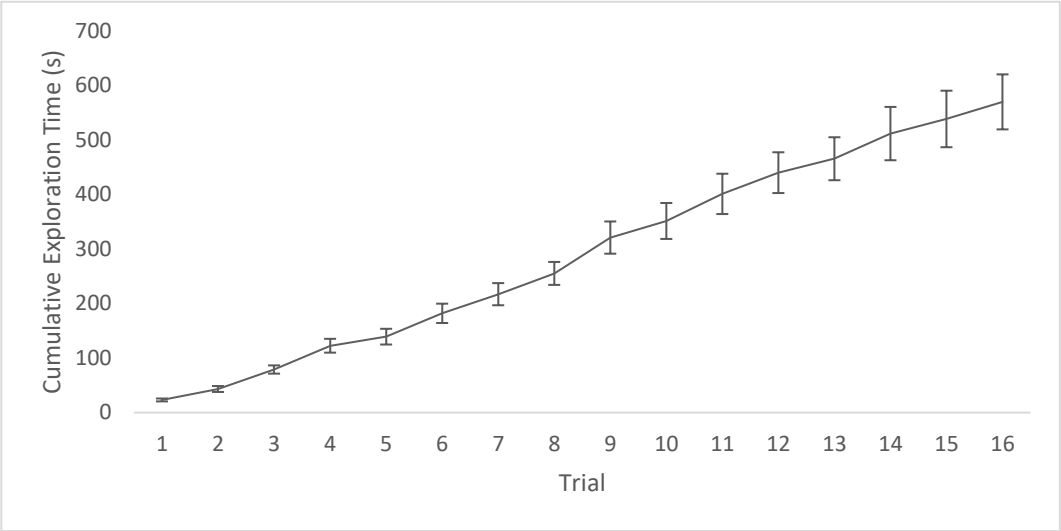



*Figure 5.10* represents the cumulative exploration times within the session. The graph shows a linear increase throughout the session, indicating that TASTPM mice continuously explored both objects until the end of the session. Cumulative exploration time by trial 16 was 570.1 seconds, which means mice spent an average of 35.63 seconds exploring both novel and familiar objects during each test trial. Vertical bars represent standard error of the mean.

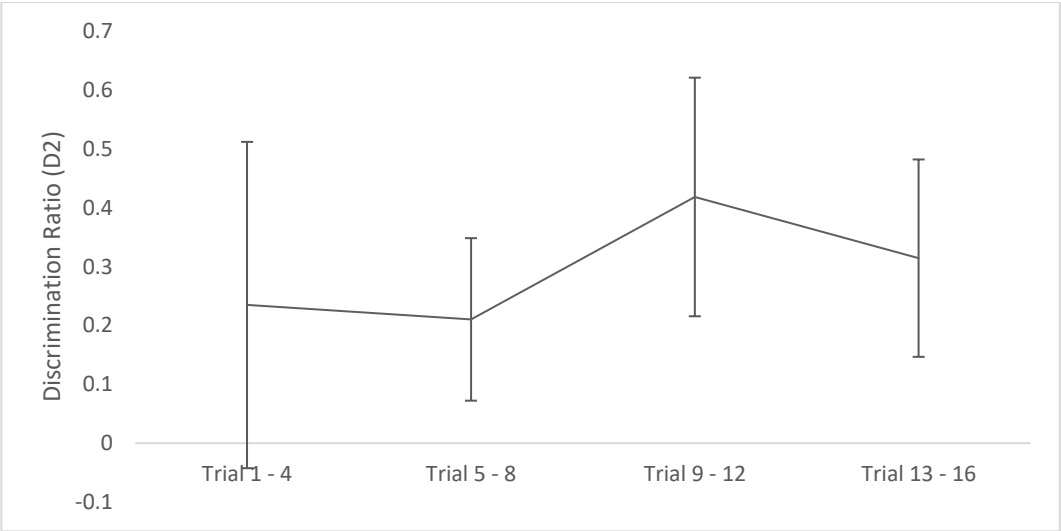



*Figure 5.11* shows the mean averaged D2 ratios of TASTPM mice performance across 16 trials, blocked into four sets of four consecutive trials. Performance levels across the blocked trials were stable and did not show significant fluctuations. Error bars indicate Standard Error of Mean.

#### 5.3.1.2 Object location task

To determine if 7-month old TASTPM mice performance was above chance in discriminating objects in the novel over familiar location, a one-sample t-test (two-tailed) was used to compare group D1 scores and averaged D2 ratio against zero. The results found that at 7 months of age, TASTPM mice performed at chance level in the object location task (mean D1 (±SEM) = 1.63 (±1.24), $t(5) = 1.31$, $p = 0.25$; mean averaged D2 (±SEM) = 0.05 (±0.03), $t(5) = 1.94$, $p = 0.11$), indicating preferential exploration towards objects in the novel location. See figure 5.9 for details.

As in the spontaneous object recognition task, performance changes across the session were measured by comparing four blocks of four trials. Blocks were obtained by calculating the mean of groups of 4 trials for each animal. Performance level changes were measured by running a repeated measures ANOVA on all four blocks and results found that animal performance changed during the session, $F(3, 15) = 6.31$, $p < 0.01$, and a Bonferroni pairwise comparison revealed different levels of performance between blocks 1 and 2 ($p = 0.005$), blocks 1 and 3 ($p = 0.005$) and trial blocks 1 and 4 ( $p < 0.05$). Group performance in block 1 (first 4 trials) was significantly higher than block 2, 3 and 4, indicating that group performance fell then stabilised during the session. Refer to figure 5.15.

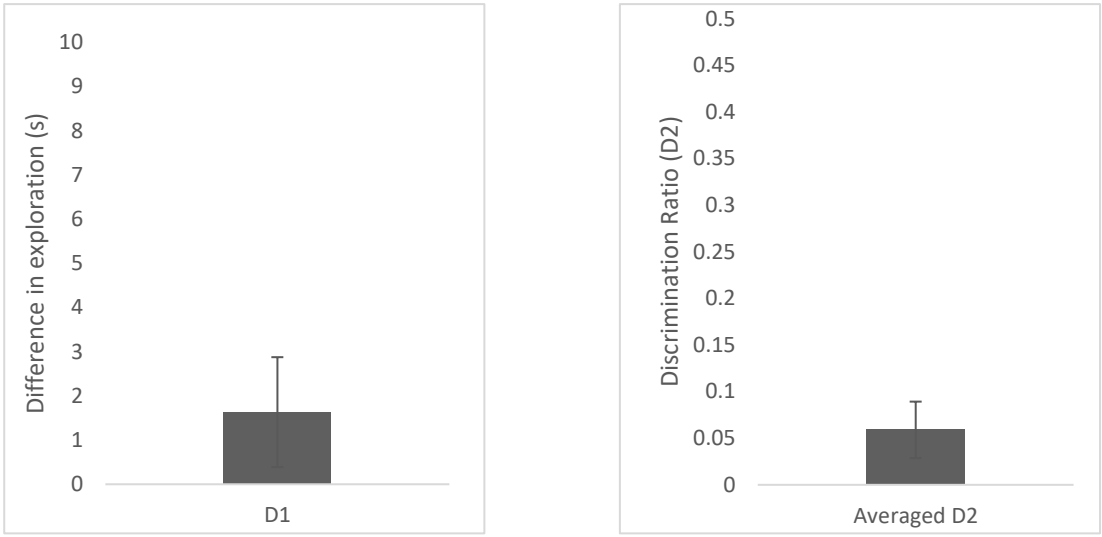



*Figure 5.12* represents performance of TASTPM mice at 7 months in the object location task based on the D1 scores (left) and averaged D2 ratio (right). Analysis on both measures found chance level performance of TASTPM mice in the object location task. Vertical bars represent the standard error of the mean.

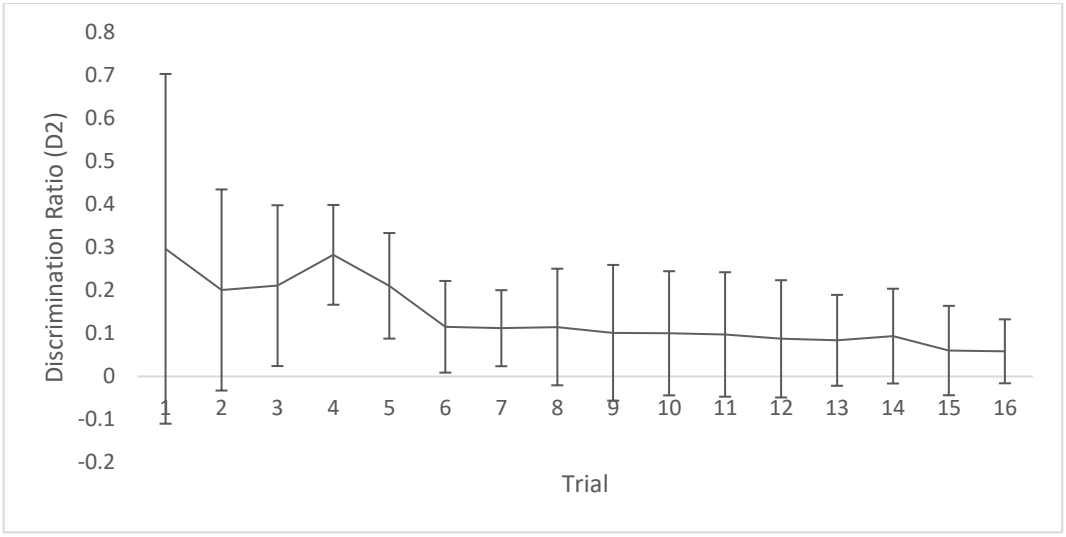



*Figure 5.13* represents the averaged D2 curve of the object location task at 7 months. At the start of the session, performance of TASTPM mice were at a D2 of 0.3, but performance gradually fell after trial 4 and remained stable until the end of the session. Averaged D2 ratios were calculated by obtaining the 'running average' of each trial within the session. Vertical bars represent the standard error of the mean.

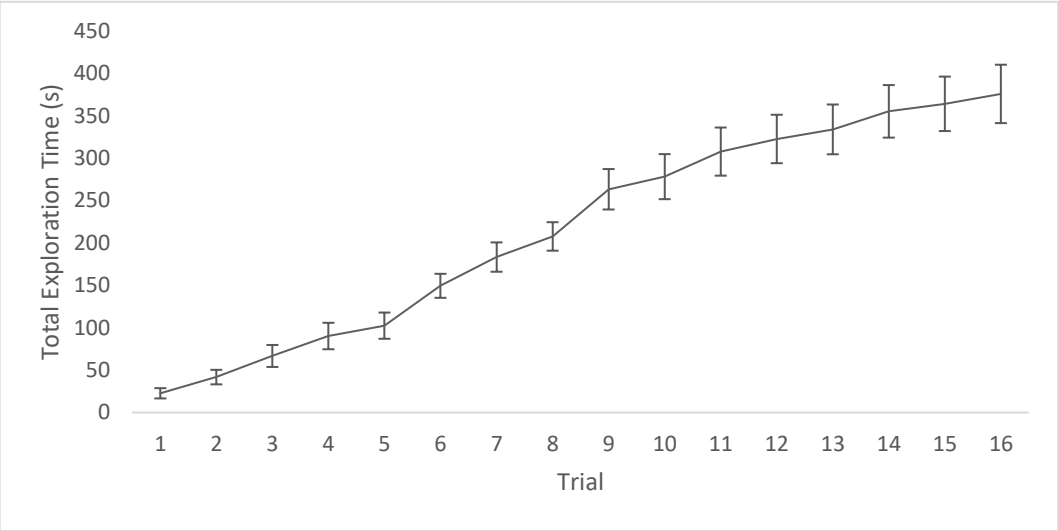



*Figure 5.14* represents the cumulative exploration times within the session. The graph shows a linear increase throughout the session, indicating that TASTPM mice continuously explored both objects until the end of the session. Cumulative exploration time by trial 16 was 375.89 seconds, which means mice spent an average of 23.49 seconds exploring both novel and familiar object locations during each test trial. Vertical bars represent standard error of the mean.

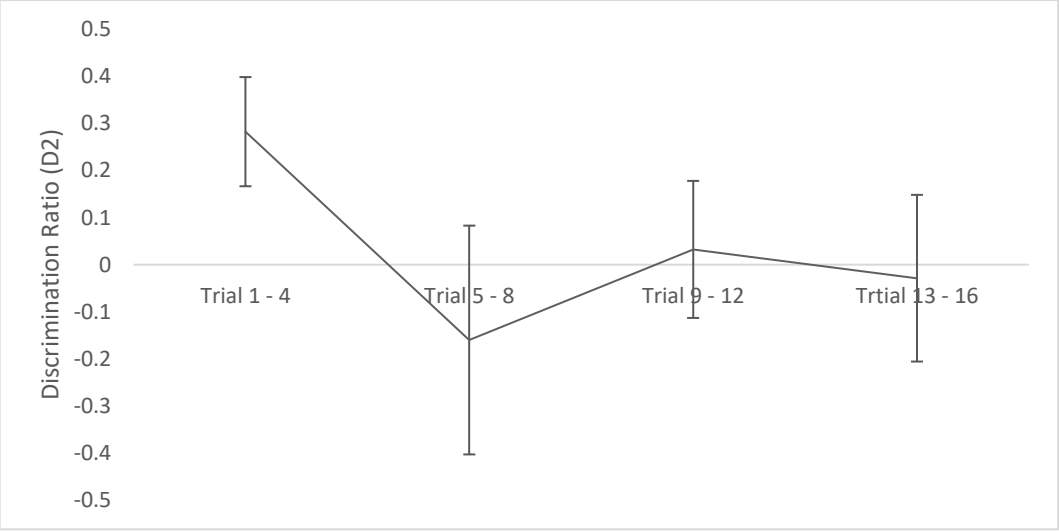



*Figure 5.15* shows the mean averaged D2 ratios of TASTPM mice performance across 16 trials, blocked into four sets of four consecutive trials.Performance level at block 1 (first 4 trials) were significantly higher than blocks 2, 3 and 4. Error bars indicate the standard error of the mean.

### 5.3.2 Performance of TASTPM mice at 10 months of age

Since experienced and naïve TASTPM mice showed similar performance in both spontaneous object recognition and object location task, animals from both groups were grouped together in the following analysis.

#### 5.3.2.1 Spontaneous object recognition

To see if 10 month old TASTPM mice show the ability to discriminate between the novel and familiar object, a one-sample t-test (two-tailed) was used to compare D1 scores and averaged D2 ratio against zero. It was found that mice successfully demonstrated recognition memory by showing preference towards the novel objects (mean D1 (± SEM) = 7.12(± 1.60), $t(9) = 4.45$, $p < 0.005$; mean averaged D2(± SEM) = 0.18(± 0.04), $t(9) = 4.58$, $p = 0.001$). See figure 5.16 for D1 and averaged D2 graphs respectively.

Change in performance levels across the session was measured by running a Repeated Measures ANOVA on four blocks of 4 trials; segregated over 16 trials. As in previous chapters, blocks were obtained by calculating the mean of the averaged D2 of the first four trials and consecutive blocks of trials of each animal. No evidence of block effects was found ($F(3, 24) = 0.43$, $p < 0.05$), indicating that 10 month old TASTPM did not show fluctuations in performance during the testing session (see figure 5.19).

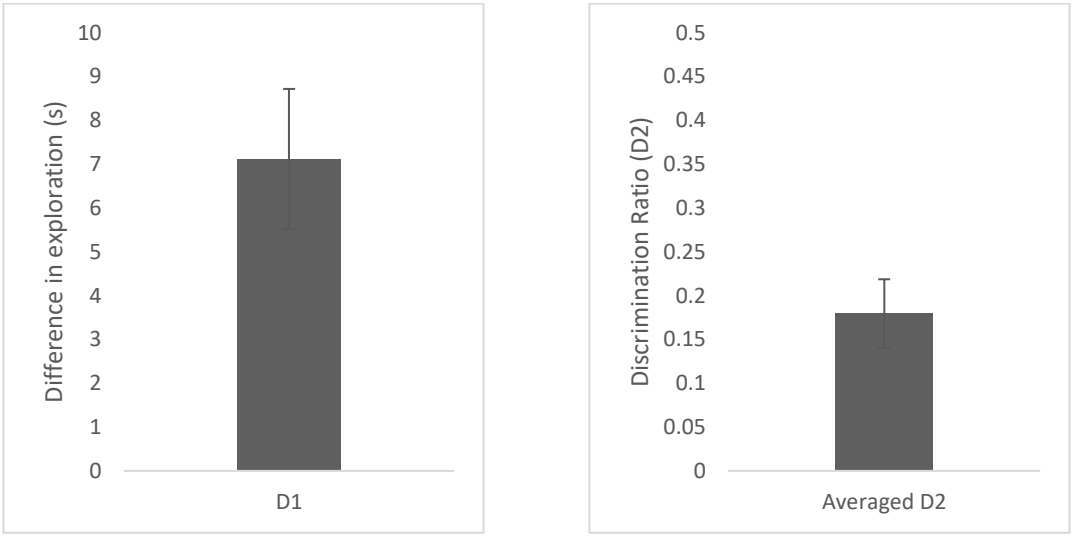



*Figure 5.16* depicts performance of TASTPM mice in the SOR task at 10 months based on D1 measures (*left)* and the averaged D2 ratio (*right)*. Both analyses were based on all animals that were tested in the SOR at 10 months of age (n = 10). TASTPM mice showed above chance performance in the SOR task for both D1 and averaged D2 ratio. Vertical bars represent the standard error of mean.

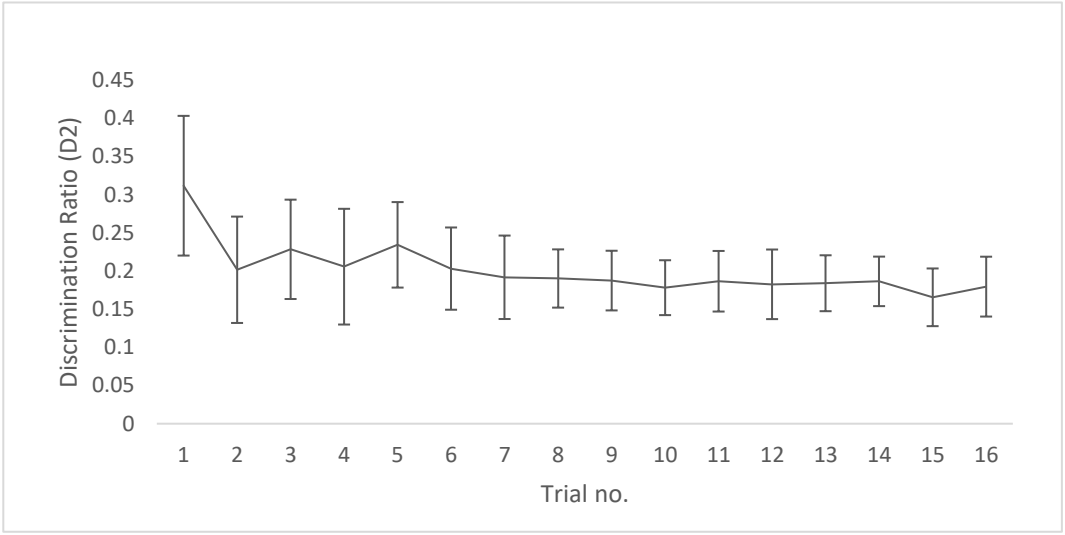



*Figure 5.17* represents the averaged D2 curve of the spontaneous object recognition task at 10 months. Performance of TASTPM mice were stable throughout the session. Averaged D2 ratios were calculated by obtaining the 'running average' of each trial within the session. Verical bars represent the standard error of the mean.

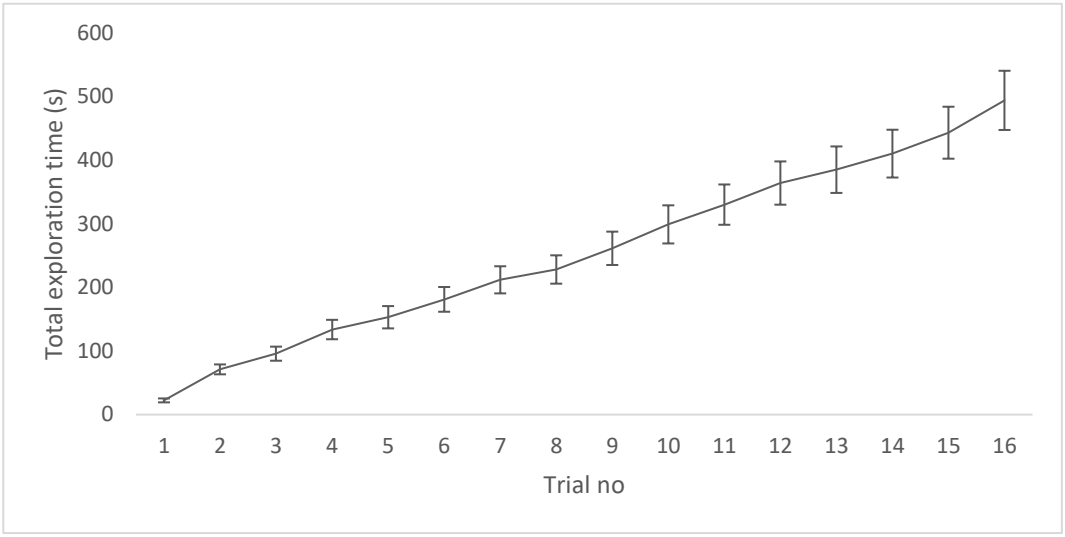



*Figure 5.18* represents the cumulative exploration times within the session. The graph shows a linear increase throughout the session, indicating that TASTPM mice continuously explored both objects until the end of the session. Cumulative exploration time by trial 16 was 494.2 seconds, which means mice spent an average of 30.89 seconds exploring both novel and familiar objects during each test trial. Vertical bars represent standard error of the mean.

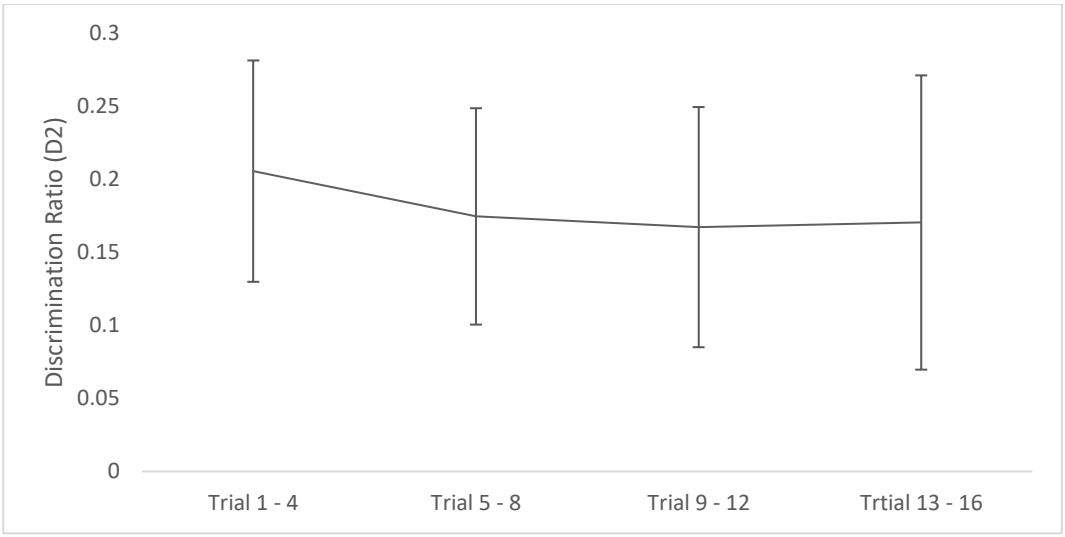



*Figure 5.19* shows the mean averaged D2 ratios of TASTPM mice performance across 16 trials, blocked into four sets of four consecutive trials. Performance levels across the blocked trials were stable and did not show significant fluctuations. Error bars indicate standard error of mean.

#### 5.3.2.2 Object location

To determine if 10 month old experienced TASTPM mice performance was above chance in discriminating objects in the novel over familiar location, a one-sample t-test (two-tailed) was used to compare group D1 scores and averaged D2 ratio against zero. The results found that at 10 months of age, performance level of TASTPM mice was above chance in the object location task when the averaged D2 scores were analysed (mean averaged D2 (±SEM) = 0.06 (±0.02), t (9) = 2.36, p = 0.042) and performance level was at chance when D1 scores were analysed (mean D1 (±SEM) = 1.96 (±0.95), t(9) = 2.06, p = 0.069), indicating mice showed preferential exploration towards objects in the novel location when analysis was based on one measure (averaged D2) but not another (D1). See figure 5.20.

As in previous sections, performance changes across the session were measured by comparing four blocks of four trials. Blocks were obtained by calculating the mean of groups of 4 trials for each animal. Performance level changes were measured by running a repeated measures ANOVA on all four blocks and results found no effect of block, F(3, 27) = 1.87, p = 0.16, indicating that performance remained stable and did not significantly change during the testing session. Refer to figure 5.23.

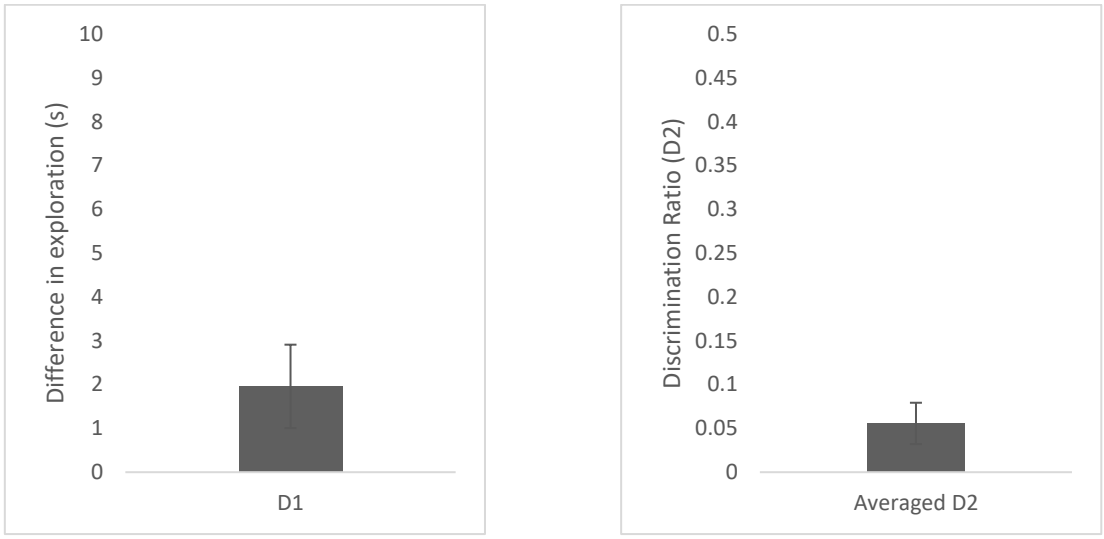



*Figure 5.20* represents performance of TASTPM mice at 10 months in the object location task based on the D1 scores (left) and averaged D2 ratio (right). Analysis on both measures found chance level performance of TASTPM mice in the object location task. Vertical bars represent the standard error of the mean.

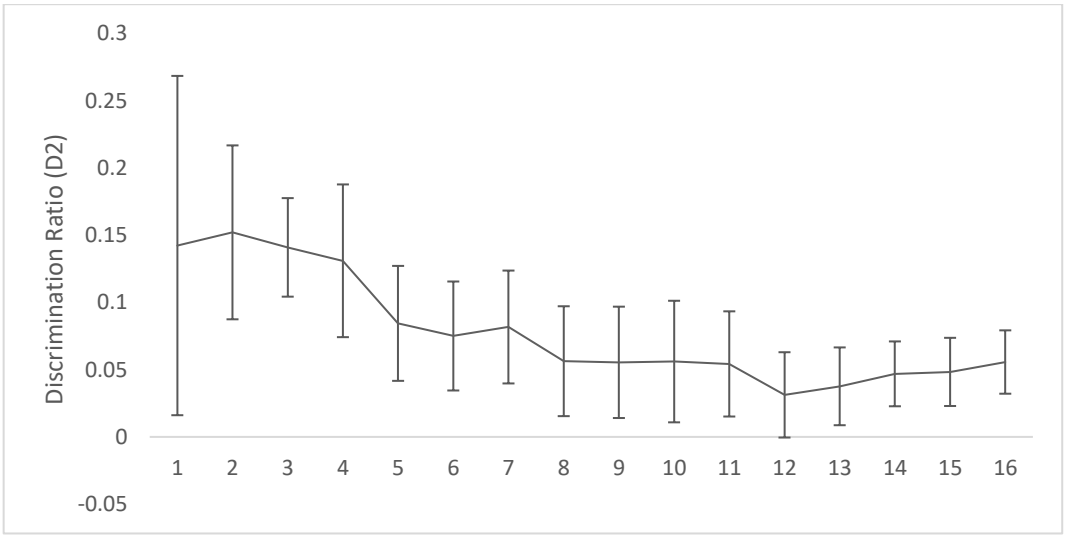



*Figure 5.21* represents the averaged D2 curve of the object location task at 10 months. Averaged D2 ratios were calculated by obtaining the 'running average' of each trial within the session. Verical bars represent the standard error of the mean.

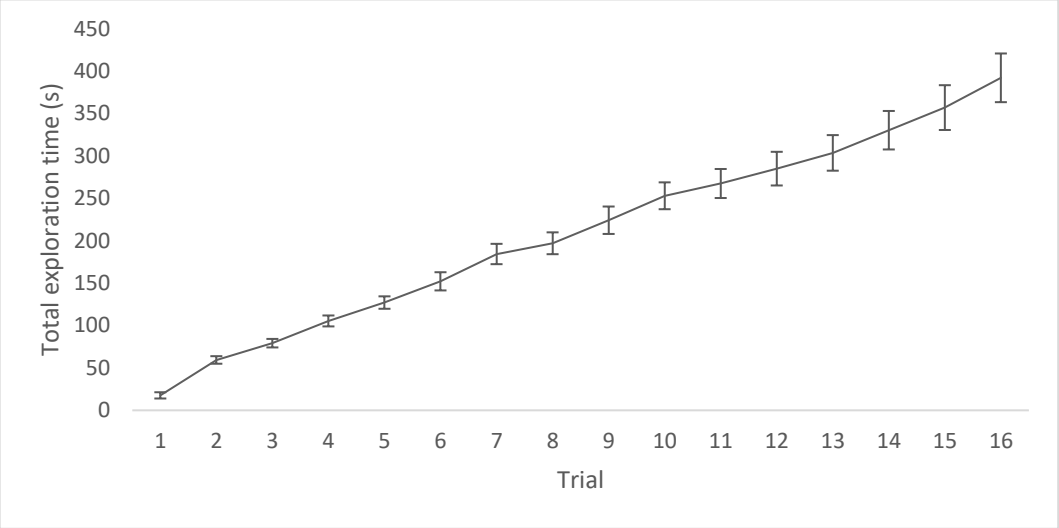



*Figure 5.22* represents the cumulative exploration times within the session. The graph shows a linear increase throughout the session, indicating that TASTPM mice continuously explored both objects until the end of the session. Cumulative exploration time by trial 16 was 392.24 seconds, which means mice spent an average of 24.52 seconds exploring both novel and familiar object locations during each test trial. Vertical bars represent standard error of the mean.

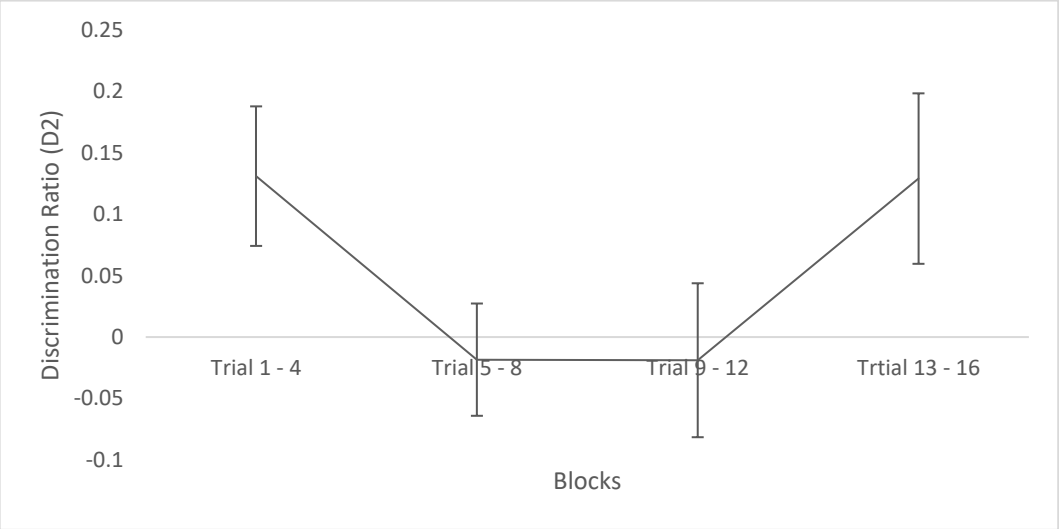



*Figure 5.23* shows the mean averaged D2 ratios of TASTPM mice performance across 16 trials, blocked into four sets of four consecutive trials. Performance level of TASTPM mice were stable throughout the session. Error bars indicate standard error of mean.

| | | Trial number | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age** | **Task** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| **7** | **SOR** | 0.31 | 0.52 | 0.12 | -0.02 | 0.20 | 0.23 | -0.14 | 0.55 | 0.26 | 0.38 | 0.66 | 0.38 | 0.45 | 0.46 | 0.11 | 0.24 |
| | **OL** | 0.30 | 0.11 | 0.23 | 0.50 | -0.08 | -0.36 | 0.09 | 0.13 | -0.005 | 0.09 | 0.07 | -0.02 | 0.04 | 0.22 | -0.41 | 0.03 |
| **10** | **SOR** | 0.20 | 0.20 | 0.28 | 0.14 | 0.35 | 0.05 | 0.12 | 0.18 | 0.17 | 0.09 | 0.27 | 0.14 | 0.20 | 0.22 | -0.13 | 0.39 |
| | **OL** | 0.14 | 0.16 | 0.12 | 0.10 | -0.10 | 0.03 | 0.12 | -0.12 | 0.05 | 0.06 | 0.04 | -0.22 | 0.11 | 0.17 | 0.07 | 0.17 |

*Table 5.3* details the performance levels (averaged D2 ratio) of TASTPM mice within the testing session in the spontaneous object recognition (SOR) and object location (OL) task at 7 and 10 months of age.

## 5.4 Discussion

This study aimed to (1) validate the continual trials approach to running the spontaneous object recognition and object location task in the TASTPM mice; and (2) to provide a behavioural paradigm that helps to clarify conflicting findings in the literature. To achieve this, we compared the performance of TASTPM mice at 7 and 10 months old in the spontaneous object recognition and object location task; and at 10 months, introduced naïve animals to compare their performance against the experienced group of TASTPM mice.

To determine if TASTPM mice show changes in recognition and location memory with age, we tested mice in the multiple trials version of the spontaneous object recognition and object location task at 7 and 10 months of age (figure 5.2 and 5.3). This study found that TASPM mice failed to show an age-related decline in both tasks. Performance in the object location task was also worse than performance in the spontaneous object recognition task, however this is only true with the averaged D2 measure. This confirmed findings by Scullion and colleagues (2011), which tested TASTPM mice at 4 – 8 months old in the object recognition task and found that mice did not exhibit an age-related decline in recognition memory. In fact, like in Scullion's study, TASTPM mice in the present study successfully discriminated the novel from the familiar object at 7 months of age. Although, Scullion et al., (2011) did not test TASTPM mice beyond 8 months old. Howlett et al., (2004) found that TASTPM from 6 months old was impaired in the object recognition task compared to wildtype mice. Furthermore, their finding showed that 10 month old TASTPM mice performance in the object recognition task was not above chance, a finding that was not confirmed in this study. However, it is important to note that the Howlett's (2004) study used naïve mice, whereas the 10 month old mice in the current study had prior experience at 7 months of age.

Previous studies examining spatial/location memory of TASTPM mice in the spatial alternation task, the Y-maze, and Morris Water Maze (Scullion et al., 2011) have found that

performance in these tasks gradually declines with age. To fill in the gaps in the literature on spatial memory, the present study examined performance of TASTPM mice in the object location task at 7 and 10 months of age. Contrary to previous studies examining spatial memory in TASTPM mice, the present study did not find an age-related decline in the object location task. However, at both time points, performance of TASTPM mice were at chance level, indicating that from 7 months, TASTPM mice was impaired in object location memory. Performance levels of TASTPM mice during the testing session dropped off after trial 4 and remained thus until the end of the session. This pattern was not observed in 10 month old TASTPM mice performance, which suggests that for some time and prior to 10 months, TASTPM mice may be susceptible to interference of object location memory.

The present study showed that the TASTPM mice exploration times did not decrease with age and exploration times between tasks were not different either. Spontaneous object recognition task and its variants are reliant on free exploration of objects based on an animals' preference towards novelty (Ennaceur and Delaceur, 1988). Previous study (Willig et al., 1987) found that aged rats spent less time exploring novel objects in an object exploration task compared to young rats. Scullion et al., (2011) have also found this to be true; that the exploratory drive of TASTPM mice decreases as the animals ages. Also, studies (Howlett et al., 2004; Scullion et al., 2011 respectively) reported that TASTPM mice spent 5 – 8 seconds and 7 month old TASTPM mice spent about 9 seconds exploring the novel objects; the current study found that mice spent an average of 19s exploring novel objects. Baker and Kim (2002) have shown that exposure to stress may impair recognition memory in animals; thus by running the multiple trials version of spontaneous tasks, exploration would not be masked by stress caused by repeated handling.

Discrepancies between performance of TASTPM mice in the object recognition task have been thought to be caused by the levels of previous experience in the task (Scullion et al., 2011; Howlett et al. 2004). To rectify that, the current study investigated the influence

of different experience levels of TASTPM mice on performance in recognition memory tasks. This study compared performance of experienced TASTPM mice (that were initially tested at 7 months old) and naïve TASTPM mice performance in the object recognition and object location task at 10 months of age; it was found that experience levels did not have an effect on performance in both tasks. Naïve and experienced TASTPM mice also spent similar amounts of time exploring the novel and familiar objects/locations.

Aside from providing evidence that the continual trials approach was simplistic enough to set up in a diseased model, the current study provided further evidence of the TASTPM mice model as a transgenic model of Alzheimer's Disease by testing object recognition and object location memory in the continual trials apparatus. The findings of Scullion et al., (2011) have been confirmed, that up to 10 months, TASTPM mice do not show an age-related decline in object recognition and location memory. Performance of TASTPM mice in the object location task has not been characterised prior to this study, and although mice did not show an age-related decline in the task, performance in this task was worse compared to the object recognition task and performance levels of TASTPM mice impaired in the task both at 7 and 10 months. Further work should aim to investigate performance of TASTPM mice compared to wildtype controls in the multiple trials version of the spontaneous object recognition and object location task to determine that the impairments of TASTPM mice were not due to floor effects, especially in the object recognition task.

# Chapter 6

## Study 5: Delay-dependent performance on recognition memory in the continual trials apparatus.

### 6.1 Introduction

The present study examined the effects of variable delays on performance of mice in the spontaneous object recognition task. To achieve this, the continual trials was adapted to incorporate variable retentions delays between sample and test phases; and mice were tested in a multiple trials version of the spontaneous object recognition task with retention delays of 1, 4 and 24 hours between sample (acquisition) and test (retrieval). Experiment 1 of this study was a pilot study to validate the separation of the sample and test phase with a 1-hour retention delay. Experiment 2 of this study, investigated the delay-dependent effect of memory with a 1-, 4- and 24-hour delay between sample and test. The findings in this study supported Hammond et al., (2004; Taglialatela et al., 2009; Hall et al., 2016) showing that mice do not exhibit delay-dependent decline in memory with retention delays of up to 24 hours.

As outlined in Chapter 1, the spontaneous object recognition task has been instrumental in the investigation of short-term and long-term recognition memory in rodents. Also, this enabled the examination of precognitive and amnesic effects of drug infusions or transgenes. This was achieved through the manipulation of the retention intervals between the sample (acquisition) and test (retrieval) phase. Previous literature on the effects of retention delay on recognition memory in the spontaneous object recognition task have produced conflicting findings, with some studies exhibiting delay-dependent effects on recognition memory (Winters & Bussey, 2005; Dodart et al., 1997; Sik et al., 2003); whilst other studies found no such effect (Hammond et al., 2004; Jessberger et al., 2009; Winters

et al., 2004; Bruin et al., 2006; de Lima et al., 2006; Hall et al., 2016; Taglialatela et al., 2009).

Work done by Sik and colleagues in 2003 compared the performance of different strains that were typically used as background for transgenic manipulations in a task of recognition memory at various retention delays. They examined the performance of Swiss, BALB/c, 129/sv, and C57BL/6J mice in the spontaneous object recognition task at 1-, 4- and 24-hour delays and found a delay-dependent effect on recognition memory. They also found that depending on different discrimination measures yielded different conclusions; based on the D1 measure (difference in exploration times between novel and familiar object), performance levels were dependent on strain but pointed out that this was due to different exploration levels of the mouse strains; with the Swiss and BALB/c showing high exploratory behaviour, the 129/sv exhibiting low exploratory behaviour and the C57s falling somewhere in between. However, when exploration behaviour between the different strains were accounted for (D2 ratio), Sik et al., found that the delay-dependent decrease in performance levels did not differ between strains.

In contrast to Sik et al., (2003), in a study by Hall et al., (2016), which investigated the performance of Tc1 (a transgenic model of Down Syndrome) and wildtype controls in the spontaneous object recognition task at immediate (30s), short-term (10-min) and long-term (24-hour) delay, found that wildtype (C57) mice performance levels at short-term (10-min) vs. long-term (24-hour) delay were similar with mean discrimination ratio of 0.75 and 0.73 respectively. Whereas immediate (30s) vs short-term (10-min) mean discrimination ratios were 0.71 and 0.76 respectively. This indicated that wildtype controls did not exhibit delay-dependent effects on performance in the spontaneous object recognition task.

Whilst delays were widely incorporated in the standard spontaneous object recognition task, retention delays have yet to be manipulated in the mouse version of the continual trials version of the spontaneous object recognition task. Further, to our knowledge, only one study (Albasser et al., 2010) actively manipulated retention delays in the bow-tie maze. Albasser et al., (2010) conducted an experiment comparing performance of Lister Hooded and Dark Agouti rats on the spontaneous object recognition performance at short, medium and long retention delays in the bow-tie maze. During the short delay, rats received a 24-trial session where performance at first half of the session (Trial 1 – 12; immediate < 1-minute delay), was compared against an increasing delay of 2 – 24 minutes at the second half of the session. For both medium (3 hour) and long (24 hour) retention delays, rats received 20 trial sessions which after the immediate delay at trial 1 – 10, animals were returned to their home cage for the duration of the retention delay, and then followed by a second session (trial 11 – 20) where the animals were tested for their memory of objects presented during trials 1 – 10. Albasser and colleagues found that both Lister Hooded and Dark Agouti rats showed a delay dependent effect; whereby performance in the < 1 min delay was superior compared to performance during the short (2 – 24 minute), medium (3 hour) and long (24 hour) delay. They also found that Lister Hooded rats performed better than Dark Agouti rats at the short and long retention delays.

The experiments in this chapter used a paradigm which combined the advantages of the delayed non-match to sample (DNMS) and the spontaneous object recognition task: multiple trials version of the spontaneous object recognition task (Ameen-Ali et al., 2012; Albasser et al., 2010). As the name suggests, this task combines the naturalistic paradigm of the SOR task and the multiple trials of DNMS task. The one trial SOR is known also for having high variance caused by day to day differences in animal behaviour, and to reduce said variance, experiments run a large number of animals over many days. By using the

multiple trials version of the OR, we managed to reduce the number of mice used in the task by 25% whilst maintaining statistical power (see chapter 3, for details).

The incorporation of retention delays in this study was different than that of Albasser et al., (2010), in Albasser and colleagues study, an experimental session was separated into two halves, the first half of the session examined recognition memory at a retention interval of less than 1 minute, after which the animals were returned to their home cages for 3- or 24-hours to await the second half of the session. The objects from the first half of the session acted as the sample exposure and baseline for testing after the retention delay. Furthermore, at the second half of the session, the objects that were initially presented in the first half were presented in reverse order, which meant that if object A was the first object to be presented in the first half of the session, object A would be tested last in the second half of the session. Unlike Albassers and colleagues procedure, the current study utilised a relatively simple design, whereby testing sessions were divided into two phases: a sample phase, where pairs of identical objects were presented sequentially, and a test phase, in which the corresponding familiar and novel objects were presented.

The aim of this study was to investigate delay dependent memory at 1, 4 and 24 hours across different delays in the recognition memory task by the method of multiple trials in mice. Experiment 1 of this study was a pilot experiment investigating the effects of 1-hour retention delay on recognition memory in mice, using the modified method as explained above. Experiment 2 of this study aimed to investigate the effects of various retention delays (1-, 4- and 24-hour) on recognition memory in the multiple trials apparatus. We expect to find a delay-dependent effect of recognition memory as seen in Sik et al., (2003) study.

## 6.2 Experiment 1: Spontaneous object recognition task with 1-hour retention delay.

## 6.3 Materials and Methods

### 6.3.1 Subjects

Twelve experimentally naïve female C57BL/6J sourced from Envigo (formerly Harlan, UK) were used as subjects in this experiment. The animals were housed in groups of 4 in open top cages. Sawdust bedding, nesting material, cardboard rolls and hammock were provided in cages as enrichment. Animals were food deprived to 90 – 95% their free feeding weight. Water was freely available throughout the study except during habituation and testing. Mice were 12 weeks old and weighed between 16.0 – 18.0 grams at the start of the experiment.

### 6.3.2 Apparatus

The apparatus used in this experiment was used in previous chapters and details of the apparatus were described in General Methods, Chapter 2, section 2.1 (see figure 2.1). Objects were placed in the top-left and right hand corner of the testing area at 3cm equidistant from the walls to ensure optimal object exploration (see figure 2.1 for object placement).

### 6.3.3 Objects

Various types of junk objects were used in this study. Objects had different shapes, sizes, colours and textures. In order to prevent bias caused by olfactory cues, multiple copies of objects were used in this study. Animals did not re-encounter specific objects during the experiment. Refer to Chapter 2, Section 2.2 for further details and examples of objects used in this study.

### 6.3.4 Behavioural analysis

Behaviour analysis details were listed in General Methods Chapter 2, Section 2.5. As in the previous chapter, analyses in this chapter will focus on the D1 measure and averaged D2 ratio. Task performance was analysed by comparing the group D1 measure and D2 ratio in the one sample t-test (two-tailed) against 0. Changes in performance levels within the session were determined by a Repeated Measures ANOVA on four blocks of 2 trials. Blocks were obtained by averaging 2 consecutive trials until the end of the session.

### 6.3.5 Habituation and pre-training

The experiments in this study were conducted in a dark room illuminated by diffused lighting originating from a table lamp (50w lightbulb) which was positioned to shine to a wall. White noise was continuously played in the background in order to mask noises that came from outside the room. These conditions were maintained all throughout habituation and experiment proper.

Prior to the start of pre-training, all animals received five handling sessions to ensure that the animals were accustomed to being handled by the experimenter; and to minimize anxiety from subsequent handling. Pre-training consisted of four stages and the stages are as follows: Stage 1, cage mates freely exploring the apparatus for 30 minutes; Stage 2, a single mouse freely exploring the apparatus for 20 minutes; Stage 3, shuttling training for 10 minutes; and Stage 4, exposure to 2 pairs of objects for 5 minutes. Further details of pre-training were described in General methods Chapter 2, Section 2.3.

### 6.3.6 Testing protocol

All animals in this experiment received an 8-trial testing session with an hour delay between the sample and test phases.

Initially, mice were placed in the holding area of the continual trials apparatus. After 1 minute, the central door opened to allow the mouse to shuttle into the object area. The animal was given 2 minutes to explore a pair of identical objects located in the top-left and top-right hand corner of the apparatus. At the end of the sample trial 1, the side arm doors were opened to allow the mouse to return to the holding area for 1 minute. While the mouse waits in the holding area, the experimenter changes the objects in the test area to prepare for the next sample trial. This was done to ensure that animals were not disturbed by objects being changed around in the object area. After a minute, the central door was opened and the mouse shuttled to the object area to start sample trial 2. The object area now contained a new pair of identical objects. After 2 minutes, the side arm doors were opened once more so the mouse was able to return to the holding area. This procedure was repeated until the end of the sample phase (trial 8).

Upon completing the sample phase, the mouse was then placed back into its home cage and returned to the holding room before the start of the test phase at the end of the delay period. Mice in their home cages were brought back into the experiment room for the test phase 5 minutes before the start of the test phase. The animal was placed in the holding area to await the start of the test phase. At the end of the hour delay, the central arm door was opened to allow the mouse to move into the object area which contained a copy of the object previously seen in sample trial 1 and a novel object. After 2 minutes, the side arm doors open to allow the mouse to return to the holding area for 1 minute. The central door was opened once more to reveal a novel object and a copy of a familiar object encountered in sample trial 2 in the object area. At the end of test trial 2, the side arm doors opened and the animal

shuttled back into the holding area. This procedure was repeated until the end of the testing phase (Trial 8).

The hour delay between sample and test began at the end of sample trial 1. This was to ensure that there were 1-hour delay between each trial of the sample and test phases during the testing session.

To minimise side bias, the novel objects in this experiment were counterbalanced by being presented equally on the left and right side (4 trials on the left; 4 trials on the right) during the test session. Objects were counterbalanced between animals to minimise the effects of object salience. Further counterbalancing details are found in chapter 2, section 2.4.1.

If an animal failed to shuttle to the next compartment (area) within 3 minutes after the doors opened, the testing session would then be stopped, and all behavioural data of the animal would be excluded from the analysis.

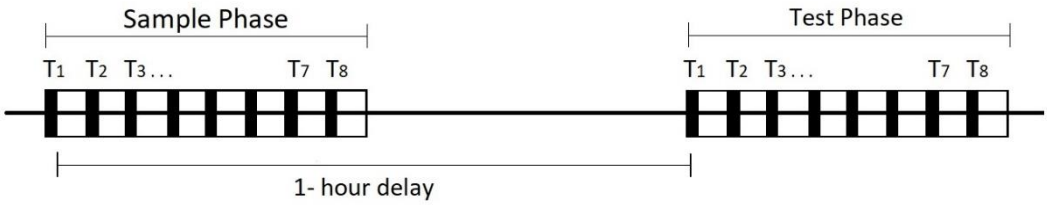



*Figure 6.1* shows the protocol structure of the current experiment with an hour delay between the sample and test phase. As mentioned in the test protocol (see above), timing for the one-hour delay started after the end of Sample Trial 1. The block on the left represents the sample phase, whereas the block on the right represents the test phase. The sample and test phases lasted for at least 20 minutes, and would take longer depending on the time taken for mice to shuttle between compartments during the session. In between the sample and test, the mouse, in its home cage would be placed in the holding room.

## 6.4 Results

To determine whether mice were able to discriminate novel from the familiar objects after an hour delay, one-sample (two-tailed) t-tests (against 0) were used to analyse group D1 and averaged D2 ratios. The analyses found that animals' performance was above chance, demonstrating memory of previously seen object an hour prior to test. Mean D1 (±SEM) = 4.24 (±1.15), $t(11) = 3.66$, $p < 0.005$; mean averaged D2 (±SEM) = 0.16 (±0.04), $t(11) = 3.96$, $p < 0.005$; see figure 6.2 left and right for respective graphs.

To investigate if performance levels changed during the session, trials in the session were divided into blocks and analysed with a repeated measures ANOVA. Unlike previous studies (for example study 1, chapter 3), whereby blocks were obtained by calculating the average of every 4 trials. However, due to the smaller length of the current session and further experiments (8 trials), blocks were obtained by calculating the mean of the first 2 trials and each subsequent pairs of trials (1 and 2, 3 and 4, 5 and 6, etc) to make up 4 blocks. The analysis found no effect of block, $F(3, 33) = 0.578$, $p = 0.634$; sphericity assumed, suggesting that performance throughout the session was stable and did not show significant fluctuations, refer to figure 6.5.

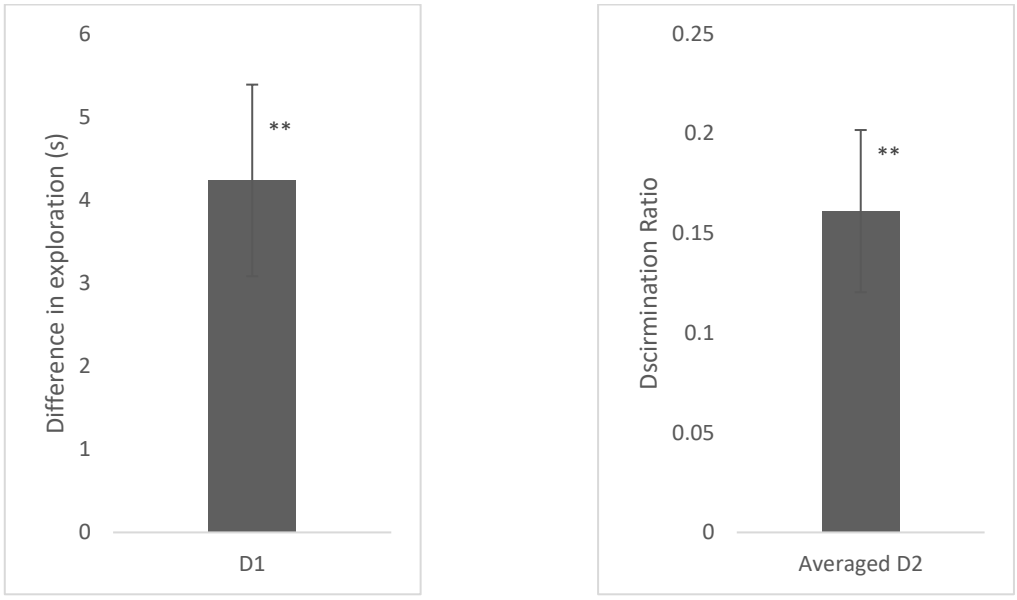



*Figure 6.2* Group means of the averaged and updated D2 ratios over an 8-trial session (n = 12 animals). D1 measure (*left*): Mice showed the ability to discriminate between the novel and familiar object at a 1-hour delay between sample and test, $t(11) = 3.66$, $p < 0.005$**, mean = 4.24 (±1.15). Averaged D2 ratio (*right*): Mice showed above chance performance in the SOR task with an hour delay between sample and test: $t(11) = t(11) = 3.96$, $p < 0.005$**, mean = 0.16 (±0.04). Error bars indicate the standard error of the mean.

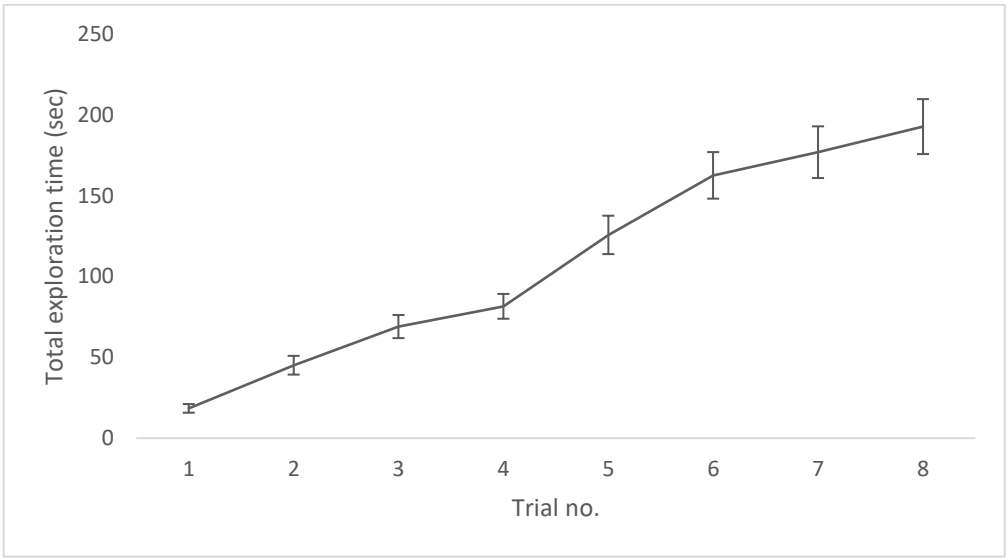



*Figure 6.3* Total exploration (novel and familiar object) during the test phase. The linear increase of total exploration times across the session showed that mice continuously explored objects across the test phase. At the end of the session (trial 8), total exploration time was 197.73 (±17.0), indicating that an animal spent an average of 24.71 seconds exploring objects at each test trial. Vertical bars represent standard error of the mean.

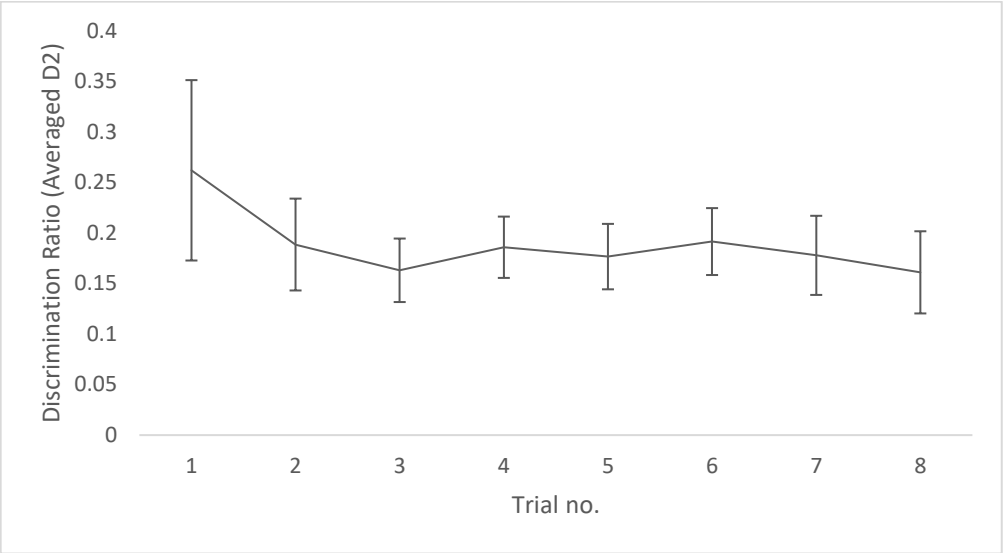



*Figure 6.4* The graph depict averaged D2 ratios across the testing session. Performance was consistent throughout the session, with a mean of 0.26 at the beginning of the session and 0.16 at the end of the session. Averaged D2 scores for each trial were obtained by calculating the 'running average' of each trial within a session. Error bars indicate the standard error of the mean.

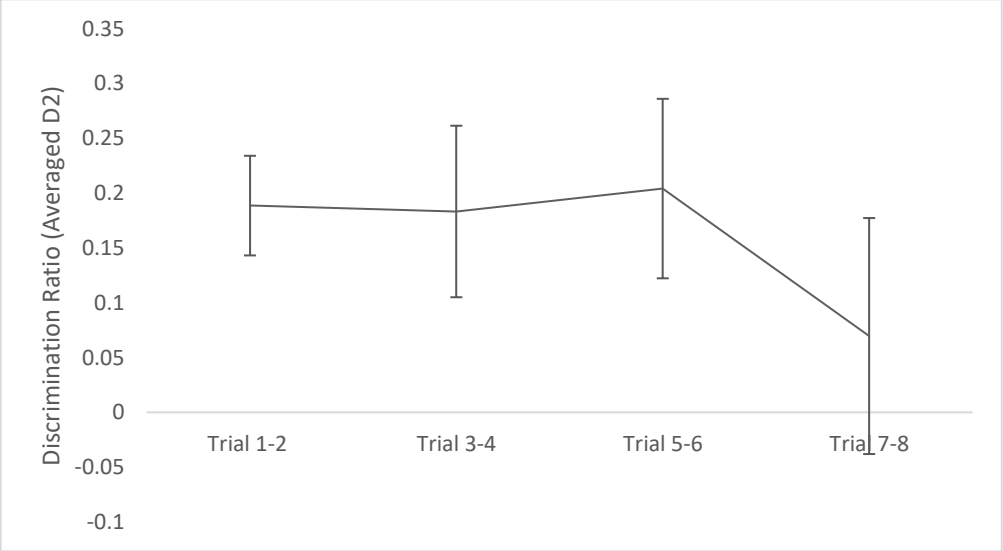



*Figure 6.5* shows the mean averaged D2 ratios of mice performance (SOR at 1-hour delay) across 8 trials, blocked into four sets of two consecutive trials. Error bars indicate the standard error of the mean.

## 6.5 Discussion

This experiment was a pilot that aimed to validate an adapted version of the continual trials spontaneous object recognition task designed to manipulate retention delays. This was achieved by separating the testing session into two halves, to comprise of a sample phase and a test phase block with an hour retention interval between both the phases. This experiment found that performance of mice was above chance at a 1-hour retention delay between acquisition and retrieval. This finding supports previous studies examining recognition memory after an hour retention delay (Sik et al., 2003; Winters et al., 2004; Dodart et al., 2007) which found that rodents showed evidence of retaining memory of the familiar object after an hour.

The risk of changes of performance levels caused by proactive interference is typical when running tasks involving multiple trials (Ameen-Ali et al., 2012; Albasser et al., 2010; Seel et al., 2017). There was no evidence of performance levels changes of mice, suggesting that the level of proactive interference was not high enough to alter performance of mice during the session.

The following experiment aimed to examine whether mice exhibit delay-dependent effects on object recognition memory. Mice received 3 testing session in the multiple trials version of the spontaneous object recognition task at 1-, 4-, and 24-hour retention delay. We predict that mice in this experiment will exhibit a delay-dependent decline of object recognition memory, similar to what Sik et al., (2003) found.

## 6.6 Experiment 2: Effects of variable retention delays (1, 4 and 24 hours) on object recognition memory.

## 6.7 Materials and Methods

### 6.7.1 Subjects

All mice used in this experiment were used in previous Experiment 1 and housing conditions were identical to that of Experiment 1.

### 6.7.2 Testing Protocol

The testing protocol of this experiment was identical to that of the previous experiment except that all animals received 3 testing sessions with delays of 1, 4 and 24 hours between sample and test phases. Testing sessions were separated into three blocks whereby each block lasted a week. This experiment used a latin square design (see figure 6.6); mice were separated into groups of based on their housing groups and either received group 1, group 2 or group 3 testing sequence. The table below illustrates the testing sequence between delays and groups.

| Week | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| 1 | 1-hour | 4-hour | 24-hour |
| 2 | 4-hour | 24-hour | 1-hour |
| 3 | 24-hour | 1-hour | 4-hour |

*Figure 6.6* represents the testing sequence of the current experiment. Utilising a Latin Square design, mice were divided into 3 groups (based on their cage groups) and were received testing orders based on the group allocated.

### 6.8 Results

Delay-dependent effects on recognition memory was measured by comparing group performance (D1 scores and averaged D2 ratio) at 1-, 4- and 24-hour delays with a Repeated Measures ANOVA. The analyses found no delay dependent effects on memory, whereby animals performance was similar across 1-, 4-, and 24-hour delay intervals. D1: $F(2, 22) = 0.395$, $p = 0.678$; and averaged D2: $F(2, 22) = 0.032$, $p = 0.968$ (see figure 6.7 for graphical representation).

As in the previous experiment, to determine if mice performance in the object recognition task at one, 4 and 24 hours delay was above chance, one sample (two-tailed) t-tests were used to compare group D1 scores against 0. It was found that mice performance at 1- and 24-hour delay were above chance level, (1 hour: mean (±SEM) = 5.18 (±1.53), $t(11) = 3.38$, $p < 0.01$; 24 hours: mean (±SEM) = 4.14 (±1.22), $t(11) = 3.38$, $p < 0.01$), and was unable to discriminate between the novel from the familiar object when the delay between sample and test was at 4 hours (mean (±SEM) = 3.18 (±1.69), $t(11) = 1.89$, $p = 0.086$), see figure 6.7 *upper*.

Analysis of animal performance using group averaged D2 ratios (one sample t-test against 0), have shown that mice showed preferential exploratory behaviour towards the novel objects, indicating memory of the familiar objects at delay intervals of 1-, 4- and 24-hours. 1-hour: mean (±SEM) = 0.19 (±0.04), $t(11) = 4.57$, $p = 0.001$; 4-hour: mean (±SEM) = 0.18 (±0.06), $t(11) = 3.05$, $p < 0.05$; 24-hour: mean (±SEM) = 0.19 (±0.05), $t(11) = 4.11$, $p < 0.005$, see figure 6.7 *lower left*.

As in the previous experiment (section 6.4), performance level changes across the session was measured by analysing four blocks of trials with a repeated measures ANOVA. Identical to the previous experiment, blocks were obtained by calculating means of the averaged D2 between the first 2 trials and subsequent pairs of trials. The analyses found that

across all delays (1, 4 and 24 hour), no block effects were found (1 hour: $F(3, 33) = 0.295$, $p = 0.829$, sphericity assumed; 4 hour: $F(3, 33) = 0.913$, $p = 0.445$, sphericity assumed; and 24 hour: $F(3, 33) = 0.722$, $p = 0.961$, sphericity assumed). This indicates that, at different delay intervals, performance levels did not change across the session (refer to figure 6.8).

A further repeated measures ANOVA was used to analyse the total exploration times (figure 6.11) of mice during the test phases across 1-, 4-, and 24-hour retention delays. It was revealed that the total time spent exploring both object did not differ across all delays, $F(2, 22) = 0.348$, $p = 0.71$.

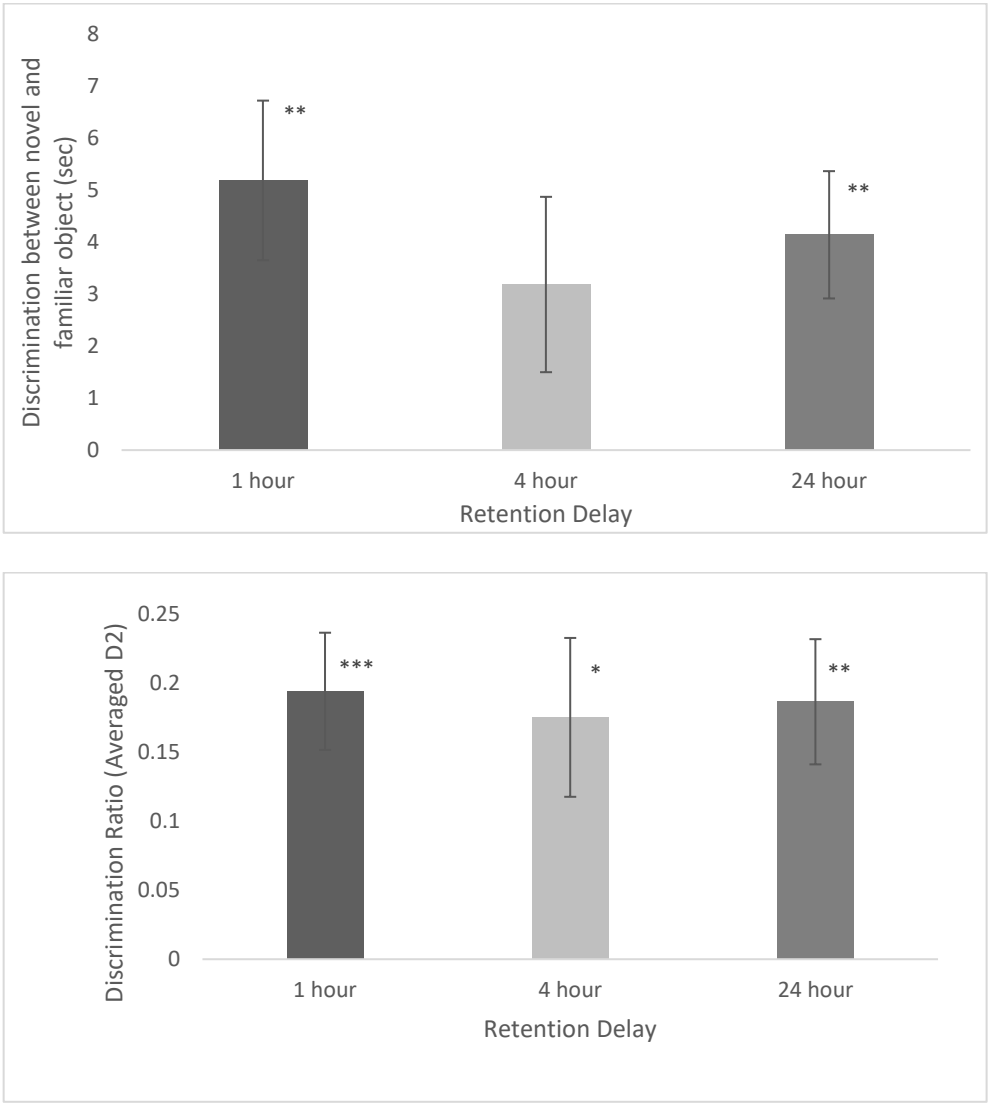



*Figure 6.7* depicts object recognition performance at 1-, 4- and 24-hour retention delays. *Upper* Difference between novel and familiar object (D1 scores). *Lower* shows the averaged D2 ratio. Mice showed above chance performance across all retention delays, but no delay dependent effect was found; $p < 0.001$***, $p < 0.01$**, $p < 0.05$*. Vertical bars represent standard error of the mean (SEM).

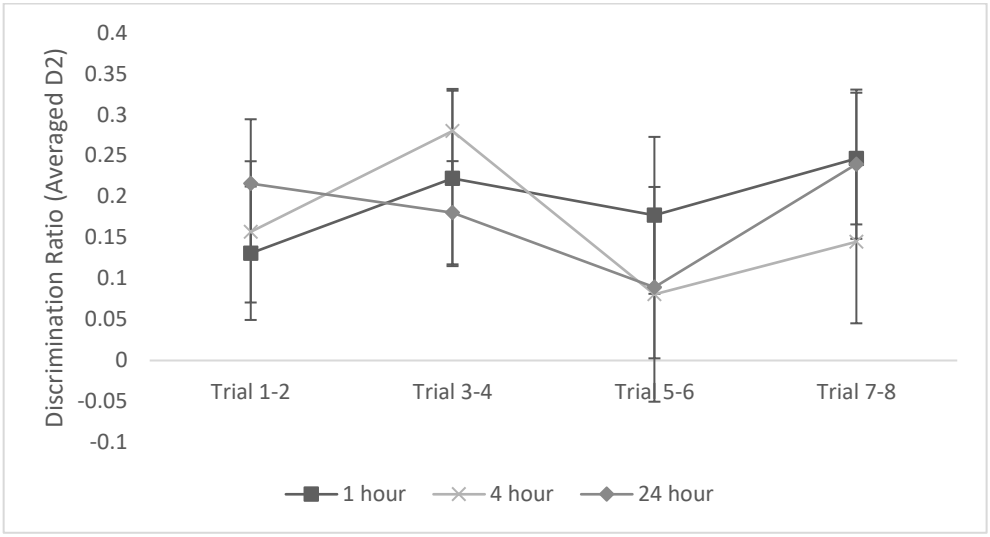



*Figure 6.8* shows the mean averaged D2 ratios of animal performance at 1-, 4- and 24 hour retention delay in the object recognition task across 8 trials. Trials were segregated into four blocks of two consecutive trials. Findings indicated that performance levels within the sessions were unchanged and there were no differences in levels of performance between all retention delays. Error bars represent standard error of the mean.

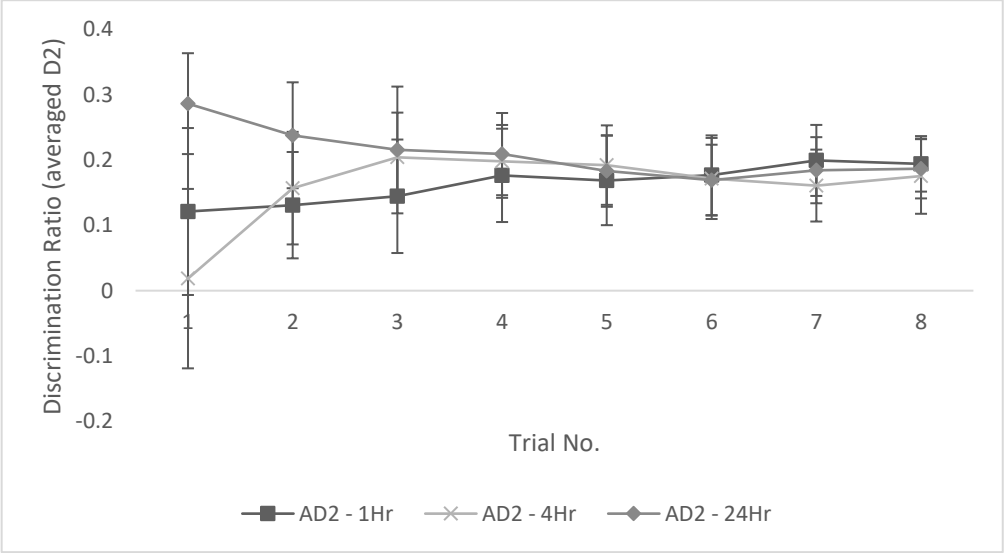



*Figure 6.9* represents the averaged D2 curve of animal performance in the object recognition task at 1-, 4- and 24 hour retention delays. Performance across all delays show that, although performance levels were different at the beginning of the session, animal performance stabilised from trial 3 onwards until the end of the session. The averaged D2 ratio for each trial was obtained by calculating the 'running average' of each trial within the session. Vertical bars represent standard error of the mean.

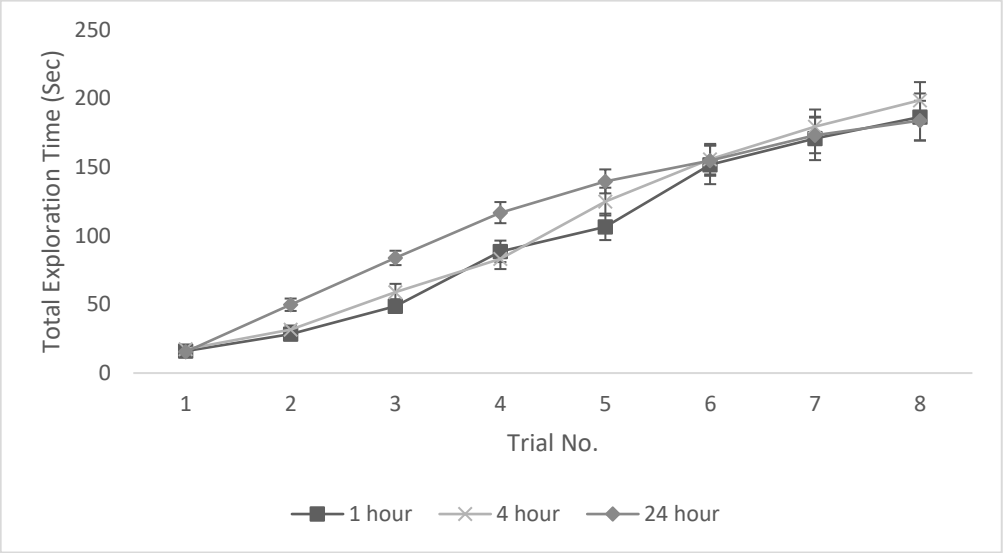



*Figure 6.10* shows the total time spent exploring the novel and familiar objects during the test phase (1-,4- and 24- hour retention delays). There was a linear increase in total exploration times across all retention delays, indicating that animals continously explored the objects within the session. Cumulative exploration by the end of the session were as follows: 1-hour = 186.56 sec, 4-hour = 198.79 and 24-hour = 183.98; which means that mice spent an average of 23.32, 24.85 and 23.00 seconds exploring objects on each trial across all retention delays respectively. Error bars represent standard error of the mean.

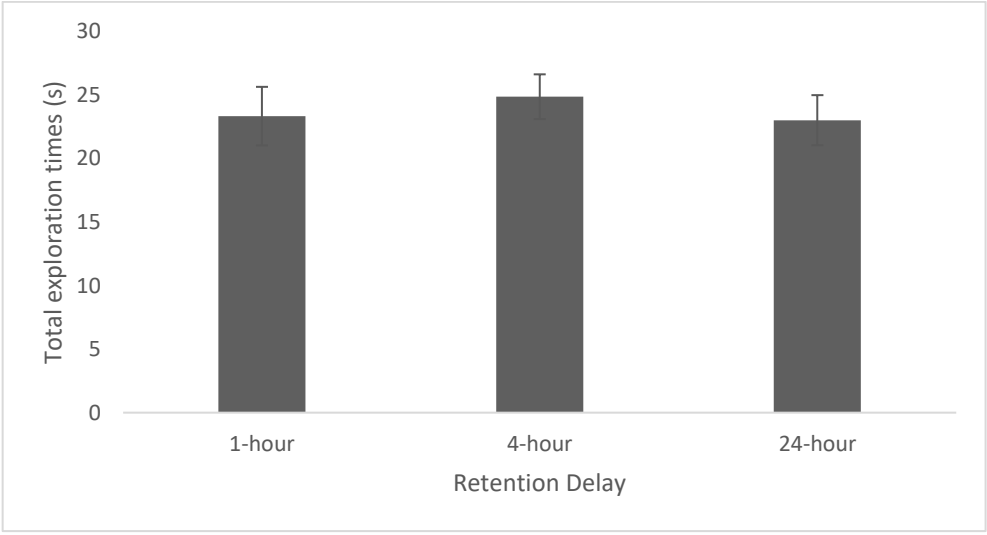



*Figure 6.11* represents the mean total exploration times at 1-, 4- and 24-hour retention delays in the sample phase of the testing session. Mice spent similar amount of time exploring the objects across all retention delays. The error bars represent the standard error of the mean.

| | | Trial number | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Delay** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| **Experiment 1** | 1-hour | 0.26 | 0.12 | 0.11 | 0.25 | 0.14 | 0.27 | 0.10 | 0.04 |
| **Experiment 2** | 1-hour | 0.12 | 0.14 | 0.17 | 0.27 | 0.14 | 0.22 | 0.34 | 0.16 |
| | 4-hour | 0.02 | 0.30 | 0.30 | 0.18 | 0.17 | 0.07 | 0.10 | 0.28 |
| | 24-hour | 0.29 | 0.19 | 0.17 | 0.19 | 0.08 | 0.10 | 0.28 | 0.20 |

*Table 6.1* details the object recognition memory (averaged D2 scores) of mice within the testing session of experiment 1 and 2 of the present study. The 1, 4 and 24 hours represent the delays between the sample phase and test phase of the experiment.

## 6.9 General Discussion

This experiment aimed to investigate whether delay-dependent forgetting occurs in object recognition memory. To achieve this, the continual trials paradigm was adapted to incorporate longer retention delays between the sample and test phases; and mice were tested in the continual trials version of the spontaneous object recognition task at 1-, 4- and 24-hour retention delays. This experiment found no delay-dependent effect on recognition memory in the continual trials task, supporting the findings of Albasser et al., (2010) and Hall et al., (2016).

Albasser and colleagues found that although rats exhibited a decline in recognition memory at immediate (1 min) vs medium- (3-hour) and long-delays (24-hours), rats had similar performance levels at 3-hour and 24-hour delay (approximate D2 value = 0.09 for both delays). This finding was similar to that of the current experiment, whereby the mean D2 ratios of the 4- and 24-hour delays were similar, averaging at around 0.1. Which suggested that over longer delays, the increased sensitivity the continual trials task was able to detect subtle changes or lack of changes in memory across long-term delays. Furthermore, work by Hall et al., in 2016 found that performance of wildtype mice at immediate (30s) delay did not differ from 10 minutes delay and performance at 10 mins delay was not different from 24-hours delay; which further supports the findings of the current study.

As in the previous experiment, performance levels of mice in the current experiment across the testing sessions did not change across all retention delays. With multiple trials task, there is often a risk of interference that may adversely affect animal performance, especially with increasing retention delays. However, this experiment found little or no evidence of interference across the session and at retention delays of 1-, 4- and 24-hours. The present study (experiment 2) found that mice demonstrated similar exploration levels across all retention delays.

To conclude, the present chapter aimed to investigate the effects of delay-dependent effects on memory in a continual trials version of the spontaneous object recognition task. To do this, experiment 1 validated the multiple trials version of the spontaneous object recognition task, and experiment 2 investigated the evidence of delay-dependent decline in recognition memory. The current study provided a baseline for performance of mice at various retention delays in the continual trials apparatus, which widens the potential of the approach to test the effects of pharmacological substances on recognition memory.

Following the successful adaptation of the continual trials paradigm to test the effects of variable retention delays in object recognition memory, the next chapter of this thesis will present a study investigating the effects of NMDA blockade on object recognition memory in mice using the continual trials approach.

# Chapter 7

## Study 5: The effects of NMDA receptor antagonist MK-801 on recognition memory in mice.

### 7.1 Introduction

The current chapter aimed to examine the role of NMDA receptors on the modulation of short-term and long-term object recognition memory in mice using the continual trials apparatus. The NMDA receptor antagonist MK-801 was systemically injected before exposure and/or test during long-term (24-hours) and immediate (1-minute) test of object recognition memory. Using a 2x2 state-dependent design, the present chapter will establish at this stage that, at a lower dose (0.01mg/kg) of MK-801, recognition memory was state-dependent and had no effect on level of activity. The state-dependent effect on recognition memory found in the lower dose was replicated in the higher dose (0.1mg/kg) of MK-801, but displayed an effect of drug (increased distance travelled in the MK groups).

The role of the neurotransmitter glutamate in the formation of memory processes has been widely studied, and a large body of evidence have shown that the N-methyl-D-aspartate (NMDA) receptors are closely linked to learning and memory processes, especially during encoding (for review see Riedel et al., 2003). The role of NMDA receptors in learning and memory have been investigated using tasks of object recognition memory (Winters and Bussey, 2005; Barker et al., 2006; King et al., 2004; Nilsson et al., 2007; de Lima et al., 2005; Pitsikas, et al., 2006; van der Staay et al., 2011) and object-location/ object-in-place task (Barker and Warbutron, 2008; Adriani et al., 1998; Han et al., 2013) by the administration of competitive and non-competitive antagonist. AP5, although low in bioavailability is one of the most widely used competitive NMDA antagonist in animal behavioural studies due to its high selectivity and limited side effects (Riedel et al., 2003).

MK-801 (dizocilpine maleate), on the other hand, is a non-competitive and selective NMDA antagonist that has a high affinity towards NMDA receptors (Wong et al., 1986) and acts by binding to NMDA receptors and blocking the channel pore in a use-dependent manner. Furthermore, the range of which MK-801 could be used therapeutically is limited, in part because higher dosages of the drug causes ataxia and large behavioural changes occur within a narrow dose range (Nilsson et al., 2007). In a study conducted by Andine et al., (1999), found that although rats injected with 0.1mg/kg of MK-801 did not display sensorimotor impairments, a higher dose of 0.2mg/kg of the drug induced ataxia in female rats and the same effect was seen in male rats at 0.5mg/kg of MK-801. Similar effects were also found in mice, whereby ataxia begins to occur at 0.3mg/kg of the drug administration (Nilsson et al., 2007). Behavioural changes in animals administered with MK-801 found increased levels of locomotor activity (Amalric et al., 1994; Mele et al., 1994; Hargraves and Cain, 1992).

The effects of the NMDA receptor antagonist, MK-801 have been examined in different tasks of learning and memory, and it is widely acknowledged that the drug impairs performance in learning and memory task during encoding. This has been found in investigations of the radial arm maze (Caramanos and Shapiro, 1994; Huang et al., 2004; White and Best, 1998), passive avoidance (Bevenga and Spaulding, 1988; Harrod et al., 2001; Venable and Kelly, 1990), t-maze alternation (Mackes and Wilner, 2006), morris water maze (McLamb et al., 1990; Filliat and Blanchet, 1995; Ahlander et al., 1999; Uekita and Okaichi, 2005). Also, there are studies which found that administration of MK-801 post-exposure or pre-test impaired performance in learning and memory tasks (Boess et al., 2004; de Lima et al., 2005; Vales et al., 2006; da Silva et al., 2009; Ko and Evenden, 2009).

Whilst administration of MK-801 has been known to impair performance in tasks of memory, some studies have instead found facilitating effects of MK-801 on memory. For example, in a step-down passive avoidance task (Mondadori et al., 1989; Mondadori and

Weiskrantz, 1993) and passive avoidance task (Mondadori et al., 1989), animals that received the drug post learning showed improved retention.

Previous studies investigating the effects of MK-801 on object recognition memory found that impairments occurred when MK-801 was administered prior to the exposure phase (de Lima et al., 2005; King et al., 2007; Nilsson et al., 2007; van der Staay et al., 2011). Investigations into the effects of MK-801 administration post-exposure or pre-test however, has been conflicting, with some studies (de Lima et al., 2005; Pichat et al., 2007) reporting impairment of performance; whilst other studies reported facilitating effects of the drug (Nilsson et al., 2007) in the object recognition task.

Work by de Lima and colleagues (2005) which examined dose dependent (0.001, 0.01 and 0.1 mg/kg) effects of MK-801 found that when the drug was administered pre-exposure, rats that received 0.01 and 0.1 mg/kg MK-801 showed impaired performance compared to the saline control group in a test of short-term (1.5-hour) and long-term (24 hour) novel object recognition memory. Further, when the 0.1mg/kg of the drug was administered immediately after the exposure phase, rats displayed impaired novel object recognition in both short-term and long-term memory relative to saline controls.

In a separate study by Nilsson et al., (2007), mice received either injections of saline or MK-801 (0.1mg/kg and 0.2mg/kg) prior to exposure, immediately after exposure, or pre-test in an object recognition task. Object recognition memory was tested 1.5 hours after exposure phase and found that when the drug was given prior to exposure, mice showed impaired performance in the task. However, when the drug was administered post-exposure or pre-test, the animals instead showed increased novel object exploration, indicates that the MK-801 has facilitating effect on retention memory and suggested that the activation of the NMDA receptors is required for encoding but not consolidation and retrieval.

To our knowledge, previous literature examining the effects of MK-801 on learning and memory, primarily the novel object recognition task, would investigate the impacts of

the drug pre-exposure, post-exposure and pre-test, but failed to include state-dependent controls in their studies. To fill in the gap in this literature, the current study examined state-dependent learning in the spontaneous object recognition task by introducing a group of mice which received drug at both sample and test.

State-dependent learning and memory is a form of information processing whereby acquisition of information is encoded during a particular state and retrieval of said information is dependent on the state in which encoding occurred (Radulvic, Jovasevic and Meyer, 2017). Initially described in the 1930s by Girden and Geller in a study which reported that dogs administered with cucare prior to being conditioned to leg flexion response, were unable to display the conditioned response when curare was no longer in the animals' system. However, the dogs elicited the leg flexion response when the animals were administered cucare again. Since then, state-dependency learning has been demonstrated in several difference species, most of which involves the administration of drugs. Research on state-dependent learning have been extensively found in the investigations of drug effects on passive avoidance and learning (Harrod et al., 2001; Overton, 1991; Koek, 2011), but not in tasks of recognition memory.

In a study by Harrod and colleagues (2001), they investigated whether the NMDA antagonist MK-801 blocked the acquisition and retention in a passive avoidance task by utilizing a state-dependent learning design. The state-dependent learning employs a 2x2 design which results in saline-saline, saline-drug, drug-saline and drug-drug groups. Rats received intraperitoneal injections of either saline or MK-801 (0.05 and 0.10 mg/kg) 30 minutes prior to training and test. The acquisition of passive avoidance response was measured two minutes and 24-hours after the end of training. They reported that, at a dose of 0.05mg/kg, rats showed only marginal state-dependent learning at 24-hour retention tests; however, when rats were administered with a higher dose of the drug (0.1mg/kg), they found that passive avoidance response was state-dependent.

The present chapter consists of four experiments which aimed to examine the effects of MK-801 on object recognition memory when administered during acquisition and retrieval phases. As briefly mentioned above, Experiment 1 aimed to investigate whether a low dose (0.01mg/kg) of MK-801 would block acquisition and retention during a long retention delay (24-hours) using a state-dependent learning design; Experiment 2 examined if state-dependency effects that were found during the low dose were present with administration of a higher (0.1mg/kg) dose of MK-801. Experiment 3 of the present chapter investigated the effects of higher dose (0.1mg/kg) MK-801 on short-term object recognition memory. Lastly, experiment 4, examined the whether an effect of state-dependency was found at a higher dose during a 24-hour retention delay in the spontaneous object recognition task.

Immediate and long-term spontaneous object recognition memory in the present chapter was examined using a novel multiple trial method which have been found to increase sensitivity and reduce within animal variance (as detailed in chapter 3). Thus far, as shown in previous studies in this thesis, the use of the continual trials method enabled further contribution to different fields in memory research, such as ageing, Alzheimer's disease, and presently, the functions of NMDA receptors in learning and memory. The continual trials task combines the advantages of the DMNS (multiple trials) and spontaneous object recognition (no-prior training and food reward) tasks, which in turn results in reduced time taken to collect data and increased sensitivity due to a decrease in the day-to-day variance in animal behaviour.

Based on the previous literature available on the effects of MK-801 on object recognition memory, the expected findings in this chapter would include impairment in performance of mice that received administration of MK-801 prior to exposure phase compared to saline controls.

## 7.2 Materials and Methods

### 7.2.1 Apparatus

The apparatus used in this experiment was identical to that used in previous chapters and details of the apparatus can be found in Chapter 2 (General methods), section 2.1 (See figure 2.1 for details).

### 7.2.2 Objects

Various junk objects with different properties were used in this study. Junk objects were described in Chapter 2, section 2.2, see figure 2.2 for examples of junk objects.

### 7.2.3 Behavioural analysis

Refer to chapter 2, section 2.5 for details of behavioural analysis. To determine if group performance is significantly different, a one-way ANOVA was used to compare means of the four different groups. Then a one-sample t-test was used to measure if individual group performance was above chance. Exploration times and locomotor activity differences between groups were measured using a one-way ANOVA.

## 7.4 Experiment 1: MK-801 0.01mg/kg and 0.1mg/kg: Object recognition (24-hour test)

## 7.5 Materials and Methods

### 7.5.1 Subjects

Thirty-two females C57BL/6J mice were used as subjects in this experiment (Charles River, UK). The animals were housed in groups of four under diurnal conditions (0700 light; 1900 dark). Sawdust bedding, cardboard tunnels and nesting material were provided as forms of enrichment. Mice were food deprived up to 85% of their free feeding weight and thus maintained throughout behavioural testing. Water was freely available throughout the study. Mice weighed between 14.6 and 19.1grams at the start of behavioural testing. The animals previously took part in a conditioning task.

### 7.5.2 Drugs and injection

(+)-MK-801 hydrogen maleate (Sigma-Aldrich, UK) was dissolved in saline (0.9% NaCl solution) and injected intraperitoneally 30 minutes before acquisition and retrieval. Doses of 0.01 and 0.1mg/ kg (10mL/kg) were used in this experiment.

### 7.5.3 Habituation and pre-training

The condition of the experimental room in this chapter was identical to that of previous chapters. Mice received habituation and pre-training protocol as described in chapter 2, section 2.3. Pre-training lasted for 7 days.

### 7.5.4 Testing protocol

Mice in this experiment received two 8-trial spontaneous object recognition task protocol which had a 24-hour retention delay between sample and test that was previously

described in chapter 6, section 6.3.6. Mice received i.p injections 30 mins prior to the acquisition phase and 30 mins prior to test phase.

In experiment 1, mice were divided into 4 treatment groups (N = 8/group) where animals either received i.p injections of (a)saline at sample and saline at test; (b)saline at sample and drug at test; (c)drug at sample and saline at test; or (d)drug at sample and drug at test. The dose used in this experiment was 0.01mg/kg. In experiment 2, mice were divided into two groups of 16 animals and received either (a)saline/saline; or (b)drug/drug injections intraperitoneally. The allocation of mice in experiment 2 were counterbalanced according to the groups that mice were assigned to in experiment 1; four mice from each group in experiment 1 were assigned to the saline/saline or MK/MK group in experiment 2. The drug dose used in experiment 2 was 0.1mg/kg 10mL/kg. The experimenter was unaware of the type of treatment that mice received during behavioural testing, as the drug treatments were administered by an assistant. The washout period for the drug was 14 days, therefore testing for experiment 2 occurred two weeks after experiment 1 (see figure 7.1 for testing protocol).

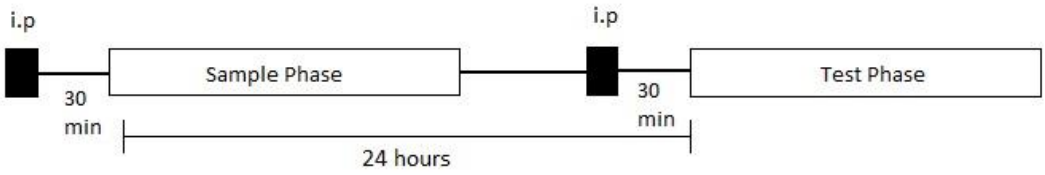



*Figure 7.1* shows a representative diagram of the experimental procedure of the spontaneous object recognition task with 24-hour retention delay and used in experiments 1, 2 and 4. Mice received either an i.p injection of saline or drug 30 minutes prior to sample and test.

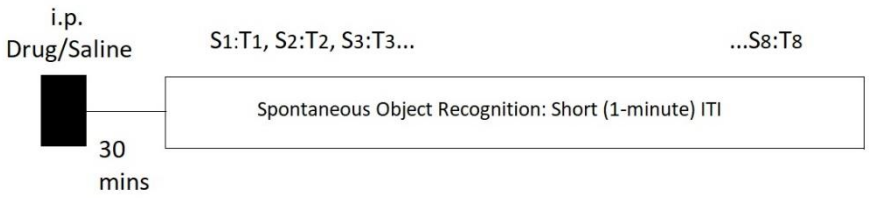



*Figure 7.2* represents the procedure of experiment 3: the spontaneous object recognition task with short (1-minute) retention interval between sample and phase. Mice received i.p injection of either drug or saline 30 minutes prior to the start of the testing session.

## 7.6 Results

### 7.6.1 Experiment 1: 0.01mg/kg MK-801 object recognition at 24-hour retention delay

The effect of MK-801 administration was analysed using a 2x2 (Exposure x Test) ANOVA on the preference scores such as the D1 measure and D2 ratios. The 2x2 ANOVA revealed a significant Exposure x Test interaction, $F(1, 28) = 8.07$, $p = 0.008$, and simple main effects revealed differences between the SAL-SAL and MK-SAL group, $F(1, 28) = 5.23$, $p = 0.03$; and MK-SAL and MK-MK group, $F(1, 28) = 6.81$, $p = 0.014$ (figure 7.3). These findings suggest that the retrieval of object recognition memory at a low dose (0.01mg/kg) of MK-801 was state-dependent. The analysis of the D2 scores revealed an Exposure x Test interaction, $F(1, 28) = 4.48$, $p = 0.043$, but no effects of exposure or test [$F(1, 28) = 0.079$, $p = 0.78$ and $F(1, 28) = 0.42$, $p = 0.525$ respectively]. Subsequent pairwise comparisons did not differ between SAL-SAL, SAL-MK, MK-SAL and MK-MK group.

To determine if individual groups show the ability to discriminate the novel from the familiar object in the current experiment, the mean D1 and D2 ratio of the groups (Sal/Sal, Sal/ Drug, Drug/Sal, Drug/Drug) were analysed using one-sample (two-tailed) t-test against zero. The results found that all groups show above chance performance in the spontaneous object recognition task, indicating that they retained memory of the familiar objects from the sample phase after 24-hours retention delay (see table 7.1 for results summary).

The effect of MK-801 administration was analysed using a 2x2 (Exposure x Test) ANOVA on the distance travelled and total exploration times. The 2x2 ANOVA on the distance travelled during the sample phase (figure 7.5), revealed a significant Exposure x Test interaction, $F(1, 28) = 0.126$, $p = 0.725$, but no effects of exposure or test [$F(1, 28) = 1.22$, $p = 0.278$ and $F(1, 28) = 0.56$, $p = 0.815$ respectively]. The 2x2 ANOVA on the distance travelled during the test phase (figure 7.5), revealed a significant Exposure x Test interaction, $F(1, 28) = 2.26$, $p = 0.144$, but no effects of exposure or test [$F(1, 28) = 0.54$, $p = 0.469$ and $F(1, 28) = 1.61$, $p = 0.215$ respectively]. The 2x2 ANOVA on the total

exploration times during the sample phase (figure 7.6), revealed a significant Exposure x Test interaction, $F(1, 28) = 0.006$, $p = 0.938$, but no effects of exposure or test [$F(1, 28) = 0.426$, $p = 0.519$ and $F(1, 28) = 0.016$, $p = 0.899$ respectively]. The 2x2 ANOVA on the total exploration times during the test phase (figure 7.6), revealed a significant Exposure x Test interaction, $F(1, 28) = 0.00$, $p = 0.988$, but no effects of exposure or test [$F(1, 28) = 0.719$, $p = 0.404$ and $F(1, 28) = 2.50$, $p = 0.125$ respectively]. These findings indicate that the administration of MK-801 had no effect on the distance travelled and total exploration times.

A Group*Block ANOVA was used to examine if performance levels changed over the testing session between groups (figure 7.7). Blocks were obtained by calculating the mean of the first two trials and subsequent trials until the end of the session, resulting in 4 blocks of two trials. The results found an evidence of block [$F(3,84) = 6.385$, $p = 0.001$] but no effect of group*block [$F(9, 84) = 0.404$, $p = 0.93$]. Post-hoc pairwise comparison (Bonferroni) revealed a change in levels of performance between Trial 1 – 2 and trial 5 – 6 ($p < 0.001$) and trials 1- 2 and trials 7 – 8 ($p = 0.029$). Performance of animals were significantly worse in trials 1 – 2 compared to performance at trials 5 – 6 and 7- 8.

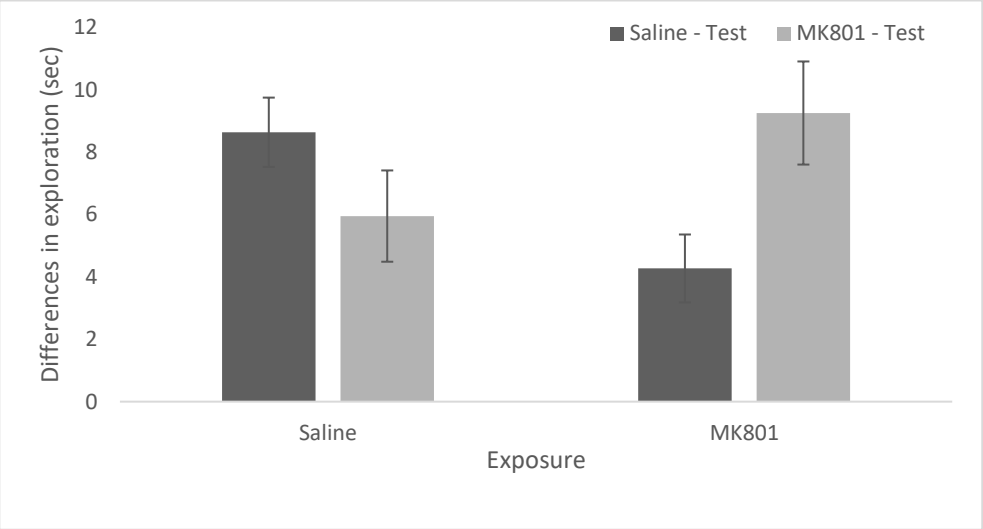



*Figure 7.3* represents the effects of MK-801 on the performance of mice in the spontaneous object recognition task. The bars and SEM represent the difference in time spent exploring the novel and familiar objects after a 24-hour retention delay. The performance of the MK/SAL group was impaired compared to the saline (SAL/SAL) and the state-dependent (MK/MK) control groups.

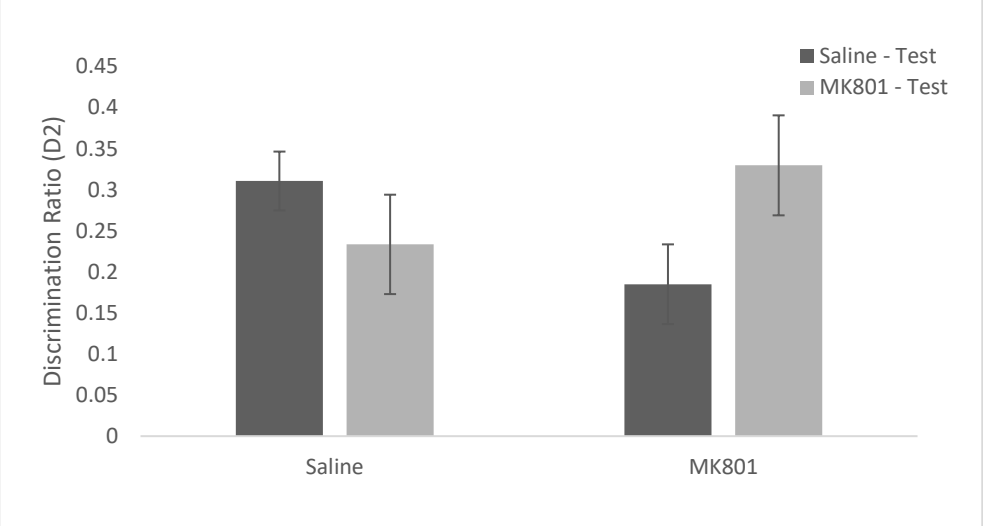



*Figure 7.4* represent the effects of MK-801 administration on the performance of the spontaneous object recognition task. The bars and SEM represent the ratio of the difference between time spent exploring the novel and familiar object and the total time spent exploring both objects. The results found no difference between performance of groups using the D2 measure.

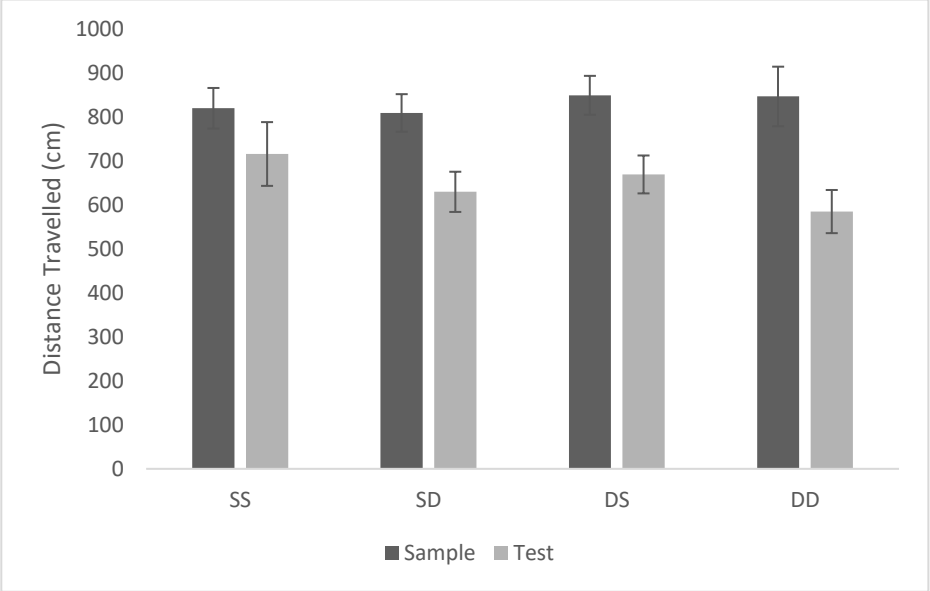



*Figure 7.5* represent the activity levels (distance travelled) by mice in the sample and test phases in the spontaneous object recognition task. The dark grey bars represent the mean distance travelled (±SEM) during the sample phase; and the light grey bars represent the mean distance travelled (±SEM) at test. All groups showed similar activity levels throughout the sample and test.

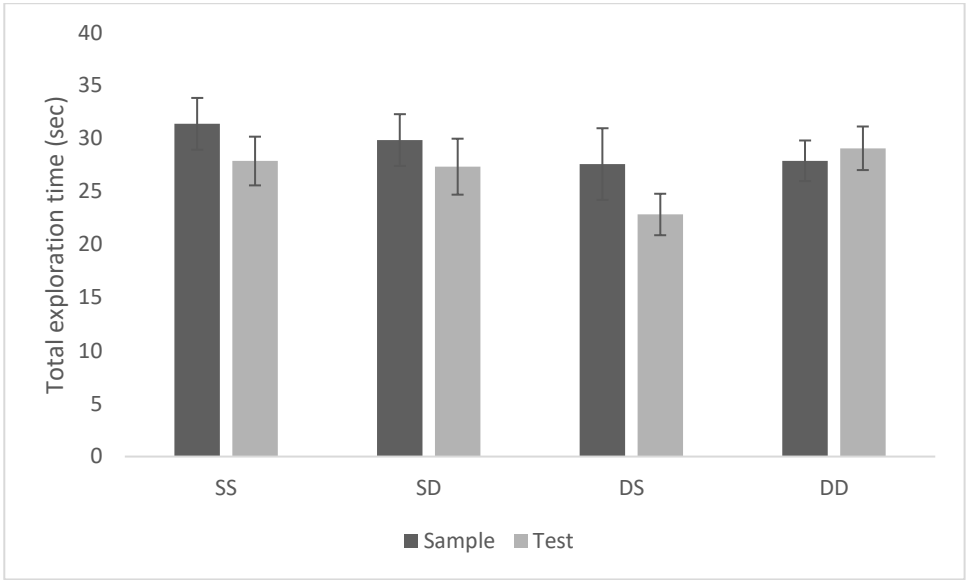



*Figure 7.6* shows the total exploration times (seconds) by the saline control, sal/drug, drug/sal, and drug/drug groups. The dark grey bars represent the mean total exploration times (±SEM) of during the sample phase; whereas the light grey bars represent the mean total exploration times (±SEM) of the test phase. There were no between group differences in total exploration times at sample and test phase.

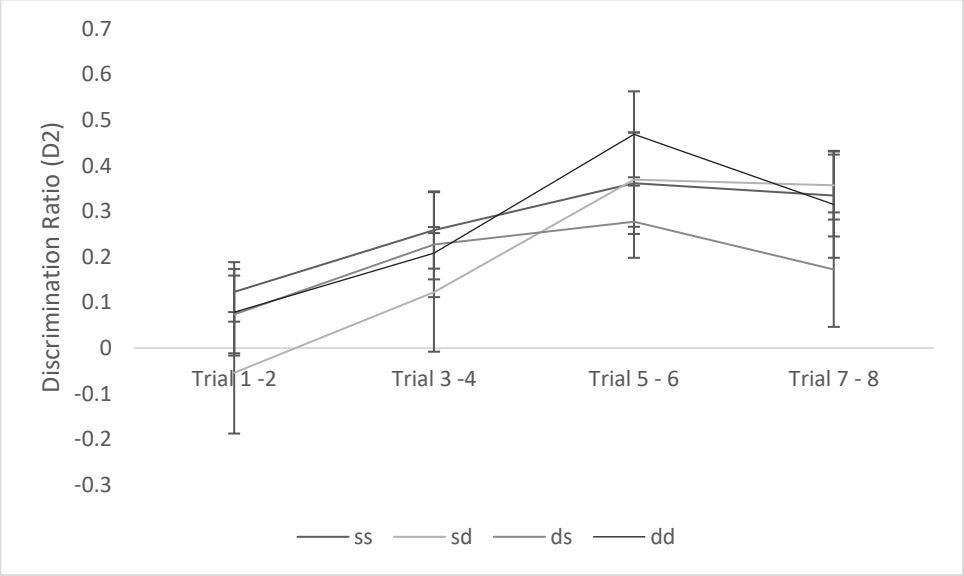



*Figure 7.7* represent the changes in performance levels of all groups throughout the test phase of the spontaneous object recognition task at 24-hour retention delay. The testing session was divided into four blocks of two trials which were obtained by calculating the mean D2 ratio of the first two trials (trial 1 and 2) and each subsequent pairs of trials until the end of the session. No between group differences were found, however an effect of block was found between the first (trials 1 and 2) and third (trial 5 and 6) block; and first and fourth (trial 7 and 8) block.

### 7.6.2 Experiment 2: 0.1mg/kg MK-801 object recognition at 24-hour retention delay

As in the previous experiment, the effects of MK-801 administration on performance in the spontaneous object recognition task at 24-hour retention delay was examined by comparing the mean performance (D1 scores and D2 ratio; see figure 7.8 and 7.9 respectively) of the saline group and drug group using an independent sample t-test. The results found that performance of mice that received 0.1mg/kg MK-801 injection at sample and test were similar to saline controls (D1: $t(30) = -0.329$, $p = 0.744$; D2: $t(30) = -0.251$, $p = 0.804$). This indicates that when controlled for state-dependency at a higher dose, the drug did not affect object recognition memory performance.

To investigate whether the saline controls and drug group exhibited object recognition memory by preferentially exploring the novel over familiar object, the means of group performance (D1 and D2 ratio) were analysed using a one-sample (two-tailed) t-test against zero. The results found that both the state-dependency controlled (MK/MK group) and saline controls showed the ability to discriminate the novel from the familiar object in the spontaneous object recognition task at 24-hour retention delay (see table 7.1 for results summary).

To determine whether the drug affected exploratory behaviour toward objects (figure 7.10) and the activity levels (figure 7.11) during the sample and test phase of the task, an independent sample t-test was used to compare the total exploration times and the distance travelled between the drug group and saline control at sample and test. The analysis found that at a dose of 0.1mg/kg, MK-801 had no effect on total exploration times at sample [$t(30) = -0.548$, $p = 0.588$] and test [$t(30) = -0.791$, $p = 0.435$], but had an effect on the animals' activity levels during the task (Sample: $t(30) = -5.065$, $p < 0.001$; Test: $t(30) = -3.715$, $p = 0.001$).

As in the previous experiment, changes in performance levels during the task was examined by analysing the effects of four blocks of two trials (figure 7.12). Blocks were

obtained by calculating the mean D2 of trial 1 and 2 and every subsequent pair of trials (trials 3 and 4; trials 5 and 6; trials 7 and 8). A block*group ANOVA revealed an effect of block [$F(2.327, 69.811) = 3.608$, $p = 0.026$, greenhouse-geisser corrected], and a post-hoc pairwise comparison showed a change in performance levels at block 1 and 2 ($p < 0.001$). The analysis did not show an interaction of group*block [$F(2.327, 69.811) = 0.284$, $p = 0.786$, greenhouse-geisser corrected].

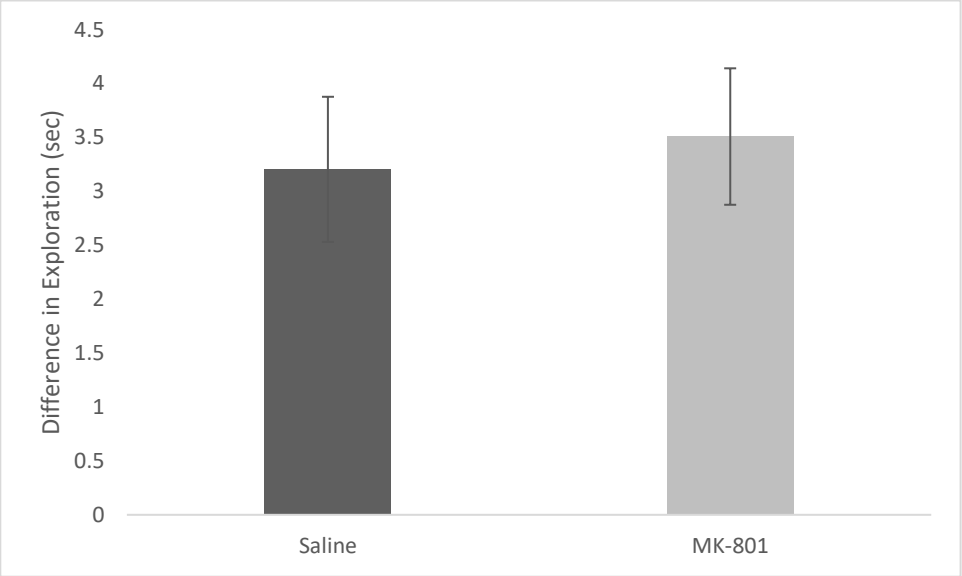



*Figure 7.8* represents the effects of state-dependency controlled administration of MK-801 (injection at sample and test) on the differences in exploration times in the spontaneous object recognition task. The bars are representative of the mean and SEM of the difference between the novel and familiar objects (D1) of the MK-801 group and saline controls.

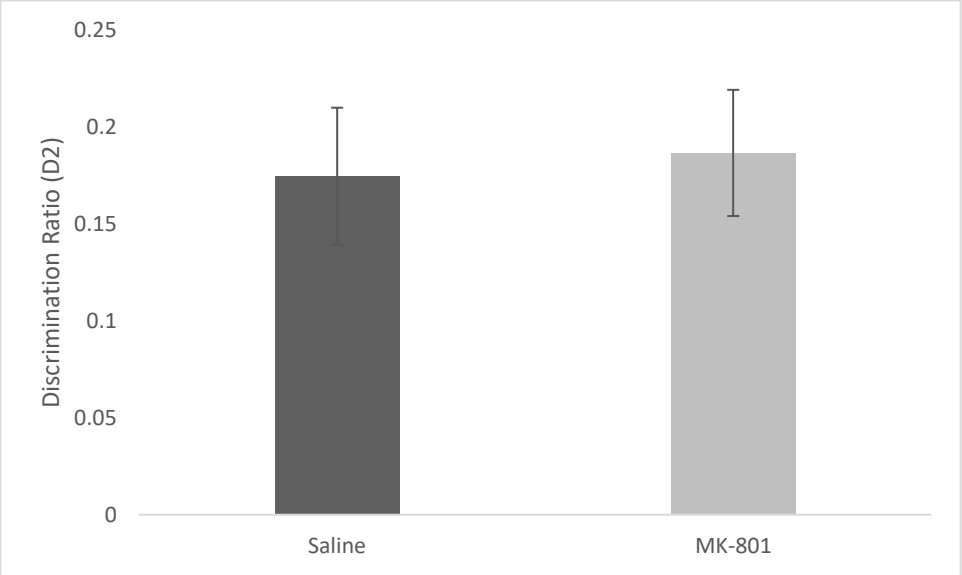



*Figure 7.9* represents the effects of state-dependency controlled administration of MK-801 (injection at sample and test) on the performance in the spontaneous object recognition task. The bars are representative of the mean and SEM of the discrimination ratio (D2) of the MK-801 group and saline controls.

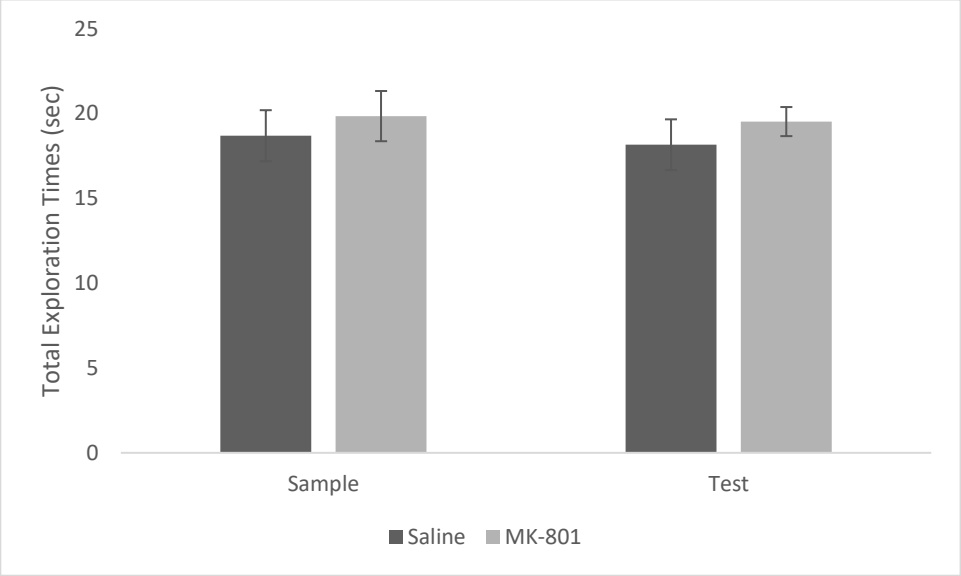



*Figure 7.10* shows the mean total exploration times of the MK-801 group and saline controls in the spontaneous object recognition task. The dark grey bars represent the mean (±SEM) total exploration times of the saline group at sample and test; whereas the light grey bars represent the total exploration times of the MK-801 group.

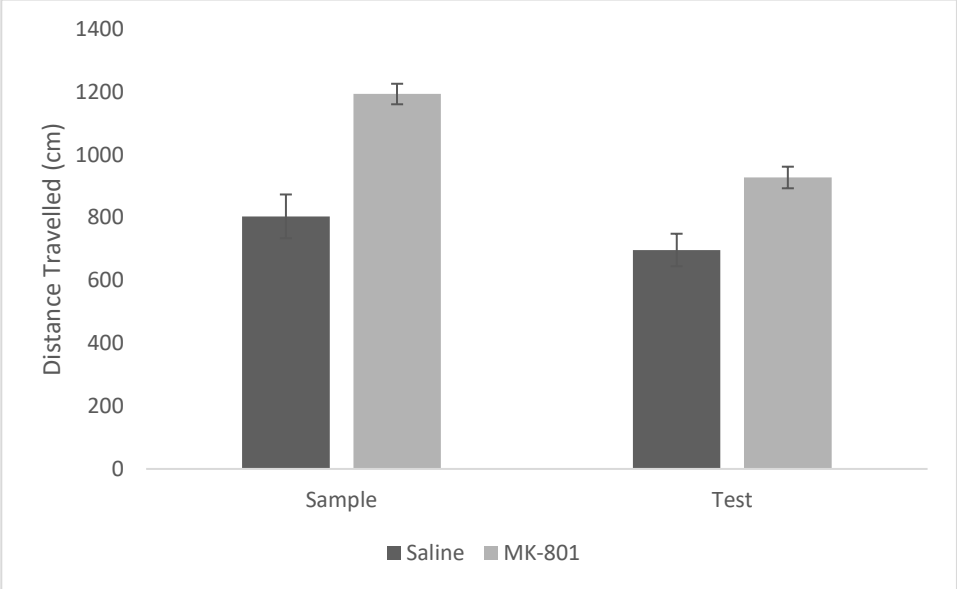



*Figure 7.11* shows the mean distance travelled of the MK-801 group and saline controls in the spontaneous object recognition task. The dark grey bars represent the mean (±SEM) distance travelled of the saline group at sample and test; whereas the light grey bars represent the mean distance travelled of the MK-801 group.


*Figure 7.12* represent the changes in performance levels of the MK-801 group and saline controls throughout the test phase of the spontaneous object recognition task at 24-hour retention delay. The testing session was divided into four blocks of two trials which were obtained by calculating the mean D2 ratio of the first two trials (trial 1 and 2) and each subsequent pairs of trials until the end of the session. No between group differences were found, however an effect of block was found between the first (trials 1 and 2) and second (trial 3 and 4) block.

### 7.7 Experiment 3 and 4: MK-801 0.1 mg/kg: Object recognition

### 7.8 Material and Methods

#### 7.8.1 Subjects

Thirty-two female C57BL/6J mice (Charles River, UK) were used as subjects in this experiment. The housing conditions of animals in the current experiment was identical to that of the previous experiment, with the exception that mice were housed in groups of 8. As in the previous experiment, mice were food deprived to 85% of their free feeding weight and thus maintained throughout the experiment. Water was freely available throughout the experiment. The animals weighed between 14.4 and 18.8 grams at the start of behavioural testing. The animals had previous experience in a conditioning task.

#### 7.8.2 Drugs and injections

As in the previous experiment, (+)-MK-801 hydrogen maleate (Sigma-Aldrich, UK) was dissolved in saline (0.9% NaCl solution). Animals were injected intraperitoneally 30 minutes prior to the testing session in experiment 3 and 30 minutes before acquisition and retrieval in experiment 4. A dose of 0.1mg/ kg (10mL/kg) were used in this experiment.

#### 7.8.3 Habituation and pre-training

The pre-training and habituation in these experiments were identical to that of the previous experiment. Pre-training for animals in experiment 3 and 4 lasted for a total of 7 and 10 days respectively.

#### 7.8.4 Testing protocol

##### 7.8.4.1 Experiment 3: 0.1mg/kg MK-801 object recognition at short delay (1-minute)

The animals (N = 16) in this experiment received an 8-trial spontaneous object recognition task as described in chapter 2, section 2.4.1. Mice were injected with either saline or 0.1mg/kg MK-801 interperitoneally 30 minutes prior to behavioural testing. To briefly

illustrate, animals were initially placed in the holding area of the apparatus, after 1 minute, the central arm door opened, and the animal shuttled through to the object area. Animals were presented with a pair of identical object for 2 minutes and after that the side arm doors opened and the animals returned to the holding area for 1 minute. During this time, the experimenter swapped the objects to prepare for the test phase. The central door opened again, and the animals returned to the object arena to explore a pair of objects, in which one object was a copy of the familiar object presented during the sample phase, and a novel object. The animal was given 2 minutes to explore the objects before the side arm doors opened and the animals returned to the holding area once more. This procedure was repeated until the end of the 8-trial testing session (see figure 7.2).

#### 7.8.4.2 Experiment 4: 0.1mg/kg MK-801 object recognition at long-delay (24-hours)

This experiment aimed to further investigate the state-dependent effects on recognition memory in mice by replicating the MK-SAL and MK-MK group in experiment 1 with a higher dose (0.1mg/kg) of MK-801. As in experiment 2, the animals (N=16) in the current experiment received an 8-trial spontaneous object recognition testing session with a 24-hour retention delay between acquisition and retrieval phases. All animals received i.p injection of the drug (0.1mg/kg MK-801) 30 minutes prior to the sample phase and an administration of either drug or saline 30 minutes prior to test. The testing protocol of this experiment was detailed in chapter 6, section 6.3.6.

## 7.9 Results

### 7.9.1 Experiment 3: 0.1mg/kg MK-801 object recognition at short delay (1-minute)

In order to determine if the performance of the drug group was significantly different from the saline/control group, independent samples t-tests were conducted on group D1 and updated D2 scores. Results found that performance of both groups were not significantly different from each other D1: $t(14) = 1.03$, $p = 0.32$; Updated D2: $t(14) = 0.79$, $p = 0.44$ (see

figure 7.13 for graphical representation); which indicated that administration of 0.1mg/kg MK-801 prior to the testing session did not affect performance of mice in the spontaneous object recognition task at short (1-minute) inter-trial intervals.

To investigate performance levels of individual groups, the D1 and D2 scores of the saline and MK-801 groups were analysed using one-sample t-tests against zero. It was found that performance of the saline group was above chance (D1 scores), $t(7) = 3.61$, $p = 0.009$; while the MK-801 group did not show the ability to discriminate between the novel and familiar objects at a short inter-trial interval, $t(7) = 2.26$, $p = 0.058$. Whereas when D2 ratios were analysed, it was revealed that performance of the saline group was above chance, $t(7) = 3.01$, $p = 0.02$; while the MK-801 group were unable to discriminate the novel from the familiar object at an immediate delay, $t(7) = 2.14$, $p = 0.069$ (see table 7.1).

As in the previous experiment, the effect of MK-801 on exploration times at sample and test was analysed with an independent sample t-test between saline and drug group (see figure 7.14). The analysis found that administration of 0.1mg/kg of MK-801 did not influence time spent exploring objects in both the sample [$t(14) = -0.1$, $p = 0.92$] and test phases [$t(14) = -0.91$, $p = 0.038$]. However, analyses of distanced moved (cm) in the apparatus during sample and test (figure 7.15) revealed that mice injected with MK-801 showed an increase in distance moved compared to saline controls during sample [$t(14) = -5.16$, $p < 0.001$] and test [$t(14) = -6.02$, $p < 0.001$].

As in the previous experiment, changes in performance levels between the saline and MK-801 groups were investigated by comparing blocks of performance across the testing session. Blocks were obtained by calculating the average of the first 2 trials and each consecutive pairs of trials to make up 4 blocks. An analysis of between (groups) and within (block) subjects ANOVA found no effect of block [$F(1.66, 23.2) = 0.968$, $p = 0.379$] and block*group [$F(1.66, 23.2) = 0.389$, $p = 0.643$]. These findings indicate that performance

levels of both groups were stable throughout the testing session and were not different from each other (see figure 7.16).

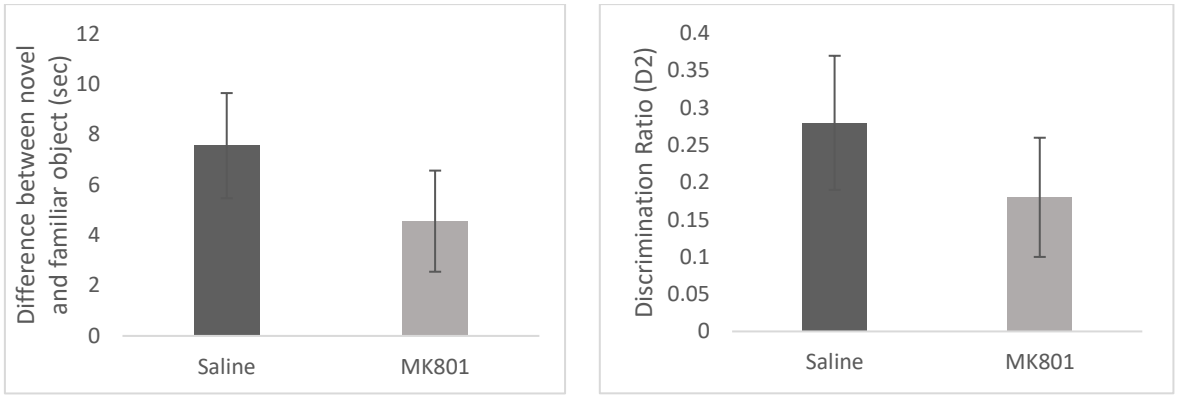



*Figure 7.13* representation of the performance of saline and MK-801 mice in the spontaneous object recognition task with a short (1-minute) ITI based on the *left* D1 scores and *right* D2 ratio. Analysis of both measures found that systemic administration of MK-801 did not have an effect on short-term spontaneous object recognition memory.

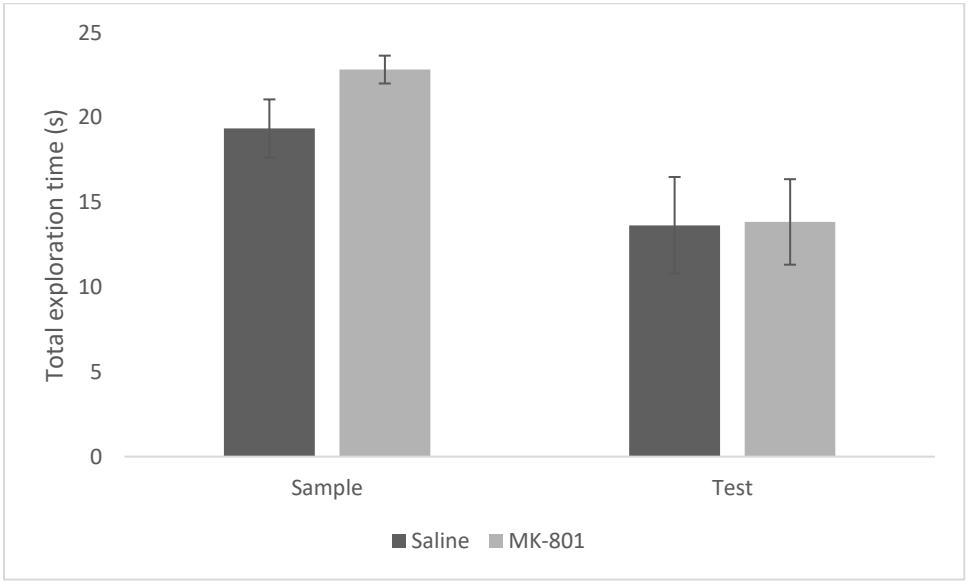



*Figure 7.14* represents the mean total time spent (±SEM) exploring the pairs of objects presented during the sample and test phases by the saline and MK-801 mice. The drug group did not show differential exploration times during both sample and test phases in comparison to the saline controls.

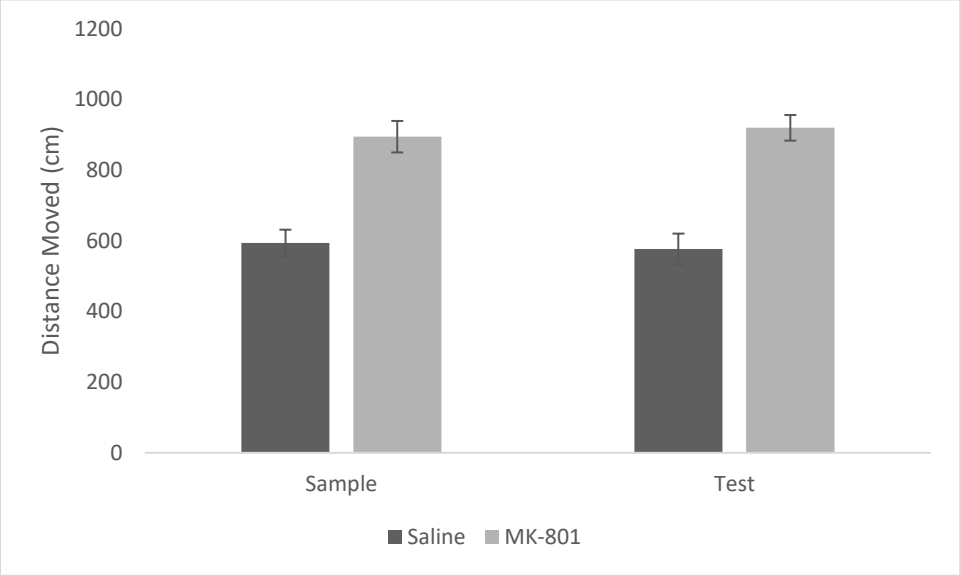



*Figure 7.15* shows the distance travelled (±SEM) of drug and saline controls during the sample and test phases of the spontaneous object recognition task. Animals injected with MK-801 showed increased locomotion during sample and test compared to saline controls.

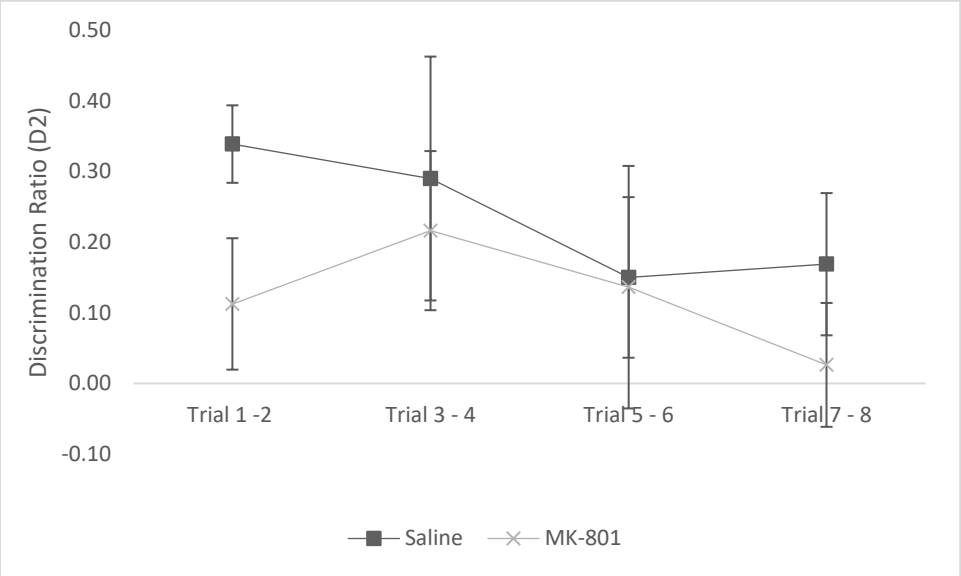



*Figure 7.16* is representative of the performance levels between the MK-801 group and saline controls across the testing session. Performance levels across the session remained relatively constant and both groups showed similar performance.

### 7.9.2 Experiment 4: 0.1mg/kg MK-801 object recognition at long-delay (24-hours)

As in the previous experiment, the effect of state-dependency was examined using an independent samples t-test on the mean D1 and D2 ratio of the MK/MK and MK/Sal group. The analysis found that performance of both groups were similar (figure 7.17), in both D1 [$t(14) = -0.021$, $p = 0.983$] and D2 ratio [$t(14) = 0.141$, $p = 0.89$]. This indicated that at a higher dose (0.1mg/kg), performance of both groups were impaired by the drug.

To find out if individual groups show the ability to discriminate between the novel and familiar object at 24-hours, mean D1 and D2 ratio of both groups were analysed with a one-sample (two-tailed) t-test and the results found that both the MK/MK and MK/SAL group showed above chance performance [D1 MK/MK $t(7) = 2.95$, $p = 0.021$; D1 MK/SAL $t(7) = 2.81$, $p = 0.026$; D2 MK/MK $t(7) = 3.16$, $p = 0.16$; D2 MK/SAL $t(7) = 3.13$, $p = 0.017$]. Mice from both groups showed memory of the familiar objects by preferentially exploring the novel objects at test.

To determine whether there was an effect of drug on the distance moved and total exploration times at exposure and test, independent samples t-test were used to compare the distance travelled and exploration times of the group that received MK-801 and Saline at test. It was found that both groups showed no difference in distance travelled during sample [$t(14) = -0.729$, $p = 0.478$] and test [$t(14) = 1.60$, $p = 0.131$] (figure 7.18). The analysis also found that the total exploration times (figure 7.19) of both groups at sample were different [$t(14) = -2.17$, $p = 0.048$] but not at test [$t(14) = 0.287$, $p = 0.779$].

As in the previous experiment, changes in performance levels between the saline and MK-801 groups were investigated by comparing blocks of performance across the testing session. Blocks were obtained by calculating the average of the first 2 trials and each consecutive pairs of trials to make up 4 blocks. An analysis of between (groups) and within (block) subjects ANOVA found no effect of block [$F(3, 42) = 0.307$, $p = 0.28$] and block*group [$F(3, 42) = 0.372$, $p = 0.118$]. These findings indicate that performance levels

of both groups were stable throughout the testing session and were not different from each other (see figure 7.20).

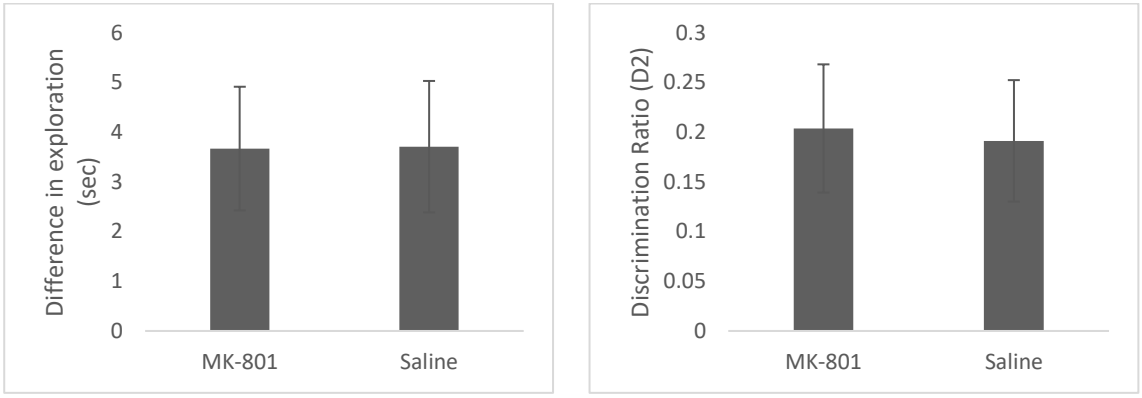



*Figure 7.17* represents performance (±SEM) of the MK-801 and saline group based on the *left* D1 scores and *right* D2 ratio. Both groups demonstrated similar performance levels between groups across the D1 scores and D2 ratio.

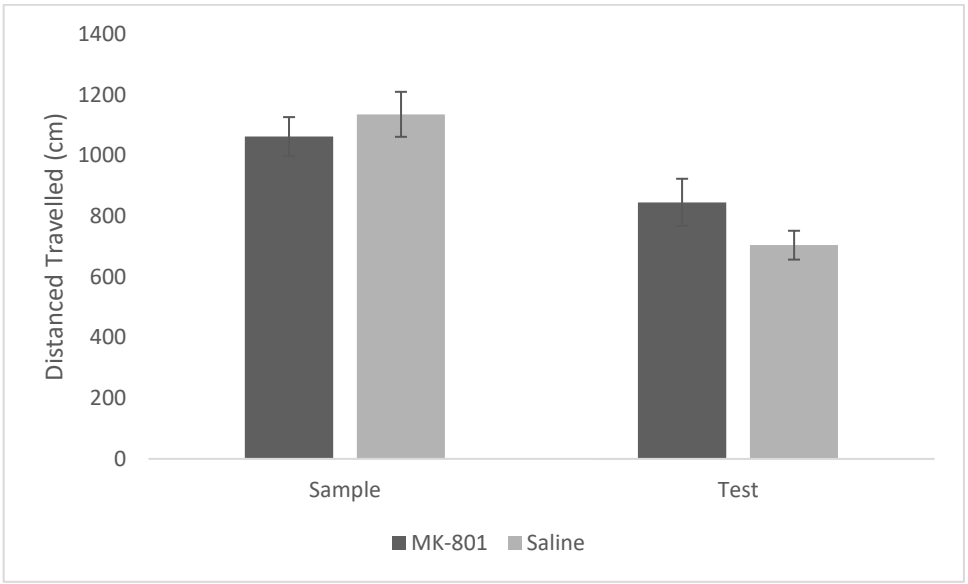



*Figure 7.18* represents the distance travelled (±SEM) within the object area of the continual trials apparatus for both saline and drug group in the sample and test phase. Groups showed similar distance travelled during the test phase, which indicated that the administration of MK-801 at test did not affect the distance travelled within the apparatus.

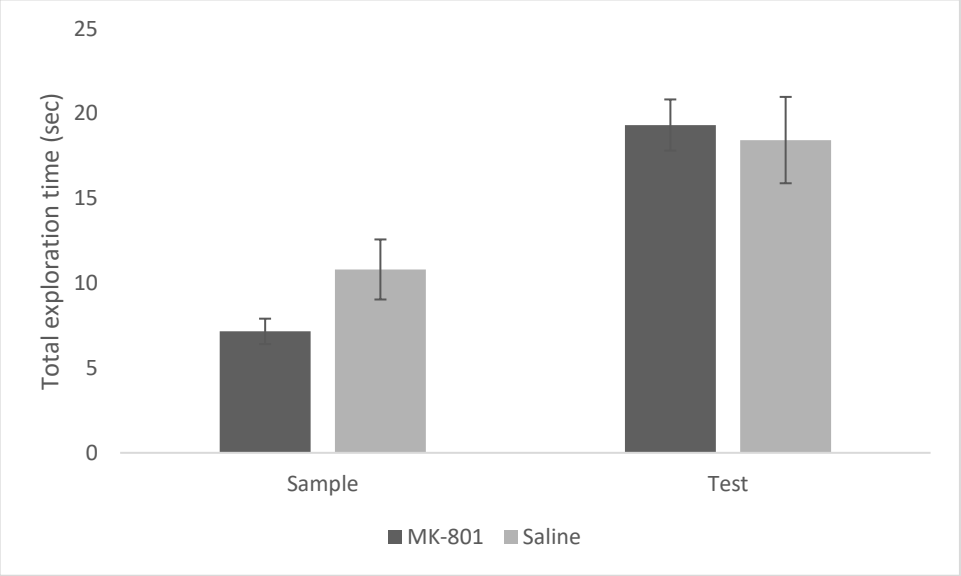



*Figure 7.19* represents the total exploration times (±SEM) of saline and drug group at sample and test. The drug group spent a significantly less time exploring the pairs of objects in the sample phase compared to saline group; however both groups showed similar exploration levels at test.

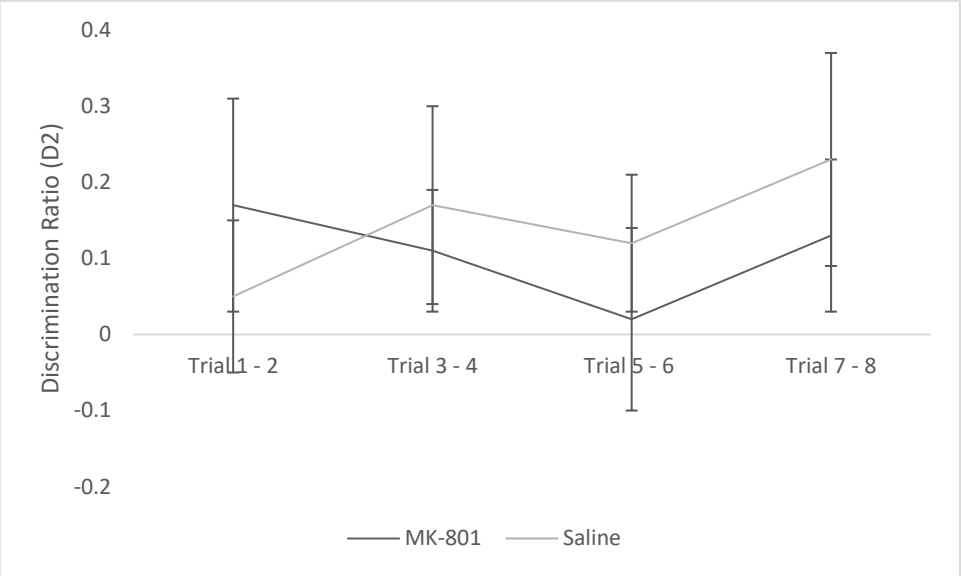



*Figure 7.20* shows the changes in performance levels of drug and saline groups across the test phase. Changes in performance levels were examined by comparing blocks of performance across the session between drug and saline group. The results found that performance levels of drug and saline group were not different from each other and showed consistent performance throughout the testing session.

| Experiment | Group | D1 | D2 | Total Exploration Times (sec) | | Distance travelled (cm) | | N |
|---|---|---|---|---|---|---|---|---|
| | | | | Sample | Test | Sample | Test | |
| **1** | SAL | 8.64*** | 0.31*** | 31.39 | 27.88 | 819.44 | 715.55 | 8 |
| | SAL + MK | 5.95** | 0.23** | 29.86 | 27.35 | 808.86 | 629.59 | 8 |
| | MK + SAL | 4.27** | 0.18** | 27.60 | 22.84 | 848.90 | 669.08 | 8 |
| | MK + MK | 9.26** | 0.33** | 27.91 | 29.08 | 846.33 | 584.73 | 8 |
| **2** | SAL | 3.21*** | 0.17*** | 18.70 | 18.17 | 803.69 | 696.59 | 16 |
| | MK + MK | 3.51*** | 0.19*** | 19.86 | 19.53 | 1192.97 | 927.32 | 16 |
| **3** | SAL | 7.55** | 0.28* | 13.64 | 19.35 | 593.80 | 576.61 | 8 |
| | MK-801 | 4.56 | 0.18 | 13.84 | 22.82 | 894.72 | 919.82 | 8 |
| **4** | MK + SAL | 3.71* | 0.19* | 10.81 | 18.44 | 1135.61 | 704.39 | 8 |
| | MK + MK | 3.67* | 0.20* | 7.16 | 19.33 | 1062.01 | 845.60 | 8 |

*Table 7.1* provides a summary of the findings and results of all experiments from the current study which assessed the effects of MK-801 administration on long-term and short-term memory in the multiple trials version of the spontaneous object recognition task. Behavioural parameters such as the total exploration times (sec), the distance travelled (cm) and the number of animals (N) used in all experimental groups. Experimental group performance of the spontaneous object recognition task was represented by the difference between novel and familiar object exploration (D1) and the discrimination ratio (D2), ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

| | | Trial number | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Group** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| **Experiment 1** | SAL/SAL | 0.23 | 0.018 | 0.22 | 0.30 | 0.27 | 0.46 | 0.22 | 0.45 |
| | SAL/MK | 0.06 | -0.17 | 0.14 | 0.11 | 0.23 | 0.51 | 0.26 | 0.45 |
| | MK/SAL | 0.03 | 0.08 | 0.22 | 0.16 | 0.27 | 0.22 | 0.09 | 0.25 |
| | MK/MK | 0.05 | 0.11 | 0.11 | 0.31 | 0.52 | 0.41 | 0.20 | 0.43 |
| **Experiment 2** | SAL/SAL | 0.03 | 0.07 | 0.42 | 0.11 | 0.04 | 0.10 | 0.11 | 0.12 |
| | MK/MK | 0.02 | 0.02 | 0.36 | 0.16 | 0.05 | 0.09 | 0.26 | 0.17 |
| **Experiment 3** | SAL | 0.24 | 0.44 | 0.40 | 0.18 | 0.11 | 0.17 | 0.28 | 0.06 |
| | MK-801 | 0.13 | 0.10 | 0.18 | 0.26 | -0.06 | 0.30 | 0.28 | -0.23 |
| **Experiment 4** | MK/SAL | 0.07 | 0.02 | 0.05 | 0.30 | -0.06 | 0.29 | 0.13 | 0.33 |
| | MK/MK | -0.05 | 0.40 | 0.15 | 0.08 | -0.01 | 0.05 | 0.24 | 0.03 |

*Table 7.2* details the object recognition memory (averaged D2 scores) of mice within the testing session of experiment 1, 2, 3 and 4 of the present study.

## 7.10 General Discussion

The aim of the current study in this thesis was to investigate the effects of the NMDA receptor blockade on recognition memory in mice. To achieve this, a series of experiments were conducted to assess the effects of an NMDA antagonist MK-801 administered either prior to exposure or test in a novel approach to assessing object recognition memory. Experiment 1 used a state-dependent design to assess the effects of 0.01mg/kg MK-801 in performance of mice in the multiple trials spontaneous object recognition task with a 24-hour (long-term) retention delay; Experiment 2 examined if state-dependent memory that was found in the lower (0.01mg/kg) dose could be replicated in a higher dose (0.1mg/kg) of the drug in the object recognition task with a long-term delay; Experiment 3 investigated the effects of 0.1mg/kg MK-801 on immediate memory mice in the object recognition task. Finally, Experiment 4 examined the effects of 0.1mg/kg MK-801 on the performance of mice when animals were administered the drug pre-exposure and received either saline or the drug at test.

This study found that at a low dose (0.01mg/kg), administration of MK-801 at pre-exposure but not pre-test impaired long-term object recognition memory in mice, however the effect of MK-801 injection at exposure was reversed when mice were injected with MK-801 again during test, suggesting state-dependent effect on recognition memory. These findings conflict with results from previous studies (de Lima et al., 2005; van der Staay et al., 2011; King et al., 2004) of MK-801 which reported that object recognition performance was impaired when the drug was administered prior to the exposure phase, suggesting that MK-801 impacts memory at the encoding stage, but not during consolidation or retrieval. Also, when mice were given a low and higher dose (0.01 and 0.1mg/kg) MK-801 prior to exposure, and then prior to test (state-dependent controls), the animals performance levels were similar to saline controls. The state-dependent effect found in this study using the low dose (0.01mg/kg) of MK-801 conflicted the findings of Harrod et al., (2001) which found

little evidence of state-dependent learning in a passive avoidance task at a dose of 0.05mg/kg. State-dependent memory found at the higher dose (0.1mg/kg) of the drug was confirmed the findings of Harrod et al., (2001). State-dependent memory is implicated in memory retention when animals that were injected with either MK-801 or saline prior to exposure and test demonstrated intact recognition memory; whereas mice that have initially been given the drug during pre-exposure then saline at test showed impairment in task performance. Harrod et al., (2001) suggested that the internal state during acquisition is discriminable under the influence of MK-801, and that administration of the drug at test would reproduce identical internal cues which in turn drives animal performance.

When object recognition memory was assessed at short (1-minute) intervals, mice that were given 0.1mg/kg MK-801 prior to the testing session were not impaired relative to saline controls, showing that the drug had no effect on immediate object recognition memory. There was, however, an effect of drug, with the MK-801 group showing increased levels of distance travelled during the task. Previous studies have found that administration of higher doses of MK-801 induced increased locomotor activity in animals (Amalric et al., 1994; Mele et al., 1994; Hargraves and Cain, 1992). Although the current study found that the group that were given MK-801 pre-exposure and saline at test were impaired relative to state-dependent controls (drug-drug group) at a lower dose, this effect was not found in the experiment that used a higher (0.1mg/kg) dose of the drug. This suggested that at a higher dose, there was little evidence of state-dependent learning, which conflicts the findings of experiment 2. The lack of state-dependent effect on memory may be attributed to impairments in the task at higher doses of MK-801. In a study by Nakagawa and Iwasaki (1996), using a 2x2 state-dependent learning design, when animals were given a higher dose of MK-801, they found that the drug impaired performance of mice that received MK-801 at pre-exposure and the state-dependent control (MK-MK) group. Also, the conflicting findings from experiment 2 may be caused by differences in experience levels of the animals,

whereby most of the animals from experiment 2 had prior drug experience from the spontaneous tasks at experiment 1, whereas mice from experiment 4 were completely drug naïve. Furthermore, both groups showed evidence of similar levels of locomotor activity, which suggests either there was no effect of drug, or prior administration of MK-801 pre-exposure affected animal behaviour at test, irrespective of whether animals received saline or the drug. The conflicting findings in the present chapter between experiment 2 and experiment 4 on the presence of state-dependency effect on memory may be rectified by testing naïve mice with on the dose dependent (0.01 and 0.1mg/kg) effects of MK-801 using the 2x2 design in the spontaneous object recognition task.

To conclude, the present chapter presented a novel evidence of state-dependent learning with the NMDA receptor antagonist MK-801 in the spontaneous object recognition task. Previous research on the effect of MK-801 on state-dependency learning have been conducted in passive avoidance (Harrod et al., 2001; Nakagawa and Iwasaki, 1996) and fear conditioning (Baker and Azorlosa, 1996). The novel multiple trials method of testing spontaneous object recognition used in the present study provided a refined method to assess recognition memory. The running of multiple trials reduced within animal variance over time, and this is crucial because drug studies often have higher variability. The results of the present chapter showed when controlled for state-dependency, the administration of MK-801 prior to the exposure phase did no impair the encoding of long-term object recognition memory, but suggests that state-dependency may play a role in the process of recognition memory retrieval. The findings show, that internal cue states which occurred as a result of a drug, may affect the recall of the memory.

# Chapter 8

# General discussion

## 8.1 Introduction

The primary objective of this thesis was to address methodological issues often found in spontaneous recognition tasks in mice, this was achieved by adapting an existing novel continual trials approach of examining spontaneous recognition task in mice. The second objective of this thesis was to further validate and generalise the novel continual trials approach in aging mice and a diseased mouse model. The third aim of the thesis was to apply the continual trials approach to investigate the effect of a pharmacological substance on recognition memory in mice.

This chapter aims to give a summary of the findings, conclusions and to discuss the possibility of future work that were suggested by these findings that were presented in the thesis. An overview of the main results is provided in section 8.2 followed by discussions of the implications and possible future work suggested by the broad findings presented in the thesis. Section 8.3 considers the implications of ageing on recognition memory in rodents. Section 8.4 examines the evidence presented for memory of objects and locations of objects in transgenic mouse models of Alzheimer's disease. Section 8.5 briefly examines the findings of the involvement of NMDA receptors in recognition memory (chapter 7) and the implications of MK-801 induced deficits in recognition memory. Section 8.6 examines the implications of the novel continual trials approach to examining recognition tasks in mice. Finally, section 8.7 concludes the broad findings of the work, provides an outline, future direction and applications of the findings suggested in the thesis.

## 8.2 Summary of Results (impact of the continual trials on spontaneous tasks)

The adaptation of the novel continual trials approach to assess recognition memory in mice was successful (as reported in chapter 3). Previously, this methodology was only utilised in rat studies (Ameen-Ali et al., 2012; Seel et al., 2017) and work presented in this thesis has shown that the continual trials approach of running spontaneous tasks is applicable to mice, as previously discussed in the introductory chapter (chapter 1), because of the behavioural differences between rats and mice (anxiety and stress levels, and erratic behaviour), it was unclear how mice would perform in the continual trials apparatus. Object recognition and object location memory were assessed using the continual trials method and performance levels of mice were found to be comparable to previous studies that assessed object recognition and location memory using the standard 'one-trial' approach (Sanderson et al., 2011; Davis et al., 2013). By running continual trials within a single session, within session variance were reduced, thus resulting in a more sensitive and refined task of spontaneous recognition. The method used in this thesis is similar to that of the Bow-tie maze (Albasser et al., 2010), but the compartmentalisation of the apparatus into separate object and holding arena with distinctive features allow for the examination of tasks involving spatial and contextual representations (Ameen-Ali et al., 2012; Seel et al., 2017). Furthermore, by increasing the number of trials within a single testing session, the increased reliability as a result of the approach, would in turn reduce the number of animals needed to produce findings with high statistical power. Using the continual trials approach, the study found that the number of mice used within spontaneous tasks may be reduced by a total of 30%. The promising findings presented in study 1 (chapter 3) provides a potential application across the field of memory research and in industry, however, further research should be conducted on the transferability of the task to extend it to different strains of mice, such as aged or diseased models.

If the task is to be of widespread use, then there is a need to fully understand the effects of different situations that might be applied to testing in the apparatus. For example, ageing studies are an important component of understanding disease and ageing effects on memory, and the reduced behavioural noise and number of animals required by the current procedure has distinct advantages for studies that involve the long term holding of animals. However, it was possible that age would impact on the ability of animals to perform multiple consecutive trials, or that previous experience in the apparatus would interact with performance at different ages. However, the results from Chapter 4 show no such concerns, with animal performance unaffected by age or experience in terms of overall performance and in terms of overcoming the effects of interference arising from the reuse of objects.

The prediction that an APP/PS1 (TASTPM) transgenic mouse model of Alzheimer's Disease would show an age-related impairment of object recognition and object-location memory in the continual trials apparatus was not supported by the findings presented in Study 3 (reported in chapter 5). TASTPM mice that were tested at 7 and 10 months of age using the multiple trials approach of the spontaneous object recognition task did not show an age-related impairment. This was also true in the examination of object-location memory in 7 and 10 months old TASPM mice. Performance of TASTPM mice that were task naïve were similar to mice that had prior experience in the spontaneous object recognition and object location task.

Based on previous findings by Sik et al., (2003), which found a delay-dependent effect on recognition memory, Study 4 predicted that mice would show a delay-dependent decline of recognition memory when using the continual trials approach to examine object recognition memory; this prediction was not supported by the findings reported in the study (Chapter 6). The continual trials methodology was adapted to enable the manipulation of longer delays. When mice were tested at 1-, 4- and 24-hour delays, performance levels at these delays were similar, suggesting an absence of delay-dependent decline in recognition memory.

Study 5 aimed to investigate the effects of NMDA receptor antagonist MK-801 on recognition memory. Using a traditional 2x2 state-dependent design, the study found that at low doses (0.01 mg/kg) of MK-801, state-dependency influenced retrieval of long-term object recognition memory and this finding was extended to a higher dose (0.1mg/kg) of the drug. Furthermore, administration of MK-801 had no adverse effects on immediate object recognition memory in mice.

### 8.3 Recognition Memory in aged mice

Study 2 provided evidence that mice showed no age-related impairments of object recognition and object location memory between 7 and 16 months of age using the continual trials methodology. This was supported by work done by Falhström et al., (2011) that investigated female C57BL6J female mice at 3, 8 and 28 months of age and found no evidence of age-dependent decline in performance in the object recognition task with an immediate (1-minute) delay between sample and test. Furthermore, research by Cavoy and Delaceur (1993) have shown that age-dependent decline in recognition memory does not occur at retention delays of 5 minutes. Studies investigating age-dependent changes in recognition memory at delays of over 15 minutes however, have found that ageing impaired recognition memory (Burke et al., 2012; Burke and Barnes, 2010; Falhström et al., 2011). Age-related impairments that are evident at longer delays may indicate deficits in consolidation of representation of memory which is widely debated (Dere et al., 2007); although Falhström and colleagues (2011) suggested that because object recognition tests are driven by exploratory behaviour, the sharp decline of exploratory drive in older mice may contribute to the age-dependent decline of object recognition performance.

The findings in chapter 4 has shown little possible interference caused by object set reuse was investigated at 14 month old mice. When a set of objects that were used in the object recognition memory task was reused in the object location task, interference from the

old memories formed during the object recognition task may interfere with the encoding and retrieval of the object location task. To reduce the possible interference levels resulting from the reuse of object sets, the object location task 7 days after the object recognition task; however, because memory of an object have been found to last for up to weeks, interference may affect performance levels of the object location task. Furthermore, interference disproportionately affects older populations of monkeys and rats (Moss, Rosene and Peters, 1988; Dunnett et al., 1997). In a human study by Lustig and colleagues (2001), they presented young and old adults with a span task that was either standard format or designed to reduce interference, and found that performance of the span task was influenced by interference and the age difference in performance was a result of the ability to overcome interference. Possible future work may investigate the increasing interference levels of ageing mice using the continual trials approach.

The study presented in chapter 4 utilized a longitudinal design, which has significant advantages, including teasing out progressive behavioural changes of a group of animals (Markowska and Savonenko, 2002; Joyal et al., 2000) and prior experience on behavioural performance (Dellu et al., 1997). Furthermore, the longitudinal design used in the experiment and resulting increased experience of the mice more closely resemble the experiences of the human population. Future work could further extend the age range to examine age-related changes in cognition and memory of a group of mice (3 months to 22 months of age), the extension of the age-range would provide a comprehensive picture of age-related changes in recognition memory in mice. Although mice show a decrease in memory that resembles changes within the ageing human population (Jucker et al., 1994), mice do not develop neurogenerative diseases such as Alzheimer's disease (Vanhooren and Libert, 2013). The following section discusses the implications of findings of study 3 and suggests possible future work.

### 8.4 Recognition memory in transgenic models of Alzheimer's disease

Following the successful validation of the continual trial approach in aged mice, this approach was further used to evaluate recognition memory in a transgenic model of Alzheimer's disease. Based on the findings presented in the thesis (chapter 5), the TASTPM (APP/PS1) mouse model of Alzheimer's disease showed no evidence of age-related decline of object recognition and object location memory. Previous literature examining recognition memory in TASTPM mice yielded conflicting findings (Howlett et al., 2004; Scullion et al., 2011), which may be a result of procedural differences and differences in experience levels. Howlett et al., (2004) tested naïve TASTPM mice, whereas Scullion and colleagues (2011) tested a single group of TASTPM mice at different age points. The work presented in this thesis on the memory of object location is a novel contribution to the cognition of TASTPM mice. Previous research on the spatial memory of TASPTM have focused on tasks of spatial alternation, Y-maze, and Morris water maze (Scullion et al., 2011). A downside to the work in Chapter 5 was that the study was absent of wildtype controls, as a result of this, it is unclear what the performance levels of TASTPM mice are relative to their littermate controls. Further work should aim to compare performance between TASTPM mice and littermate controls to tease out recognition memory impairments.

One of the main symptom of Alzheimer's disease is early episodic memory loss. Although considered a trait that is uniquely human (Tulving, 1983), recent work on birds (Clayton and Dickinson, 1998) and rats (Eacott and Norman, 2004) have revealed episodic-like memory in animals. Davis and colleagues (2013) reported that when 3xTgAD mice were tested in the What-Where-Which test of episodic-like memory, performance of 3xTgAD mouse were impaired compared to wildtype controls. Further work should aim to develop the continual trials paradigm to enable investigations of episodic-like memory in transgenic mouse models of AD; because while interference had no impact on performance of TASTPM mice in the spontaneous object recognition and object location task, this inference

should not be made in tests of episodic-like memory. The following section discusses the implications of the findings of study 5 and possible future work to build on those findings.

### 8.5 NMDA receptors in recognition memory

Following successful validation of the continual trials approach to testing recognition memory in normal and diseased mouse models, the paradigm was adapted to enable the manipulation of retention delays. The development of the behavioural paradigm to include longer retention delays allowed for the study of the effects of pharmacological substances on recognition memory.

The role of NMDA receptors in learning and memory have been widely studied, it was initially thought that NMDA receptors are involved in spatial and contextual representations that were hippocampal dependent, studies very quickly showed that the NMDA receptors were more general and implicated neural structures and behavioural assays (Robbins and Murphy, 2006), especially in learning and memory processes of encoding (for review see Riedel et al., 2003). Recently, studies have shown the role that NMDA receptors play in recognition memory (Winters and Bussey, 2005; Barker et al., 2006; King et al., 2004; Nilsson et al., 2007; de Lima et al., 2005; Pitsikas et al., 2006; van der Staay et al., 2011) using competitive and non-competitive NMDA receptor antagonists (AP5 and MK-801 respectively.

As discussed in section 8.2, work presented in chapter 7 (study 5) demonstrated that when state-dependency was controlled for, the administration of (0.01 and 0.1mg/kg) of MK-801 did not impair encoding, but the expression of memory was dependent on the state of the animal during the administration of the drug during sample and test, suggesting a state-dependent effect on memory. Findings from previous studies investigating the effects of MK-801 on recognition memory performance reported memory impairment when the drug

was given prior to encoding (de Lima et al., 2005; King et al., 2004; van der Staay et al., 2011) was not supported in the findings of study 5, the findings presented in this thesis found that the effects of the MK-801 was reversed when the drug was administered again at test, suggesting a state-dependent effect of memory. This study made a significant contribution to the investigations of the effects of MK-801 on recognition memory by being the first study that demonstrated state-dependent memory in the object recognition task using the drug. Despite this, there were shortcomings in the study which produced results that were unclear (chapter 7, study 5, experiment 4) that could be rectified by further work investigating the dose-dependent (0.01 and 0.1 mg/kg) effect of MK-801 in naïve mice using the 2x2 state-dependent design of the spontaneous object recognition task.

### 8.6 The continual trials approach to running recognition tasks

This thesis primarily sought to address the methodological issues often associated with spontaneous tasks of recognition memory in mice, such as increased behavioural variability compared to rats, and to adapt an existing continual trials paradigm that was initially developed to run recognition task in rats to test recognition memory in mice. The application of continual trials methodology in studies involving rats has been shown to be highly sensitive and a more reliable alternative to the 'standard' one-trial a day spontaneous object recognition task and its variants (Ameen-Ali et al., 2012; Seel et al., 2017). The findings reported in study 1 (reported in chapter 3) of this thesis have shown successful validation of the mouse version of the continual trials methodology in the spontaneous object recognition task and a more complex variant of object recognition: the object location task (Eacott and Norman, 2004; Langston and Wood, 2010; Davis et al., 2013).

Following the successful development and validation of the mouse version of the continual trials approach to assessing recognition memory in mice, the secondary aim of this thesis was to further examine whether the methodology was generalisable to different

research areas or strains of mice, such as: ageing or diseased models. This objective was accomplished by the investigation age-related changes of C57BL6J mice in the spontaneous object recognition and object location task using the continual trials paradigm (Study 2; reported in chapter 4); and the examination of age-related changes of object recognition and object location memory of a transgenic mouse model of Alzheimer's disease: TASTPM mice (study 3, reported in chapter 5).

The third aim of the thesis was to extend the application of the mouse version of the continual trials approach to running spontaneous tasks to test pharmacological substances. To achieve this, the behavioural paradigm of the continual trials apparatus was adapted to incorporate longer retention delays (study 4, reported in chapter 6); instead of running sample and test phases in sequential orders in a single testing session (i.e. sample trial 1, test trial 1, sample trial 2, test trial 2), the adapted methodology consisted of two testing sessions in which the sample trials were presented in the first testing session and test trials in the second testing session. This separation of the sample and test phases into two blocks enabled easier manipulation of retention delays. Following the successful adaptation of the continual trials apparatus to test longer retention delays, the next step was to examine the effects of pharmacological substances on recognition memory. Study 5 (chapter 7) examined the effects of NMDA receptor blockade by the MK-801 antagonist on object recognition memory using the continual trials approach.

The continual trials methodology allows for multiple trials to be run per animal, which increases task reliability by limiting the day-to-day behavioural variability of mice. Also, the handling of mice was vastly reduced in the continual trial task, with animals being handled only twice throughout a single testing session (once at the start and once more at the end of the session). The reduction of handling by experimenters would in turn reduce the amount of stress and this enables the animal to display true recognition abilities (Ameen-Ali et al., 2012; Hurst and West, 2010). As a result of the reduction of handling, day to day

behavioural variabilities, and the increased trial number within the session, the continual trials approach to running spontaneous tasks is much more reliable and refined.

In standard spontaneous object recognition tasks, an animal typically receives several bouts of handling by the experimenter: two bouts at sample and two at test (when the animal is placed into the open field and taken out of the open field at sample and test respectively), totalling to 4 handling bouts per trial. This demonstrates that, across an 8-trial session, which was the lowest number of trials used in studies reported in the present thesis (Chapter 6 and Chapter 7), an animal would receive 32 bouts of handling by the experimenter.

It is crucial to note that the experimenters' handling method may affect an animals' level of anxiety. If the experimenter uses handling methods that induce higher levels of anxiety, this may in turn affect the animals' behavioural response. Because the object recognition task is reliant on an animals' spontaneous behaviour and propensity to novelty, increased anxiety may induce behaviour that may mask recognition memory of the animal (Yuan et al., 2009). In a paper by Hurst & West (2010), they demonstrated that different types of handling methods affected voluntary interaction of mice with the experimenter. They found that mice engaged in more voluntary interaction with the experimenter when the animals were handled using the 'tunnel' (where mice voluntarily walked into a tunnel) and the 'cup' (where the experimenters loosely cup their hands around the mouse for 30s) method compared to animals that were handled with the more traditional 'tail' method (where the mice are picked up by their tails).

In contrast to the standard spontaneous object recognition tasks, the experimenters are not required to handle the animals as much in the continual trials approach. Because the trials are conducted within a session (interleaved sample and phases; see chapter 3, 4 and 5), an animal would only be handled twice, once at the beginning of the session and once at the end of the session. Even when the session is split between blocks of sample and test phases (see Chapter 6 and 7), animals receives a maximum of 4 bouts of handling: twice during the

sample phase and two times more during the test phase. The introduction of the continual trials approach resulted in the massive reduction of handling that the animals receives.

Thus, performance of mice in the continual trials approach is not as susceptible to possible poor handling by experimenter compared to the standard version of the spontaneous object recognition task. with its reduced number of handling. Also, the variability exacerbated by poor (or problematic) handling is diminished by the reduction of handling in the continual trials approach – increased refinement and reliability of the task.

In addition, as shown by findings presented in this thesis (Study 1, Chapter 2) by increasing the number of trials within a single testing session, a 30% reduction of mice number typically used in spontaneous object recognition and object location tasks was achieved whilst maintaining substantial statistical power compared to previous studies (Sanderson et al., 2011; Davis et al., 2013; see table 3.3 for further details). Although this is true in study 2, one should be aware that consideration should be placed when aiming to reduce the number of subjects used in experiments involving diseased mice and pharmacological substances. This is because, apart from within animal behavioural variability, one has to consider the increased between animal variability, which could be reduced with a larger sample, and this is especially true for diseased mouse models. For example, in research investigating variability within the ageing population, with some studies reporting that performance of some older adults being comparable to the younger population, creating a bimodal distribution of performance in the older population (Rapp and Amaral, 1992; Hedden and Park, 2003).

Whilst the main effect sizes (chapter 3) are comparable to previous studies using the object recognition and object location task, it is unclear whether the same conclusions can be drawn from the findings of chapter 4 and 5 examining the effects of experience on performance in the object recognition and object location. The population of the naïve groups (N = 4) in both chapter 4 and 5, was derived from older 10 month old C57ML/6J

mice and the TASTPM mouse model, and as mentioned above, the animals would naturally have a higher variability in their spontaneous behaviour. Hence, having a small sample size of 4 in the naïve group compared against an experienced group with a larger sample size, renders it difficult to draw any firm conclusion due to the unequal effect sizes between both groups.

The effects of sex differences on object recognition and object location memory was not evaluated in this thesis. Similar to the effects of experience, this is due to the small sample sizes of males and females at the end of the studies (see chapter 4 and 5), thus any effects that may be found might not be conclusive in part due to the small effect sizes of the group. This may be further exacerbated due to the age and the transgenic strain of the mouse model used in the studies.

The use of the continual trial apparatus to assess recognition memory in mice has enabled investigations into the changes of performance levels during a single testing session (proactive interference) and between testing sessions. Proactive interference often occurs when memory load is taxed as a result of previous memory interfering with the ability to form new memories (Still, 1969; Lustig et al., 2001). The findings presented in this thesis have provided little evidence of proactive interference influencing the performance levels of mice during an 8-, 12- and 16-trial testing session. Furthermore, the use of the continual trials method allows the investigations of neurological pathways and its effects on interference (Seel et al., 2017). As discussed above (section 8.3), there was little evidence of possible interference between the testing sessions caused by reuse of objects.

The current continual trial apparatus consisted of only one available context; therefore, it was not possible to investigate experiments which required contextual change during the experiment. A potential improvement to the continual trial apparatus could include the introduction of different contexts to develop tasks of object-context (what-which), location-

context (Where-which) and recollection based memory tasks such as the investigation of episodic-like memory (What-where-which) in mice (Easton et al., 2010).

## 8.7 Conclusion

In conclusion, the work presented in this thesis primarily addressed the methodological issues often associated with recognition tasks in mice. As discussed above, the continual trials approach has been validated and applied in several different areas of research which involve the use of the spontaneous object recognition and its variants, such as gerontology, Alzheimer's disease and pharmacological research. The findings in this thesis shows the potential of the continual trials approach in the field of neuroscience and in industry. Further work on the continual trials apparatus is currently ongoing, such as the developing an automated version of the continual trials task in collaboration with an industrial partner.

## REFERENCES


Adriani, W., Felici, A., Sargolini, F., Roullet, P., Usiello, A., Oliverio, A., & Mele, A. (1998). N-methyl-D-aspartate and dopamine receptor involvement in the modulation of locomotor activity and memory processes. *Experimental Brain Research*, *123*(1–2), 52–59. https://doi.org/10.1007/s002210050544

Aggleton, J. P. (1985). One-Trial Object Recognition by Rats. *The Quarterly Journal of Experimental Psychology Section B*, *37*(4b), 279–294. https://doi.org/10.1080/14640748508401171

Aggleton, J. P., Blindt, H. S., & Candy, J. M. (1989). Working memory in aged rats. *Behavioral Neuroscience*, *103*(5), 975–983. https://doi.org/10.1037/0735-7044.103.5.975

Aggleton, J. P., Vann, S. D., Denby, C., Dix, S., Mayes, A. R., Roberts, N., & Yonelinas, A. P. (2005). Sparing of the familiarity component of recognition memory in a patient with hippocampal pathology. *Neuropsychologia*, *43*(12), 1810–1823. https://doi.org/10.1016/j.neuropsychologia.2005.01.019

Åhlander, M., Misane, I., Schött, P. A., & Ögren, S. O. (1999). A Behavioral Analysis of the Spatial Learning Deficit Induced by the NMDA Receptor Antagonist MK-801 (Dizocilpine) in the Rat. *Neuropsychopharmacology*, *21*(3), 414–426. https://doi.org/10.1016/S0893-133X(98)00116-X

Ainge, J. A., Heron-Maxwell, C., Theofilas, P., Wright, P., de Hoz, L., & Wood, E. R. (2006). The role of the hippocampus in object recognition in rats: Examination of the influence of task parameters and lesion size. *Behavioural Brain Research*, *167*(1), 183–195. https://doi.org/10.1016/J.BBR.2005.09.005

Aitken, D. H., & Meaney, M. J. (n.d.). Temporally graded, age-related impairments in spatial memory in the rat. *Neurobiology of Aging*, *10*(3), 273–276. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2747831

Albasser, M. M., Amin, E., Iordanova, M. D., Brown, M. W., Pearce, J. M., & Aggleton, J. P. (2011). Separate but interacting recognition memory systems for different senses: The role of the rat perirhinal cortex. *Learning & Memory*, *18*(7), 435–443. https://doi.org/10.1101/lm.2132911

Albasser, M. M., Davies, M., Futter, J. E., & Aggleton, J. P. (2009). Magnitude of the object recognition deficit associated with perirhinal cortex damage in rats: Effects of varying the lesion extent and the duration of the sample period. *Behavioral Neuroscience*, *123*(1), 115–124. https://doi.org/10.1037/a0013829

Albasser, M. M., Olarte-Sánchez, C. M., Amin, E., Brown, M. W., Kinnavane, L., & Aggleton, J. P. (2015). Perirhinal cortex lesions in rats: Novelty detection and sensitivity to interference. *Behavioral Neuroscience*, *129*(3), 227–243. https://doi.org/10.1037/bne0000049

Albasser, M. M., Chapman, R. J., Amin, E., Iordanova, M. D., Vann, S. D., & Aggleton, J. P. (2010). New behavioral protocols to extend our knowledge of rodent object

recognition memory. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *17*(8), 407–419. https://doi.org/10.1101/lm.1879610

Alvarez-Royo, P., Clower, R. P., Zola-Morgan, S., & Squire, L. R. (1991). Stereotaxic lesions of the hippocampus in monkeys: determination of surgical coordinates and analysis of lesions using magnetic resonance imaging. *Journal of Neuroscience Methods*, *38*(2–3), 223–232. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1784125

Amalric, M., Ouagazzal, A., Baunez, C., & Nieoullon, A. (1994). Functional interactions between glutamate and dopamine in the rat striatum. *Neurochemistry International*, *25*(2), 123–131. https://doi.org/10.1016/0197-0186(94)90031-0

Ameen-Ali, K. E., Eacott, M. J., & Easton, A. (2012). A new behavioural apparatus to reduce animal numbers in multiple types of spontaneous object recognition paradigms in rats. *Journal of Neuroscience Methods*, *211*(1), 66–76. https://doi.org/10.1016/J.JNEUMETH.2012.08.006

Ameen-Ali, K. E., Easton, A., & Eacott, M. J. (2015). Moving beyond standard procedures to assess spontaneous recognition memory. *Neuroscience & Biobehavioral Reviews*, *53*, 37–51. https://doi.org/10.1016/J.NEUBIOREV.2015.03.013

Ando, S., & Ohashi, Y. (1991). Longitudinal study on age-related changes of working and reference memory in the rat. *Neuroscience Letters*, *128*(1), 17–20. https://doi.org/10.1016/0304-3940(91)90750-N

Baddeley, A. D., & Hitch, G. (1974). Working Memory. *Psychology of Learning and Motivation*, *8*, 47–89. https://doi.org/10.1016/S0079-7421(08)60452-1

Baker, J. D., & Azorlosa, J. L. (1996). The NMDA antagonist MK-801 blocks the extinction of Pavlovian fear conditioning. *Behavioral Neuroscience*, *110*(3), 618–620. https://doi.org/10.1037//0735-7044.110.3.618

Baker, K. B., & Kim, J. J. (2002). Effects of Stress and Hippocampal NMDA Receptor Antagonism on Recognition Memory in Rats. *Learning & Memory*, *9*(2), 58–65. https://doi.org/10.1101/lm.46102

Balducci, C., & Forloni, G. (2011). App transgenic mice: Their use and limitations. *NeuroMolecular Medicine*. https://doi.org/10.1007/s12017-010-8141-7

Barbosa, F. F., de Oliveira Pontes, I. M., Ribeiro, S., Ribeiro, A. M., & Silva, R. H. (2012). Differential roles of the dorsal hippocampal regions in the acquisition of spatial and temporal aspects of episodic-like memory. *Behavioural Brain Research*, *232*(1), 269–277. https://doi.org/10.1016/J.BBR.2012.04.022

Barker, G. R. I., Bird, F., Alexander, V., & Warburton, E. C. (2007). Recognition Memory for Objects, Place, and Temporal Order: A Disconnection Analysis of the Role of the Medial Prefrontal Cortex and Perirhinal Cortex. *Journal of Neuroscience*, *27*(11), 2948–2957. https://doi.org/10.1523/JNEUROSCI.5289-06.2007

Barker, G. R. I., & Warburton, E. C. (2011). When Is the Hippocampus Involved in Recognition Memory? *Journal of Neuroscience*, *31*(29), 10721–10731. https://doi.org/10.1523/JNEUROSCI.6413-10.2011

Barker, G. R. I., & Warburton, E. C. (2008). NMDA receptor plasticity in the perirhinal and prefrontal cortices is crucial for the acquisition of long-term object-in-place associative memory. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(11), 2837–2844. https://doi.org/10.1523/JNEUROSCI.4447-07.2008

Barker, G. R. I., Warburton, E. C., Koder, T., Dolman, N. P., More, J. C. A., Aggleton, J. P., … Brown, M. W. (2006). The different effects on recognition memory of perirhinal kainate and NMDA glutamate receptor antagonism: implications for underlying plasticity mechanisms. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(13), 3561–3566. https://doi.org/10.1523/JNEUROSCI.3154-05.2006

Barker, G. R. I., & Warburton, E. C. (2011). When Is the Hippocampus Involved in Recognition Memory? *The Journal of Neuroscience*, *31*(29), 10721–10731. https://doi.org/10.1523/JNEUROSCI.6413-10.2011

Bartolini, L., Casamenti, F., & Pepeu, G. (1996). Aniracetam restores object recognition impaired by age, scopolamine, and nucleus basalis lesions. *Pharmacology, Biochemistry, and Behavior*, *53*(2), 277–283. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8808132

Bartus, R. T., & Dean, R. L. (1979). Recent memory in aged non-human primates: Hypersensitivity to visual interference during retention. *Experimental Aging Research*, *5*(5), 385–400. https://doi.org/10.1080/03610737908257214

Benvenga, M. J., & Spaulding, T. C. (1988). Amnesic effect of the novel anticonvulsant MK-801. *Pharmacology Biochemistry and Behavior*, *30*(1), 205–207. https://doi.org/10.1016/0091-3057(88)90445-5

Bierley, R. A., Rixen, G. J., Tröster, A. I., & Beatty, W. W. (1986). Preserved spatial memory in old rats survives 10 months without training. *Behavioral and Neural Biology*, *45*(2), 223–229. https://doi.org/10.1016/S0163-1047(86)90794-6

Bilkei-Gorzo, A. (2014). Genetic mouse models of brain ageing and Alzheimer's disease. *Pharmacology and Therapeutics*. https://doi.org/10.1016/j.pharmthera.2013.12.009

Boess, F. G., Hendrix, M., van der Staay, F.-J., Erb, C., Schreiber, R., van Staveren, W., … Koenig, G. (2004). Inhibition of phosphodiesterase 2 increases neuronal cGMP, synaptic plasticity and memory performance. *Neuropharmacology*, *47*(7), 1081–1092. https://doi.org/10.1016/J.NEUROPHARM.2004.07.040

Broadbent, N. J., Gaskin, S., Squire, L. R., & Clark, R. E. (2010). Object recognition memory and the rodent hippocampus. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *17*(1), 5–11. https://doi.org/10.1101/lm.1650110

Brown, M. W., & Xiang, J. Z. (1998). Recognition memory: neuronal substrates of the judgement of prior occurrence. *Progress in Neurobiology*, *55*(2), 149–189. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9618747

Brown, M. W., Wilson, F. A. W., & Riches, I. P. (1987). Neuronal evidence that inferomedial temporal cortex is more important than hippocampus in certain

processes underlying recognition memory. *Brain Research*, *409*(1), 158–162. https://doi.org/10.1016/0006-8993(87)90753-0

Brown, M. W., & Aggleton, J. P. (2001). Recognition memory: What are the roles of the perirhinal cortex and hippocampus? *Nature Reviews Neuroscience*, *2*(1), 51–61. https://doi.org/10.1038/35049064

Brown, M. W., Warburton, E. C., & Aggleton, J. P. (2010). Recognition memory: Material, processes, and substrates. *Hippocampus*, *20*(11), 1228–1244. https://doi.org/10.1002/hipo.20858

Buffalo, E. A., Reber, P. J., & Squire, L. R. (1998). The Human Perirhinal Cortex and Recognition Memory. *Hippocampus*, *8*, 330–339. Retrieved from https://pdfs.semanticscholar.org/5dc6/e8a8a2c136d6e131d1ddbb0da820e6f4f42a.pdf

Burke, S. N., Maurer, A. P., Hartzell, A. L., Nematollahi, S., Uprety, A., Wallace, J. L., & Barnes, C. A. (2012). Representation of three-dimensional objects by the rat perirhinal cortex. *Hippocampus*, *22*(10), 2032–2044. https://doi.org/10.1002/hipo.22060

Burke, S. N., & Barnes, C. A. (2010). Senescent synapses and hippocampal circuit dynamics. *Trends in Neurosciences*, *33*(3), 153–161. https://doi.org/10.1016/J.TINS.2009.12.003

Burke, S. N., Wallace, J. L., Nematollahi, S., Uprety, A. R., & Barnes, C. A. (2010). Pattern separation deficits may contribute to age-associated recognition impairments. *Behavioral Neuroscience*, *124*(5), 559–573. https://doi.org/10.1037/a0020893

Calhoun, M. E., Wiederhold, K.-H., Abramowski, D., Phinney, A. L., Probst, A., Sturchler-Pierrat, C., … Jucker, M. (1998). Neuron loss in APP transgenic mice. *Nature*, *395*(6704), 755–756. https://doi.org/10.1038/27351

Cansino, S. (2009). Episodic memory decay along the adult lifespan: A review of behavioral and neurophysiological evidence. *International Journal of Psychophysiology*, *71*(1), 64–69. https://doi.org/10.1016/J.IJPSYCHO.2008.07.005

Caprioli, A., Ghirardi, O., Giuliani, A., Ramacci, M. T., & Angelucci, L. (1991). Spatial learning and memory in the radial maze: A longitudinal study in rats from 4 to 25 months of age. *Neurobiology of Aging*, *12*(5), 605–607. https://doi.org/10.1016/0197-4580(91)90093-Y

Caramanos, Z., & Shapiro, M. L. (1994). Spatial memory and N-methyl-D-aspartate receptor antagonists APV and MK-801: memory impairments depend on familiarity with the environment, drug dose, and training duration. *Behavioral Neuroscience*, *108*(1), 30–43. https://doi.org/10.1037//0735-7044.108.1.30

Cavoy, A., & Delacour, J. (1993). Spatial but not object recognition is impaired by aging in rats. *Physiology & Behavior*, *53*(3), 527–530. https://doi.org/10.1016/0031-9384(93)90148-9

Cipolotti, L., Bird, C., Good, T., Macmanus, D., Rudge, P., & Shallice, T. (2006). Recollection and familiarity in dense hippocampal amnesia: A case study.

*Neuropsychologia*, *44*(3), 489–506. https://doi.org/10.1016/j.neuropsychologia.2005.05.014

Clark, R. E., Zola, S. M., & Squire, L. R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(23), 8853–8860. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11102494

Clark, R. E., & Martin, S. J. (2005). Interrogating rodents regarding their object and spatial memory. *Current Opinion in Neurobiology*, *15*(5), 593–598. https://doi.org/10.1016/J.CONB.2005.08.014

Clark, R. E., & Squire, L. R. (2010). An animal model of recognition memory and medial temporal lobe amnesia: history and current issues. *Neuropsychologia*, *48*(8), 2234–2244. https://doi.org/10.1016/j.neuropsychologia.2010.02.004

Clarke, J. R., Cammarota, M., Gruart, A., Izquierdo, I., & Delgado-García, J. M. (2010). Plastic modifications induced by object recognition memory processing. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(6), 2652–2657. https://doi.org/10.1073/pnas.0915059107

Clayton, N. S., & Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. *Nature*, *395*(6699), 272–274. https://doi.org/10.1038/26216

Colacicco, G., Welzl, H., Lipp, H.-P., & Würbel, H. (2002). Attentional set-shifting in mice: modification of a rat paradigm, and evidence for strain-dependent variation. *Behavioural Brain Research*, *132*(1), 95–102. https://doi.org/10.1016/S0166-4328(01)00391-6

Craik, F. I. M., & Bialystok, E. (2006). Cognition through the lifespan: mechanisms of change. *Trends in Cognitive Sciences*, *10*(3), 131–138. https://doi.org/10.1016/J.TICS.2006.01.007

Czech, C., & Grueninger, F. (2013). Animal models for Alzheimer's disease - the industry perspective. *Drug Discovery Today: Therapeutic Strategies*, pp. e73–e78. https://doi.org/10.1016/j.ddstr.2013.07.001

Davis, K. E., Eacott, M. J., Easton, A., & Gigg, J. (2013). Episodic-like memory is sensitive to both Alzheimer's-like pathological accumulation and normal ageing processes in mice. *Behavioural Brain Research*, *254*, 73–82. https://doi.org/10.1016/j.bbr.2013.03.009

Davis, K. E., Easton, A., Eacott, M. J., & Gigg, J. (2013). Episodic-like memory for what-where-which occasion is selectively impaired in the 3xTgAD mouse model of Alzheimer's disease. *Journal of Alzheimer's Disease*, *33*(3), 681–698. https://doi.org/10.3233/JAD-2012-121543

de Bruin, N., & Pouzet, B. (2006). Beneficial effects of galantamine on performance in the object recognition task in Swiss mice: Deficits induced by scopolamine and by prolonging the retention interval. *Pharmacology Biochemistry and Behavior*, *85*(1), 253–260. https://doi.org/10.1016/J.PBB.2006.08.007

de Lima, M. N. M., Laranja, D. C., Bromberg, E., Roesler, R., & Schröder, N. (2005). Pre- or post-training administration of the NMDA receptor blocker MK-801 impairs object recognition memory in rats. *Behavioural Brain Research*, *156*(1), 139–143. https://doi.org/10.1016/J.BBR.2004.05.016

Dede, A. J. O., Squire, L. R., & Wixted, J. T. (2014). A novel approach to an old problem: Analysis of systematic errors in two models of recognition memory. *Neuropsychologia*. https://doi.org/10.1016/j.neuropsychologia.2013.10.012

Dellu, F., Fauchey, V., Le Moal, M., & Simon, H. (1997). Extension of a new two-trial memory task in the rat: influence of environmental context on recognition processes. *Neurobiology of Learning and Memory*, *67*(2), 112–120. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9075239

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2005). Integrated memory for objects, places, and temporal order: Evidence for episodic-like memory in mice. *Neurobiology of Learning and Memory*, *84*(3), 214–221. https://doi.org/10.1016/j.nlm.2005.07.002

Deshmukh, S. S., & Knierim, J. J. (2011). Representation of Non-Spatial and Spatial Information in the Lateral Entorhinal Cortex. *Frontiers in Behavioral Neuroscience*, *5*. https://doi.org/10.3389/FNBEH.2011.00069

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behavioural Brain Research*, *99*(2), 191–200. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10512585

Dodart, J. C., Mathis, C., & Ungerer, a. (1997). Scopolamine-induced deficits in a two-trial object recognition task in mice. *Neuroreport*, *8*(5), 1173–1178. https://doi.org/10.1097/00001756-199703240-00023

Donaldson, W. (1996). The role of decision processes in remembering and knowing. *Memory & Cognition*, *24*(4), 523–533. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8757500

Dong, H., Goico, B., Martin, M., Csernansky, C. A., Bertchume, A., & Csernansky, J. G. (2004). Modulation of hippocampal cell proliferation, memory, and amyloid plaque deposition in APPsw (Tg2576) mutant mice by isolation stress. *Neuroscience*, *127*(3), 601–609. https://doi.org/10.1016/J.NEUROSCIENCE.2004.05.040

Dunn, J. C. (2004). Remember-know: a matter of confidence. *Psychological Review*, *111*(2), 524–542. https://doi.org/10.1037/0033-295X.111.2.524

Dunnett, S. B., Evenden, J. L., & Iversen, S. D. (1988). Delay-dependent short-term memory deficits in aged rats. *Psychopharmacology*, *96*(2), 174–180. https://doi.org/10.1007/BF00177557

Dunnett, S. B., Martel, F. L., & Iversen, S. D. (1990). Proactive interference effects on short-term memory in rats: II. Effects in young and aged rats. *Behavioral Neuroscience*, *104*(5), 666–670. https://doi.org/10.1037/0735-7044.104.5.666

Eacott, M. J., Gaffan, D., & Murray, E. A. (1994). Preserved recognition memory for small sets, and impaired stimulus identification for large sets, following rhinal cortex

ablations in monkeys. *The European Journal of Neuroscience*, *6*(9), 1466–1478. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8000570

Eacott, M. J., & Norman, G. (2004). Integrated Memory for Object, Place, and Context in Rats: A Possible Model of Episodic-Like Memory? *Journal of Neuroscience*, *24*(8), 1948–1953. https://doi.org/10.1523/JNEUROSCI.2975-03.2004

Eacott, M. J., & Easton, A. (2010). Episodic memory in animals: Remembering which occasion. *Neuropsychologia*, *48*(8), 2273–2280. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2009.11.002

Easton, A., Douchamps, V., Eacott, M., & Lever, C. (2012). A specific role for septohippocampal acetylcholine in memory? *Neuropsychologia*, *50*(13), 3156–3168. https://doi.org/10.1016/j.neuropsychologia.2012.07.022

Edhouse, W. V., & White, K. G. (1988). Cumulative proactive interference in animal memory. *Animal Learning & Behavior*, *16*(4), 461–467. https://doi.org/10.3758/BF03209387

Eichenbaum, H., Yonelinas, A. P., & Ranganath, C. (2007). The Medial Temporal Lobe and Recognition Memory. *Annual Review of Neuroscience*, *30*(1), 123–152. https://doi.org/10.1146/annurev.neuro.30.051606.094328

Eichenbaum, H., Stewart, C., & Morris, R. G. (1990). Hippocampal representation in place learning. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *10*(11), 3531–3542. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2230943

Eichenbaum, H., Otto, T., & Cohen, N. J. (1994). Two functional components of the hippocampal memory system. *Behavioural and Brain Sciences*, *17*, 449–518. https://doi.org/10.1017/S0140525X00035391

Ellenbroek, B., & Youn, J. (2016). Rodent models in neuroscience research: is it a rat race? *Disease Models & Mechanisms*, *9*(10), 1079–1087. https://doi.org/10.1242/dmm.026120

Ennaceur, A. (2010). One-trial object recognition in rats and mice: Methodological and theoretical issues. *Behavioural Brain Research*, *215*(2), 244–254. https://doi.org/10.1016/j.bbr.2009.12.036

Ennaceur, A., Michalikova, S., & Chazot, P. L. (2009). Do rats really express neophobia towards novel objects? Experimental evidence from exposure to novelty and to an object recognition task in an open space and an enclosed space. *Behavioural Brain Research*, *197*(2), 417–434. https://doi.org/10.1016/j.bbr.2008.10.007

Ennaceur, A., & Aggleton, J. P. (1997). The effects of neurotoxic lesions of the perirhinal cortex combined to fornix transection on object recognition memory in the rat. *Behavioural Brain Research*, *88*(2), 181–193. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9404627

Ennaceur, A., & Delacour, J. (1988). A new one - trial test for neurobiological studies of memory in rats . 1 " Behavioral data. *Behavioural Brain Research*, *31*, 47–59. https://doi.org/10.1016/0166-4328(88)90157-X

Ennaceur, A., Neave, N., & Aggleton, J. P. (1996). Neurotoxic lesions of the perirhinal cortex do not mimic the behavioural effects of fornix transection in the rat. *Behavioural Brain Research*, *80*, 9–25. Retrieved from http://psych.cf.ac.uk/home2/aggleton/Behav Brain Res - 80 - 9-25.pdf

Ennaceur, A., Neave, N., & Aggleton, J. P. (1997). Spontaneous object recognition and object location memory in rats: The effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research*, *113*(3), 509–519. https://doi.org/10.1007/PL00005603

Fahlström, A., Yu, Q., & Ulfhake, B. (2011). Behavioral changes in aging female C57BL/6 mice. *Neurobiology of Aging*, *32*(10), 1868–1880. https://doi.org/10.1016/j.neurobiolaging.2009.11.003

Fahy, F. L., Riches, I. P., & Brown, M. W. (1993). Neuronal activity related to visual recognition memory: long-term memory and the encoding of recency and familiarity information in the primate anterior and medial inferior temporal and rhinal cortex. *Experimental Brain Research*, *96*(3), 457–472. https://doi.org/10.1007/BF00234113

Fan, L., Zhao, Z., Orr, P. T., Chambers, C. H., Lewis, M. C., & Frick, K. M. (2010). Estradiol-induced object memory consolidation in middle-aged female mice requires dorsal hippocampal extracellular signal-regulated kinase and phosphatidylinositol 3-kinase activation. *J Neurosci*, *30*(12), 4390–4400. https://doi.org/10.1523/JNEUROSCI.4333-09.2010

Filliat, P., & Blanchet, G. (1995). Effects of TCP on Spatial Memory: Comparison with MK-801. *Pharmacology Biochemistry and Behavior*, *51*(2–3), 429–434. https://doi.org/10.1016/0091-3057(95)00002-E

Frick, K. M., Stillner, E. T., & Berger-Sweeney, J. (2000). Mice are not little rats: species differences in a one-day water maze task. *Neuroreport*, *11*(16), 3461–3465. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11095500

Frick, K. M., & Gresack, J. E. (2003). Sex differences in the behavioral response to spatial and object novelty in adult C57BL/6 mice. *Behavioral Neuroscience*, *117*(6), 1283–1291. https://doi.org/10.1037/0735-7044.117.6.1283

Gaffan, D. (1974). Recognition impaired and association intact in the memory of monkeys after transection of the fornix. *Journal of Comparative and Physiological Psychology*, *86*(6), 1100–1109. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4209603

Gage, F. H., Dunnett, S. B., & Bjorklund, A. (1989). Age-related impairments in spatial memory are independent of those in sensorimotor skills. *Neurobiology of Aging*, *10*(4), 347–352. https://doi.org/10.1016/0197-4580(89)90047-X

Gamiz, F., & Gallo, M. (2012). Spontaneous object recognition memory in aged rats: Complexity versus similarity. *Learning & Memory*, *19*(10), 444–448. https://doi.org/10.1101/lm.027003.112

Gerfen, C. R., Paletzki, R., & Heintz, N. (2013). GENSAT BAC Cre-recombinase driver lines to study the functional organization of cerebral cortical and basal ganglia circuits. *Neuron*, *80*(6), 1368–1383. https://doi.org/10.1016/J.NEURON.2013.10.016

Giovanello, K. S., Alexander, M., & Verfaellie, M. (2003). Differential impairment of person-specific knowledge in a patient with semantic dementia. *Neurocase*, *9*(1), 15–26. https://doi.org/10.1076/neur.9.1.15.14369

Girden, E., & Culler, E. (1937). Conditioned responses in curarized striate muscle in dogs. *Journal of Comparative Psychology*, *23*(2), 261–274. https://doi.org/10.1037/h0058634

Good, M. a, Hale, G., & Staal, V. (2007). Impaired "episodic-like" object memory in adult APPswe transgenic mice. *Behavioral Neuroscience*, *121*(2), 443–448. https://doi.org/10.1037/0735-7044.121.2.443

Grant, D. S. (1975). Proactive interference in pigeon short-term memory. *Journal of Experimental Psychology: Animal Behavior Processes*, *1*(3), 207–220. https://doi.org/10.1037/0097-7403.1.3.207

Grillo, S. L., Duggett, N. A., Ennaceur, A., & Chazot, P. L. (2013). Non-invasive infra-red therapy (1072 nm) reduces β-amyloid protein levels in the brain of an Alzheimer's disease mouse model, TASTPM. *Journal of Photochemistry and Photobiology B: Biology*, *123*, 13–22. https://doi.org/10.1016/J.JPHOTOBIOL.2013.02.015

Hale, G., & Good, M. (2005). Impaired Visuospatial Recognition Memory but Normal Object Novelty Detection and Relative Familiarity Judgments in Adult Mice Expressing the APPswe Alzheimer's Disease Mutation. *Behavioral Neuroscience*, *119*(4), 884–891. https://doi.org/10.1037/0735-7044.119.4.884

Hall, J. H., Wiseman, F. K., Fisher, E. M. C., Tybulewicz, V. L. J., Harwood, J. L., & Good, M. A. (2016). Tc1 mouse model of trisomy-21 dissociates properties of short- and long-term recognition memory. *Neurobiology of Learning and Memory*, *130*, 118–128. https://doi.org/10.1016/J.NLM.2016.02.002

Hallé, M., Tribout-Jover, P., Lanteigne, A.-M., Boulais, J., St-Jean, J. R., Jodoin, R., … Larocque, D. (2015). Methods to monitor monocytes-mediated amyloid-beta uptake and phagocytosis in the context of adjuvanted immunotherapies. *Journal of Immunological Methods*, *424*, 64–79. https://doi.org/10.1016/j.jim.2015.05.002

Hammond, R. S., Tull, L. E., & Stackman, R. W. (2004). On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiology of Learning and Memory*, *82*(1), 26–34. https://doi.org/10.1016/J.NLM.2004.03.005

Han, R.-W., Zhang, R.-S., Xu, H.-J., Chang, M., Peng, Y.-L., & Wang, R. (2013). Neuropeptide S enhances memory and mitigates memory impairment induced by MK801, scopolamine or Aβ1–42 in mice novel object and object location recognition tasks. *Neuropharmacology*, *70*, 261–267. https://doi.org/10.1016/J.NEUROPHARM.2013.02.002

Hardy, J. A., & Higgins, G. A. (1992). Alzheimer's Disease: The Amyloid Cascade Hypothesis. *Science*, *256*(5054). Retrieved from https://search.proquest.com/docview/213544666/fulltextPDF/9492A8C823D4438PQ/1?accountid=14533

Hargreaves, E. L., & Cain, D. P. (1992). Hyperactivity, hyper-reactivity, and sensorimotor deficits induced by low doses of the N-methyl-d-aspartate non-competitive channel

blocker MK801. *Behavioural Brain Research*, *47*(1), 23–33. https://doi.org/10.1016/S0166-4328(05)80249-9

Harrod, S. ., Flint, R. ., & Riccio, D. . (2001). MK-801 induced retrieval, but not acquisition, deficits for passive avoidance conditioning. *Pharmacology Biochemistry and Behavior*, *69*(3–4), 585–593. https://doi.org/10.1016/S0091-3057(01)00565-2

Hasher, L., Chung, C., May, C. P., & Foong, N. (2002). Age, time of testing, and proactive interference. *Canadian Journal of Experimental Psychology = Revue Canadienne de Psychologie Experimentale*, *56*(3), 200–207. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12271750

Hauser, E., Tolentino, J. C., Pirogovsky, E., Weston, E., & Gilbert, P. E. (2009). The effects of aging on memory for sequentially presented objects in rats. *Behavioral Neuroscience*, *123*(6), 1339–1345. https://doi.org/10.1037/a0017681

Hedden, T., & Park, D. (2003). Contributions of source and inhibitory mechanisms to age-related retroactive interference in verbal working memory. *Journal of Experimental Psychology. General*, *132*(1), 93–112. https://doi.org/10.1037/0096-3445.132.1.93

Hedden, T., & Gabrieli, J. D. E. (2004). Insights into the ageing mind: a view from cognitive neuroscience. *Nature Reviews Neuroscience*, *5*(2), 87–96. https://doi.org/10.1038/nrn1323

Heyser, C. J., & Chemero, A. (2012). Novel object exploration in mice: Not all objects are created equal. *Behavioural Processes*, *89*(3), 232–238. https://doi.org/10.1016/j.beproc.2011.12.004

Hofer, S. M., & Sliwinski, M. J. (2001). Understanding Ageing. *Gerontology*, *47*(6), 341–352. https://doi.org/10.1159/000052825

Hok, V., Poucet, B., Duvelle, É., Save, É., & Sargolini, F. (2016). Spatial cognition in mice and rats: similarities and differences in brain and behavior. *Wiley Interdisciplinary Reviews: Cognitive Science*, *7*(6), 406–421. https://doi.org/10.1002/wcs.1411

Holdstock, J. S. (2005). The role of the human medial temporal lobe in object recognition and object discrimination. *The Quarterly Journal Of Experimental Psychology*, *58*(34), 326–339. https://doi.org/10.1080/02724990444000177

Holdstock, J. S., Mayes, A. R., Cezayirli, E., Isaac, C. L., Aggleton, J. P., & Roberts, N. (2000). A comparison of egocentric and allocentric spatial memory in a patient with selective hippocampal damage. *Neuropsychologia*, *38*(4), 410–425. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10683392

Howlett, D. R., Bowler, K., Soden, P. E., Riddell, D., Davis, J. B., Richardson, J. C., … Hussain, I. (2008). Abeta deposition and related pathology in an APP x PS1 transgenic mouse model of Alzheimer's disease. *Histology and Histopathology*, *23*(1), 67–76. https://doi.org/10.14670/HH-23.67

Howlett, D. R. (2011). APP transgenic mice and their application to drug discovery. *Histology and Histopathology*, *26*, 1611–1632.

Howlett, D. R., Richardson, J. C., Austin, A., Parsons, A. A., Bate, S. T., Davies, D. C., & Gonzalez, M. I. (2004). Cognitive correlates of Aβ deposition in male and female mice bearing amyloid precursor protein and presenilin-1 mutant transgenes. *Brain Research*, *1017*(1–2), 130–136. https://doi.org/10.1016/j.brainres.2004.05.029

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., … Cole, G. (1996). Correlative Memory Deficits, Aβ Elevation, and Amyloid Plaques in Transgenic Mice. *Science*, *274*(5284), 99–103. https://doi.org/10.1126/SCIENCE.274.5284.99

Huang, Y.-W., Hu, W.-W., Chen, Z., Zhang, L.-S., Shen, H.-Q., Timmerman, H., … Yanai, K. (2004). Effect of the histamine H3-antagonist clobenpropit on spatial memory deficits induced by MK-801 as evaluated by radial maze in Sprague–Dawley rats. *Behavioural Brain Research*, *151*(1–2), 287–293. https://doi.org/10.1016/J.BBR.2003.09.002

Hurst, J. L., & West, R. S. (2010). Taming anxiety in laboratory mice. *Nature Methods*, *7*(10), 825–826. https://doi.org/10.1038/nmeth.1500

Jankowsky, J. L., Fadale, D. J., Anderson, J., Xu, G. M., Gonzales, V., Jenkins, N. A., … Borchelt, D. R. (2004). Mutant presenilins specifically elevate the levels of the 42 residue β-amyloid peptide in vivo: evidence for augmentation of a 42-specific γ secretase. *Human Molecular Genetics*, *13*(2), 159–170. https://doi.org/10.1093/hmg/ddh019

Jaramillo, S., & Zador, A. M. (2014). Mice and rats achieve similar levels of performance in an adaptive decision-making task. *Frontiers in Systems Neuroscience*, *8*, 173. https://doi.org/10.3389/fnsys.2014.00173

Jessberger, S., Clark, R. E., Broadbent, N. J., Clemenson, G. D., Consiglio, A., Lie, D. C., … Gage, F. H. (2009). Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learning & Memory*, *16*(2), 147–154. https://doi.org/10.1101/lm.1172609

Johnson, A. P., Woods-Fry, H., & Wittich, W. (2017). Effects of Magnification on Emotion Perception in Patients With Age-Related Macular Degeneration. *Investigative Opthalmology & Visual Science*, *58*(5), 2520. https://doi.org/10.1167/iovs.16-21349

Joyal, C. C., Beaudin, S., & Lalonde, R. (2000). Longitudinal Age-Related Changes In Motor Activities And Spatial Orientation In CD-1 Mice. *Archives of Physiology and Biochemistry*, *108*(3), 248–256. https://doi.org/10.1076/1381345520000710831ZFT248

Jucker, M., Walker, L. C., Schwarb, P., Hengemihle, J., Kuo, H., Snow, A. D., … Ingram, D. K. (1994). Age-related deposition of glia-associated fibrillar material in brains of c57BL/6 mice. *Neuroscience*, *60*(4), 875–889. https://doi.org/10.1016/0306-4522(94)90269-0

Kane, M. J., & Engle, R. W. (2000). Working-memory capacity, proactive interference, and divided attention: limits on long-term memory retrieval. *Journal of Experimental Psychology. Learning, Memory, and Cognition*, *26*(2), 336–358. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10764100

Kang, J., Cirrito, J., Dong, H., Csernansky, J., & Holtzman, D. (2007). Acute stress increases interstitial fluid amyloid-β via corticotropin-releasing factor and neuronal activity. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(25), 10673–10678. https://doi.org/10.1073/PNAS.0700148104

Karran, E., Mercken, M., & Strooper, B. De. (2011). The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nature Reviews Drug Discovery*, *10*(9), 698–712. https://doi.org/10.1038/nrd3505

Kelly, P. ., Bondolfi, L., Hunziker, D., Schlecht, H.-P., Carver, K., Maguire, E., … Sommer, B. (2003). Progressive age-related impairment of cognitive behavior in APP23 transgenic mice. *Neurobiology of Aging*, *24*(2), 365–378. https://doi.org/10.1016/S0197-4580(02)00098-2

Kesner, R. P., Bolland, B. L., & Dakis, M. (1993). Memory for spatial locations, motor responses, and objects: triple dissociation among the hippocampus, caudate nucleus, and extrastriate visual cortex. *Experimental Brain Research*, *93*(3), 462–470. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8519335

Kim, J., Delcasso, S., & Lee, I. (2011). Neural Correlates of Object-in-Place Learning in Hippocampus and Prefrontal Cortex. *Journal of Neuroscience*, *31*(47), 16991–17006. https://doi.org/10.1523/JNEUROSCI.2859-11.2011

Kim, J., & Frick, K. M. (2017). Distinct effects of estrogen receptor antagonism on object recognition and spatial memory consolidation in ovariectomized mice. *Psychoneuroendocrinology*, *85*, 110–114. https://doi.org/10.1016/j.psyneuen.2017.08.013

Kimura, R., & Ohno, M. (2009). Impairments in remote memory stabilization precede hippocampal synaptic and cognitive failures in 5XFAD Alzheimer mouse model. *Neurobiology of Disease*, *33*(2), 229–235. https://doi.org/10.1016/J.NBD.2008.10.006

King, M. ., Sleight, A. ., Woolley, M. ., Topham, I. ., Marsden, C. ., & Fone, K. C. . (2004). 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation—an effect sensitive to NMDA receptor antagonism. *Neuropharmacology*, *47*(2), 195–204. https://doi.org/10.1016/J.NEUROPHARM.2004.03.012

Kinnavane, L., Amin, E., Horne, M., & Aggleton, J. P. (2014). Mapping parahippocampal systems for recognition and recency memory in the absence of the rat hippocampus. *European Journal of Neuroscience*, *40*(12), 3720–3734. https://doi.org/10.1111/ejn.12740

Ko, T., & Evenden, J. (2009). The effects of psychotomimetic and putative cognitive-enhancing drugs on the performance of a n-back working memory task in rats. *Psychopharmacology*, *202*(1–3), 67–78. https://doi.org/10.1007/s00213-008-1314-5

Koek, W. (2011). Drug-induced state-dependent learning. *Behavioural Pharmacology*, *22*(5 and 6), 430–440. https://doi.org/10.1097/FBP.0b013e328348ed3b

Laatu, S., Revonsuo, A., Jaykka, H., Portin, R., & Rinne, J. O. (2003). Visual object recognition in early Alzheimer's disease: deficits in semantic processing. *Acta*

*Neurologica Scandinavica*, *108*(2), 82–89. https://doi.org/10.1034/j.1600-0404.2003.00097.x

Langston, R. F., & Wood, E. R. (2010). Associative recognition and the hippocampus: Differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. *Hippocampus*, *20*(10), 1139–1153. https://doi.org/10.1002/hipo.20714

Larkin, M. C., Lykken, C., Tye, L. D., Wickelgren, J. G., & Frank, L. M. (2014). Hippocampal output area CA1 broadcasts a generalized novelty signal during an object-place recognition task. *Hippocampus*, *24*(7), 773–783. https://doi.org/10.1002/hipo.22268

Lee, I., Hunsaker, M. R., & Kesner, R. P. (2005). The Role of Hippocampal Subregions in Detecting Spatial Novelty. *Behavioral Neuroscience*, *119*(1), 145–153. https://doi.org/10.1037/0735-7044.119.1.145

Lee, I., & Park, S.-B. (2013). Perirhinal cortical inactivation impairs object-in-place memory and disrupts task-dependent firing in hippocampal CA1, but not in CA3. *Frontiers in Neural Circuits*, *7*, 134. https://doi.org/10.3389/fncir.2013.00134

Lipp, H. P., & Wolfer, D. P. (1998). Genetically modified mice and cognition. *Current Opinion in Neurobiology*, *8*(2), 272–280. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9635213

Lustig, C., May, C., & Hasher, L. (2001). Working memory span and the role of proactive interference. *Journal of Experimental Psychology. General*, *130*(2), 199–207. https://doi.org/10.1037//0096-3445.130.2.199

Maasberg, D. W., Shelley, L. E., & Gilbert, P. E. (2012). Age-related changes in detection of spatial novelty. *Behavioural Brain Research*, *228*(2), 447–451. https://doi.org/10.1016/J.BBR.2011.12.024

Mackes, J. L., & Willner, J. (2006). NMDA antagonist MK-801 impairs acquisition of place strategies, but not their use. *Behavioural Brain Research*, *175*(1), 112–118. https://doi.org/10.1016/J.BBR.2006.08.011

Madisen, L., Zwingman, T. A., Sunkin, S. M., Oh, S. W., Zariwala, H. A., Gu, H., … Zeng, H. (2010). A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nature Neuroscience*, *13*(1), 133–140. https://doi.org/10.1038/nn.2467

Manns, J. R., & Squire, L. R. (1999). Impaired recognition memory on the doors and people test after damage limited to the hippocampal region. *Hippocampus*, *9*(5), 495–499. https://doi.org/10.1002/(SICI)1098-1063(1999)9:5<495::AID-HIPO2>3.0.CO;2-O

Manns, J. R., Hopkins, R. O., & Squire, L. R. (2003). Semantic memory and the human hippocampus. *Neuron*, *38*(1), 127–133. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12691670

Markowska, A. L. (1999). Sex dimorphisms in the rate of age-related decline in spatial memory: relevance to alterations in the estrous cycle. *The Journal of Neuroscience :*

*The Official Journal of the Society for Neuroscience*, *19*(18), 8122–8133. https://doi.org/10.1523/JNEUROSCI.19-18-08122.1999

Markowska, A. L., & Savonenko, A. V. (2002). Protective Effect of Practice on Cognition during Aging: Implications for Predictive Characteristics of Performance and Efficacy of Practice. *Neurobiology of Learning and Memory*, *78*(2), 294–320. https://doi.org/10.1006/NLME.2002.4064

Markowska, A. L., & Savonenko, A. (2002). Retardation of cognitive aging by life-long diet restriction: Implications for genetic variance. *Neurobiology of Aging*, *23*(1), 75–86. https://doi.org/10.1016/S0197-4580(01)00249-4

May, C. P., Hasher, L., & Kane, M. J. (1999). The role of interference in memory span. *Memory & Cognition*, *27*(5), 759–767. https://doi.org/10.3758/BF03198529

McLamb, R. L., Williams, L. R., Nanry, K. P., Wilson, W. A., & Tilson, H. A. (1990). MK-801 impedes the acquisition of a spatial memory task in rats. *Pharmacology Biochemistry and Behavior*, *37*(1), 41–45. https://doi.org/10.1016/0091-3057(90)90038-J

Mele, A., Castellano, C., Felici, A., Cabib, S., Caccia, S., & Oliverio, A. (1996). Dopamine-N-methyl-d-aspartate interactions in the modulation of locomotor activity and memory consolidation in mice. *European Journal of Pharmacology*, *308*(1), 1–12. https://doi.org/10.1016/0014-2999(96)00266-X

Meunier, M., Bachevalier, J., Mishkin, M., & Murray, E. A. (1993). Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *13*(12), 5418–5432. https://doi.org/10.1523/JNEUROSCI.13-12-05418.1993

Miller, E. K., Erickson, C. A., & Desimone, R. (1996). Neural mechanisms of visual working memory in prefrontal cortex of the macaque. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *16*(16), 5154–5167. https://doi.org/10.1523/JNEUROSCI.16-16-05154.1996

Miller, E. K., Li, L., & Desimone, R. (1993). Activity of neurons in anterior inferior temporal cortex during a short-term memory task. *The Journal of Neuroscience*, *13*(4), 1460–1478. https://doi.org/10.1523/JNEUROSCI.13-04-01460.1993

Mishkin, M. (1978). Memory in monkeys severely impaired by combined but not by separate removal of amygdala and hippocampus. *Nature*, *273*(5660), 297–298. https://doi.org/10.1038/273297a0

Mishkin, M., & Delacour, J. (1975). An analysis of short-term visual memory in the monkey. *Journal of Experimental Psychology. Animal Behavior Processes*, *1*(4), 326–334. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/811754

Mondadori, C., Weiskrantz, L., Buerki, H., Petschke, F., & Fagg, G. E. (1989). NMDA receptor antagonists can enhance or impair learning performance in animals. *Experimental Brain Research*, *75*(3), 449–456. https://doi.org/10.1007/BF00249896

Mondadori, C., & Weiskrantz, L. (1993). NMDA receptor blockers facilitate and impair learning via different mechanisms. *Behavioral and Neural Biology*, *60*(3), 205–210. https://doi.org/10.1016/0163-1047(93)90371-N

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, *11*(1), 47–60. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6471907

Moss, M. B., Rosene, D. L., & Peters, A. (1988). Effects of aging on visual recognition memory in the rhesus monkey. *Neurobiology of Aging*, *9*, 495–502. https://doi.org/10.1016/S0197-4580(88)80103-9

Mumby, D. G., Pinel, J. P. J., & Wood, E. R. (1990). Nonrecurring-items delayed nonmatching-to-sample in rats: A new paradigm for testing nonspatial working memory. *Psychobiology*, *18*(3), 321–326. https://doi.org/10.3758/BF03327250

Mumby, D. G., Wood, E. R., & Pinel, J. P. J. (1992). Object-recognition memory is only mildly impaired in rats with lesions of the hippocampus and amygdala. *Psychobiology*, *20*(1), 18–27. https://doi.org/10.3758/BF03327156

Mumby, D. G., Gaskin, S., Glenn, M. J., Schramek, T. E., & Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *9*(2), 49–57. https://doi.org/10.1101/lm.41302

Murray, E. A., & Mishkin, M. (1998). Object recognition and location memory in monkeys with excitotoxic lesions of the amygdala and hippocampus. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *18*(16), 6568–6582. https://doi.org/10.1523/jneurosci.0480-05.2005

Nakagawa, Y., & Iwasaki, T. (1995). Involvement of benzodiazepine/GABA-A receptor complex in ethanol-induced state-dependent learning in rats. *Brain Research*, *686*(1), 70–76. https://doi.org/10.1016/0006-8993(95)00453-W

Nanfaro, F., Cabrera, R., Bazzocchini, V., Laconi, M., & Yunes, R. (2010). Pregnenolone sulfate infused in lateral septum of male rats impairs novel object recognition memory. *Pharmacological Reports*, *62*(2), 265–272. https://doi.org/10.1016/S1734-1140(10)70265-6

Nelson, A. J. D., & Vann, S. D. (2014). Mammilliothalamic tract lesions disrupt tests of visuo-spatial memory. *Behavioral Neuroscience*, *128*(4), 494–503. https://doi.org/10.1037/bne0000001

Nemanic, S., Alvarado, M. C., & Bachevalier, J. (2004). The hippocampal/parahippocampal regions and recognition memory: insights from visual paired comparison versus object-delayed nonmatching in monkeys. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(8), 2013–2026. https://doi.org/10.1523/JNEUROSCI.3763-03.2004

Nilsson, M., Hansson, S., Carlsson, A., & Carlsson, M. L. (2007). Differential effects of the N-methyl-d-aspartate receptor antagonist MK-801 on different stages of object recognition memory in mice. *Neuroscience*, *149*(1), 123–130. https://doi.org/10.1016/J.NEUROSCIENCE.2007.07.019

Norman, G., & Eacott, M. J. (2005). Dissociable Effects of Lesions to the Perirhinal Cortex and the Postrhinal Cortex on Memory for Context and Objects in Rats. *Behavioral Neuroscience*, *119*(2), 557–566. https://doi.org/10.1037/0735-7044.119.2.557

Norman, G., & Eacott, M. . (2004). Impaired object recognition with increasing levels of feature ambiguity in rats with perirhinal cortex lesions. *Behavioural Brain Research*, *148*(1–2), 79–91. https://doi.org/10.1016/S0166-4328(03)00176-1

Oddo, S., Caccamo, A., Kitazawa, M., Tseng, B. P., & LaFerla, F. M. (2003). Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. *Neurobiology of Aging*, *24*(8), 1063–1070. https://doi.org/10.1016/J.NEUROBIOLAGING.2003.08.012

Ohno, M. (2009). Failures to reconsolidate memory in a mouse model of Alzheimer's disease. *Neurobiology of Learning and Memory*, *92*(3), 455–459. https://doi.org/10.1016/J.NLM.2009.05.001

Olarte-Sánchez, C. M., Amin, E., Warburton, E. C., & Aggleton, J. P. (2015). Perirhinal cortex lesions impair tests of object recognition memory but spare novelty detection. *European Journal of Neuroscience*, *42*(12), 3117–3127. https://doi.org/10.1111/ejn.13106

Olarte-Sánchez, C. M., Kinnavane, L., Amin, E., & Aggleton, J. P. (2014). Contrasting networks for recognition memory and recency memory revealed by immediate-early gene imaging in the rat. *Behavioral Neuroscience*, *128*(4), 504–522. https://doi.org/10.1037/a0037055

Otto, T., & Eichenbaum, H. (1992). Neuronal activity in the hippocampus during delayed non-match to sample performance in rats: Evidence for hippocampal processing in recognition memory. *Hippocampus*, *2*(3), 323–334. https://doi.org/10.1002/hipo.450020310

Overton, D. A. (1991). Historical context of state dependent learning and discriminative drug effects. *Behavioural Pharmacology*, *2*(4 And 5), 253–264. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11224069

Pardon, M.-C., Sarmad, S., Rattray, I., Bates, T. E., Scullion, G. A., Marsden, C. A., … Kendall, D. A. (2009). Repeated novel cage exposure-induced improvement of early Alzheimer's-like cognitive and amyloid changes in TASTPM mice is unrelated to changes in brain endocannabinoids levels. *Neurobiology of Aging*, *30*(7), 1099–1113. https://doi.org/10.1016/j.neurobiolaging.2007.10.002

Pezze, M.-A., Marshall, H. J., & Cassaday, H. J. (2017). Scopolamine Impairs Appetitive But Not Aversive Trace Conditioning: Role of the Medial Prefrontal Cortex. *The Journal of Neuroscience*, *37*(26), 6289–6298. https://doi.org/10.1523/JNEUROSCI.3308-16.2017

Pichat, P., Bergis, O. E., Terranova, J.-P., Urani, A., Duarte, C., Santucci, V., … Scatton, B. (2007). SSR180711, a Novel Selective α7 Nicotinic Receptor Partial Agonist: (II) Efficacy in Experimental Models Predictive of Activity Against Cognitive Symptoms of Schizophrenia. *Neuropsychopharmacology*, *32*(1), 17–34. https://doi.org/10.1038/sj.npp.1301188

Pitsikas, N., Zisopoulou, S., & Sakellaridis, N. (2006). Nitric oxide donor molsidomine attenuates psychotomimetic effects of the NMDA receptor antagonist MK-801. *Journal of Neuroscience Research*, *84*(2), 299–305. https://doi.org/10.1002/jnr.20889

Prusky, G. T., West, P. W., & Douglas, R. M. (2000). Behavioral assessment of visual acuity in mice and rats. *Vision Research*, *40*(16), 2201–2209. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10878281

Pugh, P. L., Vidgeon-Hart, M. P., Ashmeade, T., Culbert, A. A., Seymour, Z., Perren, M. J., … Sunter, D. (2007). Repeated administration of the noradrenergic neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) modulates neuroinflammation and amyloid plaque load in mice bearing amyloid precursor protein and presenilin-1 mutant transgenes. *Journal of Neuroinflammation*, *4*(48). https://doi.org/10.1186/1742-2094-4-8

Radulovic, J., Jovasevic, V., & Meyer, M. A. (2017). Neurobiological mechanisms of state-dependent learning. *Current Opinion in Neurobiology*, *45*, 92–98. https://doi.org/10.1016/J.CONB.2017.05.013

Rapp, P. R., & Amaral, D. G. (1991). Recognition memory deficits in a subpopulation of aged monkeys resemble the effects of medial temporal lobe damage. *Neurobiology of Aging*, *12*(5), 481–486. https://doi.org/10.1016/0197-4580(91)90077-W

Rattray, I. (2008). What do we know about the long-term consequences of stress on ageing and the progression of age-related neurodegenerative disorders? *Neuroscience & Biobehavioral Reviews*, *32*(6), 1103–1120. https://doi.org/10.1016/J.NEUBIOREV.2008.03.005

Rattray, I., Scullion, G. A., Soulby, A., Kendall, D. A., & Pardon, M.-C. (2009). The occurrence of a deficit in contextual fear extinction in adult amyloid-over-expressing TASTPM mice is independent of the strength of conditioning but can be prevented by mild novel cage stress. *Behavioural Brain Research*, *200*(1), 83–90. https://doi.org/10.1016/J.BBR.2008.12.037

Reed, J. M., & Squire, L. R. (1997). Impaired recognition memory in patients with lesions limited to the hippocampal formation. *Behavioral Neuroscience*, *111*(4), 667–675. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9267644

Riedel, G., Platt, B., & Micheau, J. (2003). Glutamate receptor function in learning and memory. *Behavioural Brain Research*, *140*(1–2), 1–47. https://doi.org/10.1016/S0166-4328(02)00272-3

Ringo, J. L. (1996). Stimulus specific adaptation in inferior temporal and medial temporal cortex of the monkey. *Behavioural Brain Research*, *76*(1–2), 191–197. https://doi.org/10.1016/0166-4328(95)00197-2

Robbins, T. W., & Murphy, E. R. (2006). Behavioural pharmacology: 40+ years of progress, with a focus on glutamate receptors and cognition. *Trends in Pharmacological Sciences*, *27*(3), 141–148. https://doi.org/10.1016/J.TIPS.2006.01.009

Robitsek, R. J., Fortin, N. J., Koh, M. T., Gallagher, M., & Eichenbaum, H. (2008). Cognitive Aging: A Common Decline of Episodic Recollection and Spatial Memory

in Rats. *Journal of Neuroscience*, *28*(36), 8945–8954. https://doi.org/10.1523/JNEUROSCI.1893-08.2008

Rothblat, L. a, & Hayes, L. L. (1987). Short-term object recognition memory in the rat: nonmatching with trial-unique junk stimuli. *Behavioral Neuroscience*, *101*(4), 587–590. https://doi.org/10.1037/0735-7044.101.4.587

Salthouse, T. A., & Nesselroade, J. R. (2002). An examination of the Hofer and Sliwinski evaluation. *Gerontology*, *48*(1), 18-21-9. https://doi.org/10.1159/000048919

Sanderson, D. J., Hindley, E., Smeaton, E., Denny, N., Taylor, A., Barkus, C., … Bannerman, D. M. (2011). Deletion of the GluA1 AMPA receptor subunit impairs recency-dependent object recognition memory. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *18*(3), 181–190. https://doi.org/10.1101/lm.2083411

Save, E., Poucet, B., Foreman, N., & Buhot, M.-C. (1992). Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. *Behavioral Neuroscience*, *106*(3), 447–456. https://doi.org/10.1037/0735-7044.106.3.447

Scullion, G. A., Kendall, D. A., Marsden, C. A., Sunter, D., & Pardon, M. C. (2011). Chronic treatment with the α 2-adrenoceptor antagonist fluparoxan prevents age-related deficits in spatial working memory in APP × PS1 transgenic mice without altering β-amyloid plaque load or astrocytosis. *Neuropharmacology*, *60*(2–3), 223–234. https://doi.org/10.1016/j.neuropharm.2010.09.002

Seel, S. V., Eacott, M. J., Langston, R. F., & Easton, A. (2017). Cholinergic input to the hippocampus is not required for a model of episodic memory in the rat, even with multiple consecutive events. *Behavioural Brain Research*. https://doi.org/10.1016/j.bbr.2017.06.001

Shamy, J. L. T., Buonocore, M. H., Makaron, L. M., Amaral, D. G., Barnes, C. A., & Rapp, P. R. (2006). Hippocampal volume is preserved and fails to predict recognition memory impairment in aged rhesus monkeys (Macaca mulatta). *Neurobiology of Aging*, *27*(10), 1405–1415. https://doi.org/10.1016/j.neurobiolaging.2005.07.019

Sheng, J. G., Price, D. L., Koliatsos, V. E., Sisodia, S., Shao, P., Craft, J., … Vassar, R. (2002). Disruption of corticocortical connections ameliorates amyloid burden in terminal fields in a transgenic model of Abeta amyloidosis. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(22), 9794–9799. https://doi.org/10.1523/jneurosci.1202-06.2006

Shukitt-Hale, B., Casadesus, G., Cantuti-Castelvetri, I., & Joseph, J. A. (2001). Effect of age on object exploration, habituation, and response to spatial and nonspatial change. *Behavioral Neuroscience*, *115*(5), 1059–1064. https://doi.org/10.1037//0735-7044.115.5.1059

Sik, A., van Nieuwehuyzen, P., Prickaerts, J., & Blokland, A. (2003). Performance of different mouse strains in an object recognition task. *Behavioural Brain Research*, *147*(1–2), 49–54. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14659569

Silvers, J. M., Harrod, S. B., Mactutus, C. F., & Booze, R. M. (2007). Automation of the novel object recognition task for use in adolescent rats. *Journal of Neuroscience Methods*, *166*(1), 99–103. https://doi.org/10.1016/j.jneumeth.2007.06.032

Sobotka, S., & Ringo, J. L. (1996). Mnemonic responses of single units recorded from monkey inferotemporal cortex, accessed via transcommissural versus direct pathways: a dissociation between unit activity and behavior. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *16*(13), 4222–4230. https://doi.org/10.1523/JNEUROSCI.16-13-04222.1996

Squire, L. R. (1994). Memory and forgetting: long-term and gradual changes in memory storage. *International Review of Neurobiology*, *37*, 243-69-8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7883480

Squire, L. R., & Zola, S. M. (1998). Episodic memory, semantic memory, and amnesia. *Hippocampus*, *8*(3), 205–211. https://doi.org/10.1002/(SICI)1098-1063(1998)8:3<205::AID-HIPO3>3.0.CO;2-I

Squire, L. R., Stark, C. E. L. L., & Clark, R. E. (2004). The Medial Temporal Lobe. *Annual Review of Neuroscience*, *27*(1), 279–306. https://doi.org/10.1146/annurev.neuro.27.070203.144130

Squire, L. R., Wixted, J. T., & Clark, R. E. (2007). Recognition memory and the medial temporal lobe: a new perspective. *Nature Reviews. Neuroscience*, *8*(11), 872–883. https://doi.org/10.1038/nrn2154

Still, A. W. (1969). Proactive Interference and Spontaneous Alternation in Rats. *Quarterly Journal of Experimental Psychology*, *21*(4), 339–345. https://doi.org/10.1080/14640746908400229

Stover, K. R., Campbell, M. A., Van Winssen, C. M., & Brown, R. E. (2015). Early detection of cognitive deficits in the 3xTg-AD mouse model of Alzheimer's disease. *Behavioural Brain Research*, *289*, 29–38. https://doi.org/10.1016/J.BBR.2015.04.012

Stranahan, A. M. (2011). Similarities and differences in spatial learning and object recognition between young male C57Bl/6J mice and Sprague-Dawley rats. *Behavioral Neuroscience*, *125*(5), 791–795. https://doi.org/10.1037/a0025133

Sturchler-Pierrat, C., Abramowski, D., Duke, M., Wiederhold, K.-H., Mistl, C., Rothacher, S., … Sommer, B. (1997). Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proceedings of the National Academy of Sciences*, *94*(24), 13287–13292. https://doi.org/10.1073/PNAS.94.24.13287

Taniguchi, H., He, M., Wu, P., Kim, S., Paik, R., Sugino, K., … Huang, Z. J. (2011). A Resource of Cre Driver Lines for Genetic Targeting of GABAergic Neurons in Cerebral Cortex. *Neuron*, *71*(6), 995–1013. https://doi.org/10.1016/j.neuron.2011.07.026

Tulving, E. (2002). Episodic Memory: From Mind to Brain. *Annual Review of Psychology*, *53*(1), 1–25. https://doi.org/10.1146/annurev.psych.53.100901.135114

Uekita, T., & Okaichi, H. (2005). NMDA antagonist MK-801 does not interfere with the use of spatial representation in a familiar environment. *Behavioral Neuroscience*, *119*(2), 548–556. https://doi.org/10.1037/0735-7044.119.2.548

Underwood, B. J. (1957). Interference and forgetting. *Psychological Review*, *64*(1), 49–60. https://doi.org/10.1037/h0044616

Valdés Hernández, M. D. C., Booth, T., Murray, C., Gow, A. J., Penke, L., Morris, Z., … Wardlaw, J. M. (2013). Brain white matter damage in aging and cognitive ability in youth and older age. *Neurobiology of Aging*, *34*(12), 2740–2747. https://doi.org/10.1016/j.neurobiolaging.2013.05.032

Vales, K., Bubenikova-Valesova, V., Klement, D., & Stuchlik, A. (2006). Analysis of sensitivity to MK-801 treatment in a novel active allothetic place avoidance task and in the working memory version of the Morris water maze reveals differences between Long-Evans and Wistar rats. *Neuroscience Research*, *55*(4), 383–388. https://doi.org/10.1016/J.NEURES.2006.04.007

van der Staay, F. J., Rutten, K., Erb, C., & Blokland, A. (2011). Effects of the cognition impairer MK-801 on learning and memory in mice and rats. *Behavioural Brain Research*, *220*(1), 215–229. https://doi.org/10.1016/J.BBR.2011.01.052

van Goethem, N. P., Rutten, K., van der Staay, F. J., Jans, L. A. W., Akkerman, S., Steinbusch, H. W. M., … Prickaerts, J. (2012). Object recognition testing: Rodent species, strains, housing conditions, and estrous cycle. *Behavioural Brain Research*, *232*(2), 323–334. https://doi.org/10.1016/J.BBR.2012.03.023

Vanhooren, V., & Libert, C. (2013). The mouse as a model organism in aging research: Usefulness, pitfalls and possibilities. *Ageing Research Reviews*, *12*(1), 8–21. https://doi.org/10.1016/J.ARR.2012.03.010

Venable, N., & Kelly, P. H. (1990). Effects of NMDA receptor antagonists on passive avoidance learning and retrieval in rats and mice. *Psychopharmacology*, *100*(2), 215–221. https://doi.org/10.1007/BF02244409

Vezzani, A., Serafini, R., Stasi, M. A., Caccia, S., Conti, I., Tridico, R. V, & Samanin, R. (1989). Kinetics of MK-801 and its effect on quinolinic acid-induced seizures and neurotoxicity in rats. *The Journal of Pharmacology and Experimental Therapeutics*, *249*(1), 278–283. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2540317

Volianskis, A., Køstner, R., Mølgaard, M., Hass, S., & Jensen, M. S. (2010). Episodic memory deficits are not related to altered glutamatergic synaptic transmission and plasticity in the CA1 hippocampus of the APPswe/PS1ΔE9-deleted transgenic mice model of β-amyloidosis. *Neurobiology of Aging*, *31*(7), 1173–1187. https://doi.org/10.1016/J.NEUROBIOLAGING.2008.08.005

Vorhees, C. V., & Williams, M. T. (2014). Assessing Spatial Learning and Memory in Rodents. *ILAR Journal*, *55*(2), 310–332. https://doi.org/10.1093/ilar/ilu013

Wais, P. E., Wixted, J. T., Hopkins, R. O., & Squire, L. R. (2006). The hippocampus supports both the recollection and the familiarity components of recognition memory. *Neuron*, *49*(3), 459–466. https://doi.org/10.1016/j.neuron.2005.12.020

Warburton, E. C., & Brown, M. W. (2015). Neural circuitry for rat recognition memory. *Behavioural Brain Research*, *285*, 131–139. https://doi.org/10.1016/j.bbr.2014.09.050

Weible, A. P., Rowland, D. C., Pang, R., & Kentros, C. (2009). Neural Correlates of Novel Object and Novel Location Recognition Behavior in the Mouse Anterior Cingulate Cortex. *Journal of Neurophysiology*, *102*(4), 2055–2068. https://doi.org/10.1152/jn.00214.2009

Whishaw, I. (1995). A comparison of rats and mice in a swimming pool place task and matching to place task: Some surprising differences. *Physiology & Behavior*, *58*(4), 687–693. https://doi.org/10.1016/0031-9384(95)00110-5

Whishaw, I. Q., & Tomie, J. A. (1996). Of mice and mazes: similarities between mice and rats on dry land but not water mazes. *Physiology & Behavior*, *60*(5), 1191–1197. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8916170

White, A. M., & Best, P. J. (1998). The Effects of MK-801 on Spatial Working Memory and Within-Session Spatial Learning. *Pharmacology Biochemistry and Behavior*, *59*(3), 613–617. https://doi.org/10.1016/S0091-3057(97)00483-8

Whitt, E., & Robinson, J. (2013). Improved spontaneous object recognition following spaced preexposure trials: Evidence for an associative account of recognition memory. *Journal of Experimental Psychology: Animal Behavior Processes*, *39*(2), 174–179. https://doi.org/10.1037/a0031344

Willig, F., Palacios, A., Monmaur, P., M'Harzi, M., Laurent, J., & Delacour, J. (1987). Short-term memory, exploration and locomotor activity in aged rats. *Neurobiology of Aging*, *8*(5), 393–402. https://doi.org/10.1016/0197-4580(87)90033-9

Wimmer, M. E., Hernandez, P. J., Blackwell, J., & Abel, T. (2012). Aging impairs hippocampus-dependent long-term memory for object location in mice. *Neurobiology of Aging*, *33*(9), 2220–2224. https://doi.org/10.1016/j.neurobiolaging.2011.07.007

Winters, B. D., & Bussey, T. J. (2005). Glutamate Receptors in Perirhinal Cortex Mediate Encoding, Retrieval, and Consolidation of Object Recognition Memory. *Journal of Neuroscience*, *25*(17), 4243–4251. https://doi.org/10.1523/JNEUROSCI.0480-05.2005

Winters, B. D., Saksida, L. M., & Bussey, T. J. (2008). Object recognition memory: Neurobiological mechanisms of encoding, consolidation and retrieval. *Neuroscience and Biobehavioral Reviews*, *32*(5), 1055–1070. https://doi.org/10.1016/j.neubiorev.2008.04.004

Wixted, J. T., & Squire, L. R. (2011). The medial temporal lobe and the attributes of memory. *Trends in Cognitive Sciences*, *15*(5), 210–217. https://doi.org/10.1016/j.tics.2011.03.005

Wong, E. H., Kemp, J. A., Priestley, T., Knight, A. R., Woodruff, G. N., & Iversen, L. L. (1986). The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proceedings of the National Academy of Sciences of the United States of America*, *83*(18), 7104–7108. https://doi.org/10.1073/PNAS.83.18.7104

Xiang, J.-Z., & Brown, M. W. (1998). Differential neuronal encoding of novelty, familiarity and recency in regions of the anterior temporal lobe. *Neuropharmacology*, *37*(4–5), 657–676. https://doi.org/10.1016/S0028-3908(98)00030-6

Yonelinas, a P. (2001). Components of episodic memory: the contribution of recollection and familiarity. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *356*(1413), 1363–1374. https://doi.org/10.1098/rstb.2001.0939

Yuan, H., Long, H., Liu, J., Qu, L., Chen, J., & Mou, X. (2009). Effects of infrasound on hippocampus-dependent learning and memory in rats and some underlying mechanisms. *Environmental Toxicology and Pharmacology*, *28*(2), 243–247. https://doi.org/10.1016/J.ETAP.2009.04.011

Zhu, X. O., Brown, M. W., & Aggleton, J. P. (1995). Neuronal Sianallina of Information Imoortant to Visual Recognition-Memory in Rat Rhinal aid Neighbouring Cortices. *European Journal of Neuroscience*, *7*(4), 753–765. https://doi.org/10.1111/j.1460-9568.1995.tb00679.x

Zhu, X. O., Brown, M. W., McCabe, B. J., & Aggleton, J. P. (1995). Effects of the novelty or familiarity of visual stimuli on the expression of the immediate early gene c-fos in rat brain. *Neuroscience*, *69*(3), 821–829. https://doi.org/10.1016/0306-4522(95)00320-I

Zola, S. M., Squire, L. R., Teng, E., Stefanacci, L., Buffalo, E. A., & Clark, R. E. (2000). Impaired recognition memory in monkeys after damage limited to the hippocampal region. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(1), 451–463. https://doi.org/10.1523/jneurosci.1346-04.2004

Zola-Morgan, S., Squire, L. R., Amaral, D. G., & Suzuki, W. A. (1989). Lesions of perirhinal and parahippocampal cortex that spare the amygdala and hippocampal formation produce severe memory impairment. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *9*(12), 4355–4370. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2593004

Zola-Morgan, S., Squire, L. R., & Ramus, S. J. (1994). Severity of memory impairment in monkeys as a function of locus and extent of damage within the medial temporal lobe memory system. *Hippocampus*, *4*(4), 483–495. https://doi.org/10.1002/hipo.450040410