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The Demands of Episodic Memory on Hippocampal Function in Rats and Humans

Sabrina Vanessa Seel

Thesis submitted for the Degree of Doctor of Philosophy

Durham University, Psychology Department

2017

Abstract

This thesis sought to explore episodic memory, interference caused by similar events and its demands on hippocampal function by using different methodological and practical approaches in humans and rodents. Overall, this thesis focused on three aims, which included methodological approaches to testing episodic memory, using this approach to investigate cholinergic depletion of the hippocampus, and linking animal and human behavioural research. The recent development of spontaneous recognition tasks in rats to assess multiple trials consecutively in one testing session allow an opportunity to assess the role of contextual changes and interference in episodic memory. In a series of studies, it was shown that a new continuous trials apparatus can be used in behavioural as well as lesion studies to further explore the role of acetylcholine involved in episodic memory in rats without causing any proactive interference. Furthermore, the behavioural tasks in this thesis emphasise that context, which can take various forms, plays a profound role in segmenting memory of events. Whereas increasing the number of trials happening consecutively normally did not produce interference between events remembered, contextual representation within those trials was crucial. Chapters 2-7 demonstrated that depending on the context's nature it enhances the segmentation of similar episodes and avoids interference, but it can also hinder recollection of events. Chapter 8 supplemented these findings by providing evidence in humans, where a clear deficit in recollection was found when a spatial change in a virtual environment was encountered, revealing a location updating effect. However, further validation of the human episodic memory task is necessary to make it a useful method in assessing different forms of hippocampal mechanisms involved in episodic memory.

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Declaration

I confirm that no part of the material offered has previously been submitted by me for a degree in this or any other University. Any material generated through collaboration clearly acknowledges the work of others.

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Chapter 1

Introduction

1.1. Recognition memory

Recognition memory is a form of declarative memory, which may be defined as the ability to recognise encountered people, items or events (Brown & Aggleton, 2001) (Aggleton, 2001). Some models see recognition memory as a unitary process, whereas others proposed that two distinct processes support it. According to Yonelinas (2002) recognition may reflect two distinct memory processes: recollection and familiarity. For instance, you may see a person on the street and think they are familiar but you are unable to recollect who the person is or where you have seen them before. Recollection on the other hand triggers a process of recognition where you recall this person as someone you have previously met and remember details of that event. This distinction is captured in two-process models of recognition that regard ‘knowing’ and ‘remembering’ as separate processes, but both support recognition memory. Aggleton and Brown (1999) suggested that these two processes depend on different regions in the medial temporal lobe. The hippocampus is claimed to support recollection, and regions within the parahippocampal gyrus support familiarity (see also Eichenbaum, Otto and Cohen, 1984; Ranganath et al. 2003). Recollection is said to be more severely disrupted than familiarity by specific brain injuries, which indicates that these two are dependent on different brain regions. For instance, amnesic patients show greater memory deficits on associative tests than on item recognition tests (Yonelinas, 2002; Aggleton et al., 2000). Recognition tests that can be solved using familiarity-based mechanisms are functionally distinct and depend on different neural substrates than those that involve recollection of information. If all recognition memory judgements were based on a single form of memory then this kind of dissociation should not be observed.

However, other researchers argue that recognition is a single process, where ‘knowing’ simply reflects a weaker memory and ‘remembering’ is associated with a strong memory (Squire, Stark & Clark, 2004). Squire and Knowlton (2000) have acknowledged that familiarity and recollection may be functionally distinct, but subregions of the MTL may support familiarity and recollection equally. Studies of amnesic patients with different degrees of MTL damage have yielded conflicting results. Some neuropsychological studies have shown that the hippocampus is critical for recollection, whereas the parahippocampal region supports familiarity (Ranganath et al. 2003). However, other studies have shown similar roles for the hippocampus and parahippocampal regions in familiarity and recollection (Manns et al., 2003; Ranganath et al. 2003; Stark & Squire, 2001). These inconsistent findings could be due to different testing paradigms or varying degrees of MTL damage in patients. This raises the question why there are two distinct memory processes in the first instance. It has been argued that in many laboratory experiments, familiarity (know) judgements are faster than recollection (recollect), suggesting that familiarity discrimination leads to a fast and possibly more accurate detection of novelty, which would prove an evolutionary advantage (Brown & Aggleton, 2001).

Earlier experiments testing recognition memory tasks in non-human animals have tried to reproduce the damage of the medial temporal lobe that is found in human patients by using highly selective lesions (Haist et al., 1992; Parkin & Leng, 1993; Squire & Knowlton, 1995). Damage to the MTL causes profound amnesia (see Scoville & Milner, 1957) and reproducing this impairment in animals provides us with the chance to understand the underlying neuroanatomical mechanisms of memory better. However, developing tasks that are comparable between species is challenging and we must be sure to test the same kind of memory that is lost in amnesia. The ‘delayed matching to sample’ (DMS) task that was developed by Gaffan (1974) is a test of visual recognition memory in monkeys. The task

involves showing the animal an object during the sample phase, which they are required to remember. In the test phase the same object was presented again in addition to a new object. The monkey was trained to seek out the object that matches the one in the sample phase. This task is similar to the recognition memory task, which is used in amnesic patients (Clark & Squire, 2010). The DMS task was modified by Mishkin (1978) so that the monkey now had to seek out the novel object in the test phase. This was called the 'delayed nonmatching to sample' (DNMS) task, and uses the animal's innate preference for novel configurations. However, the DNMS and the DMS tasks involved training the monkeys and providing food rewards in order for the animals to carry out the task successfully. The animal is required to understand the rules of the task prior to the testing session, so that any observed deficits are the result of damages to the MTL and not merely a failure to apply the rules to the task (Dix & Aggleton, 1999). Additionally, animals were rewarded for correct responses, which can lead them to develop strategies to increase their food intake.

Given the problems associated with those two tasks, it was necessary to develop experimental procedures, which do not involve a large amount of pre-training and food reinforcements. Furthermore, in order to verify that recognition memory consists of two components it is essential to localise the individual structures within the MTL, namely the perirhinal cortex and hippocampus. Whereas human patient studies have proven to be useful in determining the brain structures underlying recognition, most research focuses on animal models, as they are easily controlled.

1.1.1 Spontaneous object recognition

Ennaceur and Delacour (1988) original paper described an object memory test in rats, which is based on their preferential exploration of novel objects over familiar objects. The task is based on the idea that rodents have an innate preference for novelty, which serves as a measure of recognition. In a typical spontaneous object recognition (SOR) task, rats are

exposed to two identical objects (sample phase) in an open field and they are allowed to explore freely for two to three minutes (Figure 1.1). Following a delay, which can range from 1 minute to 24 hours, rats are placed back in to the open field with a familiar copy of the object and a novel object (test phase). Given the rats' natural tendency to explore novel aspects of an environment, they will spend more time exploring the object they have not seen before. This preferential exploration is then used to determine the animal's memory for environmental configurations. This protocol has several advantages: (1) it is entirely based on spontaneous behaviour (2) it allowed researchers for the first time to use similar procedures in different species (3) it does not involve any reinforcements. However, disadvantages of using spontaneous exploration are: (1) behaviour can be driven by olfactory cues rather than memory (Clark & Squire, 2010), (2) produces only short-term memories (compared to human memory which can last years) and research about the perceptual capabilities of rodents is limited and (3) despite of the easy administration of SOR task in different laboratories, the procedure of the recognition test may vary and lead to inconsistent findings compared to rewarded studies. For example, some studies (such as Norman & Eacott, 2004) had animals perform the task repeatedly over multiple days, which led to multiple trials per animal. Other studies (for example Dere, Hutson & De Souza Silva, 2005) had animals only perform a single trial. Furthermore, some experiments use three minutes of free exploration for the sample and test phases (Norman & Eacott, 2004; Barker & Warburton, 2011), but others have phases that last up to 15 minutes (Ainge et al., 2006) or even 25 minutes (Mumby, Piterkin, Lecluse, & Lehman, 2007). This difference in exposure times can have a significant effect on the performance and behaviour of the rodents. Extending the time to explore the open field in the sample phase causes increased habituation to the objects, which could influence memory at test. As such, Albasser et al. (2009) examined the length of sample periods using different durations (4, 6 and 8 minutes). Results have shown that increasing the length of the sample

phase does not only increase object exploration, but also improves recognition at a 24hr delay. However, when the time period of exploration was split up, Dix and Aggleton (1999) found that the first two minutes were the most crucial.

Memory for novel configurations is not only influenced by the time spent in the open field, but also by the delay between sample and test phase. Increasing the delay will diminish performance at test as a result of forgetting, but the nature of the object will determine the memory strength and length (Norman & Eacott, 2004). Rats can successfully discriminate between novel and familiar items for up to 24 hours when junk objects (toys, vases, and cans) clearly differ. When objects were made of very similar material, such as blocks of Duplo, animals only successfully discriminated the novel from the familiar objects for 15 minutes (Norman & Eacott, 2004). The perirhinal cortex is thought to be crucial for recognition memory. Clear evidence comes from electrophysiological (Brown & Aggleton, 2001; Brown, Wilson, & Riches, 1987) and lesion studies in monkeys and in rats (Murray & Mishkin, 1998; Zola-Morgan, Squire, & Amaral, 1989; Norman & Eacott, 2004; Winters et al., 2004). In particular it has been suggested that the perirhinal cortex is necessary for the identification of complex objects that have features in common (Norman & Eacott, 2004). Fornix and hippocampal damage in contrast have not shown to impair SOR tasks (Barker & Warburton, 2011; Langston & Wood, 2010; Langston, et al., 2010).

The simplicity of the SOR task has led to its widespread use in many different laboratories and the task has many advantages. As it has been previously explained, the task relies on spontaneous behaviour, does not require food or any other kind of reinforcement and findings are mostly consistent across species (Clark and Martin, 2005). Nevertheless, object exploration can vary from animal to animal and even from trial to trial. Therefore, a large number of animals is required to maintain statistical power (for example 220 rats in Ennaceur & Delacour, 1988). Other influences such as the environment around the open

field, the cues in the testing room, and the natural mismatch of objects may lead the animal to explore one object over another. Careful counterbalancing is crucial in these tasks in order to minimise these confounding variables. Additionally, stressed animals can show signs of neophobia and their exploration and recognition abilities may diminish (Ennaceur, Michalikova, & Chazot, 2009; Hurst and West, 2010). Neophobia in animals towards novelty has commonly been reported (Belzun & Le Pape, 1994; Hoplight, Vincow, & Neumaier, 2005). Without habituation to an open field environment animals, tend to stay against walls and corners. In most experiments, the amount of habituation animals require is determined before testing begins, but these times vary between laboratories. To investigate the effect of neophobia on discrimination based on free exploration in rodents Ennaceur et al. (2009) exposed rats to an open field (enclosed space) and an elevated platform (open space). Animals were required to perform an object recognition task in either space and their exploratory behaviour was recorded. Results have shown that performance on the OR task is affected by too little habituation to the testing arena. Rats spent more time in corners and were unable to discriminate between novel and familiar objects (despite exploring the objects). Hence, exposure to an unfamiliar environment does not cause neophobia towards novelty, but it interferes with the task performance itself (Ennaceur et al., 2009).

Spontaneous recognition tasks have successfully been used to study memory for objects in animals, but it is also important to look at other aspects of recognition. Tasks that test memory for locations and contexts are a useful way to investigate the individual parts that contribute to episodic memory. If we understand how different components of episodic memory come together, it will enhance our picture of the neural mechanisms underlying memory.

1.1.2. Object location tasks

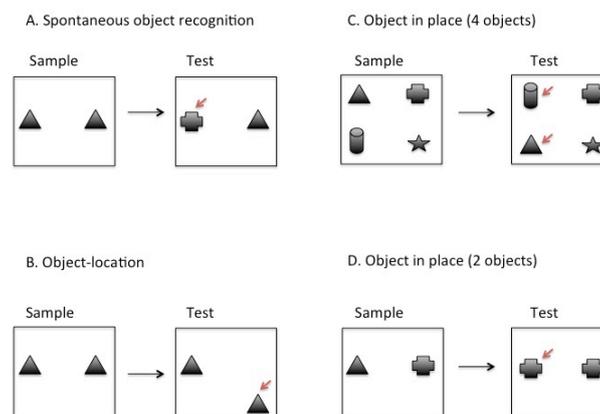
Object-location (OL) tasks allow us to test memory for an object and its place in an environment. In the OL task rats are free to explore two different objects in an open field during the sample, and at test one of the objects is moved to a new location (e.g. Save, Buhot, Foreman, Thinus-Blanc, 1992) (Figure 1.1). This leads the animal to re-explore the configuration and suggests that the animal has recognised the mismatch from the previous trial. In Save et al.'s (1992) study the behavioural response to the spatial change was assessed by comparing the time spent at the location of the missing item and a zone defined in another part of the open field. A similar task, object-in-place, involves putting rats in an open field with four different objects during the sample phase (Dix & Aggleton, 1999, Figure 1.1). The rat was allowed to freely explore the arena for five minutes (time was chosen because there were more objects to explore). In the test phase, two of the four objects swapped locations. The time spent exploring the two objects in novel locations was compared to the time spent exploring the objects that were in old locations. In other versions of this task (e.g. Davis, Eacott, & Gigg, 2013) only two different objects were used in the sample phase, and at test two copies of the same object were presented (Figure 1.1). Intact animals spent more time exploring the object that has previously not been in that location. Using two objects makes this task more comparable to the previously discussed SOR task.

The OL task has shown to be hippocampal dependent in rats. Save et al. (1992) investigated exploratory activity in response to a spatial change in rats with hippocampal and posterior parietal cortical lesions. Using a dishabituation paradigm, meaning that a stimulus was withdrawn after habituation, they showed that animals could demonstrate memory for the missing object and of its initial location. The disappearance of the stimulus induced a reaction to the change in control animals, but hippocampal and posterior parietal cortical animals did not show such a reaction, suggesting that the hippocampus may be involved in

the exploratory behaviour toward a disappeared stimulus. Detecting such a change would require memory that the stimulus was previously present.

Spatial memory tests continue to be widely used, but context also plays an important role in episodic memory.

Figure 1.1. Different test paradigms for spontaneous recognition tasks.



Each paradigm demonstrates a single trial, which consists of one sample and one test phase. The arrow indicates the novel object configuration. **A.** Spontaneous recognition (SOR). **B.** Object-location (OL). **C.** Object in place (OiP) with 4 four objects. **D.** Object in place (OiP) with two objects.

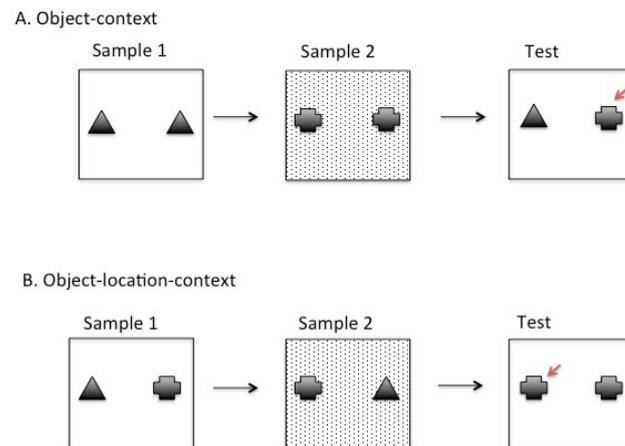
1.1.3. Object context tasks

Spontaneous recognition tasks can also be modified to test memory for contextual configurations of an environment (Figure 1.2). Contextual cues are important for memory and object-context tasks can help us understand the relationship between the episodic memory components. The role of context in memory was investigated by Dix & Aggleton (1999) and in their context condition, each session was divided up into four sample phases and a test. In the sample two identical object pairs were encountered in an arena in two different contexts. In the test phase one object was in the same context as in the sample phase and the other object was novel in that context. In other variants of this task only two sample phases are used (Figure 1.1). In the first sample rats are exposed to two copies of two different objects

(A and B) in context X, in the second sample rats see the same copies of objects but they swapped locations and are in context Y (B and A). At test, rats are placed in the open field with copies of both of the previously encountered objects in either X or Y (A and A, or B and B). Given rats' natural tendency to explore novelty, they will spend more time with the object that was previously not seen in that context.

Norman and Eacott (2005) used an object-in-context and SOR paradigm to investigate the effects of perirhinal and postrhinal cortex lesions. Sham lesioned rats explored an object that had previously been experienced in a different context more than one encountered in the same context (see also Dellu et al., 1997; Dix & Aggleton, 1999), but the postrhinal-lesioned group was severely impaired at this task. Animals with fornix lesions and perirhinal lesions were able to perform the task. However, perirhinal lesioned animals were impaired on the SOR task while postrhinal lesioned animals were not. This clearly shows a double dissociation between the perirhinal and postrhinal cortex. There have been reports of hippocampal lesions, which impair object-context memory (e.g: Mumby, Gaskin, Glenn, Schramek, Lehmann, 2002), but Norman and Eacott's (2005) fornix-lesioned group was still able to distinguish between the novel and old configuration. Langston and Wood (2010) reported a similar finding, suggesting that the hippocampal involvement may depend on the definition of 'context'. To fully understand the processes underlying contextual memory we need to be clear about the nature of what makes a 'context', which will be discussed later in this chapter. For example, context can be defined as the floor and walls of the arena, but also as the different features of the testing room. From an associative learning perspective, contextual representation is the binding and integrating of different elements (Robertson et al., 2015). In support of this, Albasser et al. (2013) have shown that hippocampal lesions impaired a learning task in rats, which relied on proximal context cues, but not when it relied on distal room cues.

Figure 1.2. Different test paradigms for spontaneous recognition tasks.



Each paradigm demonstrates a single trial, which consists of two samples and one test phase. The arrow indicates the novel object configuration. The environment in these tasks can be changed in order to test contextual memory. **A.** Object-context (OC). **B.** Object-location-context (OLC)

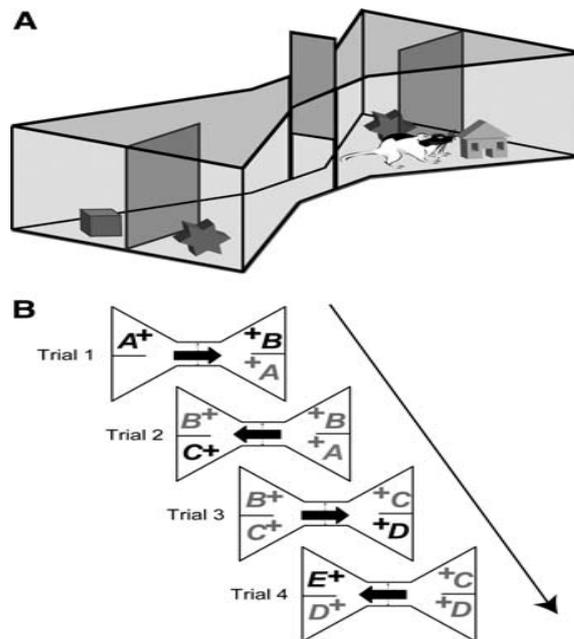
1.2. Multiple trials and statistical power

Many studies of recognition in rodents now use the SOR task and its variants to assess memory. As these tasks make use of the innate preference of rodents for novel items, they rely on spontaneous behaviour and do not require much pre-training. However, whilst the simplicity of administering SOR tasks has allowed a widespread use, there are some issues associated with them. In a standard spontaneous recognition task the animal completes one trial a day, which means that multiple sessions have to be run and data accumulation is slow and subject to behavioural noise. Furthermore, animals are constantly handled between sample and test phases by being moved in and out of the arena whilst objects and/or contexts are changed. This is problematic as behaviour of rodents can be heavily influenced through the stress of handling, alter the animal's reaction and distorting their object preferences (Hurst and West, 2010).

To address these limitations, Albasser, Chapman, Amin, Iordanova, Van, and Aggleton (2010) developed a new object recognition test, using a 'bow-tie maze' in rats. This protocol

combines features of the spontaneous recognition task with the delayed nonmatching-to-sample task. The maze consists of two compartments, shaped like a bow tie. The rat is placed in one end of the maze that contains an object (object A). After a short delay, it is allowed to shuttle to the other end of the maze where two different objects (objects A and B) are (Figure 1.3). In this scenario object A is familiar, because it has been seen before, but object B is novel. Rats will prefer to explore object B. On the next trial animals go back to the first compartment and encounter object B and C. Object B is now familiar and C novel, which means rats will preferentially explore object C. The objects are baited with pellets in order to encourage exploration, but are not used as rewards. This procedure is repeated several times and therefore yields to multiple data points within the same animal. The bow-tie maze has several advantages: (1) using multiple trials per session reduces the variance that are present in the normal SOR tasks, (2) it allows faster data collection, (3) provides high levels of discrimination and (4) counteracts the variance associated with object preference. The bow-tie maze is a good improvement on the previous object recognition tasks, but investigating spatial components of memory is not possible. Furthermore, the animal essentially sees a mirror image of what it has seen before and it cannot be determined if animals use allocentric or egocentric mechanisms to solve the task and the maze also does not allow for a context change, which excludes the possibility to test contextual configurations.

Figure 1.3. Schematic representation of the bow-tie maze by Albasser et al. (2010).

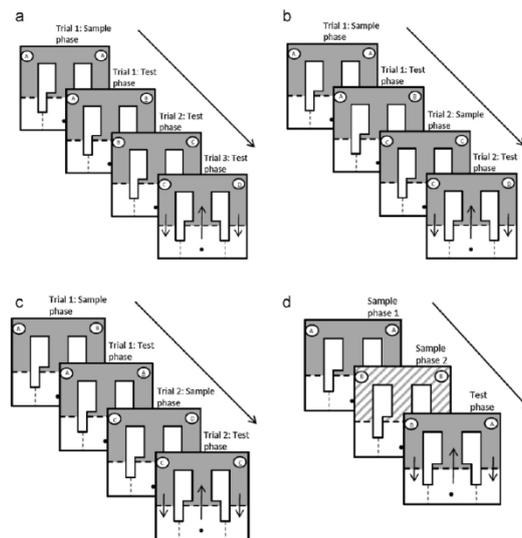


A. The sliding door separates the two compartments of the apparatus and the animal encounters two objects on each side. **B.** Presentation order of the objects in the SOR task. The arrow shows the movement of the rat (as shown in Albasser et al., 2010).

To resolve these issues Ameen-Ali, Eacott and Easton (2012) modified this approach and devised an apparatus that allows for multiple trials per session relying on measures of preferential exploration of novel objects (Figure 1.4). Rather than having test sessions every time the animal shuttled between chambers, the animal instead shuttles between a holding area (where the animal is held between phases of testing) and an object area (which is used for the presentation of objects in both samples and tests). Ameen-Ali et al. (2012) showed that in this new apparatus, SOR tasks could be run with a near 50% reduction in animal numbers, whilst maintaining statistical power. This approach was successfully used in three typical spontaneous recognition tasks, object recognition, object-location and object-in-context. Ameen-Ali et al.'s apparatus was designed to allow four contexts to be put in place for the object-context (OC) trials, through a rotating mechanism whilst the animal waited in the holding area. However, whilst Ameen-Ali et al. (2012) demonstrated that the animals could show OC memory, the design of the apparatus was not ideal, as it was very heavy and

the context change produced some noise when contexts were rotated into place which may have itself induced stress and produced the type of behavioural noise the apparatus was initially designed to remove. Additionally, tests of episodic memory have been shown to be unsuccessful (unpublished data by Ameen-Ali). However, being able to carry out spontaneous recognition tasks that involve context changes are essential for understanding the neural basis of memory.

Figure 1.4. Schematic representation of the test procedure used in Ameen-Ali et al. (2012).



The arrows indicate the rats' movement from the holding area to the testing area. The animal is presented with two objects and is given two minutes of free exploration. Following this delay the rat returns to the holding area so that the experimenter changes the objects. Once the objects have been changed for the test phase, the rat returns to the object area (as shown in Ameen-Ali et al. 2012).

The aim of Chapter 3 was to develop an apparatus that adopts the multiple trials concept of Ameen-Ali et al., but allowing for tests of context-place memory (Figure 1.5). My new continuous trials apparatus is more closely modelled on the open field, which is used for a one-trial a day what-where-which testing. It consists of a testing area and a holding area where the animal is placed at the start of testing and returns to after the completion of a phase. This area does not change. A sliding door allows the experimenter to control the movement of the animal between the two compartments. As the holding area is attached to

the open field arena, animals are trained to shuttle using pellets. The food ‘rewards’ are placed near the object to encourage exploration, and to make sure the animal keeps moving from one end of the maze to the other. After two minutes of exploration the animal is allowed to shuttle back in to the holding area and the context of the arena is changed. This process is repeated until 12 trials are completed.

Figure 1.5. Pictures of the continuous trials apparatus used in Chapters 2-7.



The apparatus consists of a testing area and a holding area. The holding area does not change, whereas the testing area has four different contexts depending on the trial.

In all of the following multiple trials tasks, the preference for novel configurations of an environment is measured through exploration times of objects. The most common measure of performance is the discrimination ratio (D2; see Ennaceur & Delacour, 1988), which is calculated as follows: $[(\text{exploration of novel item} - \text{exploration of familiar item}) / \text{total exploration}]$. It is also possible to calculate a ‘cumulative D2’, which is a running total of the D2 ratios recalculated after each trial. In the Bow-tie maze as well as in the continuous trials

apparatus by Ameen-Ali et al. (2012) a cumulative D2 was calculated. This reduces the weighting of trials where animals show less exploration. In a typical one-trial a day SOR task this is not the norm, rather each session's D2 is fed into an average D2 with each trial having equal weighting. This raises the questions whether a cumulative or average D2 score is the best approach to determine the exploration ratio for a continual trials approach to SOR tasks. The advantage of calculating a cumulative score is that any extreme values in exploration do not affect the average D2 ratio of an animal, further reducing behavioural noise. Both the average D2 (the D2 for each trial is the average of all the D2s from each trial up to that point) and the cumulative D2 (a D2 for a given trial calculated from the accumulated exploration times from all trials up to that point) will be reported in this thesis.

Based on previous studies in our lab (for example Ameen-Ali et al., 2012), it was crucial for the multiple trials apparatus to be effective in maintaining statistical power, whilst decreasing the number of animals typically used in behavioural and lesion experiments. As animal researchers we have a responsibility to justify the number of rodents used in experiments and ensure the appropriate number of animals is employed (based on the 3Rs). At times it is difficult to compute a sample size, because there is not enough information or the outcome of the experiment is highly unpredictable, such as with transgenic animals (Dell et al., 2002). Nevertheless, having clear hypotheses and questions before conducting an experiment helps to compute a sample size that will detect a significant effect (or a difference). Normally, to calculate the sample size the following factors must be known: (1) the effect size; (2) the population standard deviation; (3) the desired power of the experiment to detect the postulated effect; and (4) the significance level (for details see Dell et al., 2002).

The programme G * Power, which will be used in the data analyses by Faul, Erdfelder, Lang &, Buchner (2007), is a statistical power analysis tool for many tests, such as f-test, t-test,

chi² and others. It can also be used to calculate effect sizes and generate graphs showing the power analyses (for review see Faul et al., 2007 & 2009).

In case of the continuous trial apparatus used in most of the studies in this thesis, data was obtained from previous experiments carried out in the lab and a literature review was carried out to make an informed decision on the number of animals required. In all studies animals were randomly assigned to groups and testing protocols were counterbalanced to avoid bias and to reduce variability.

1.3. Episodic memory in animals

Episodic memory, the recollection of past events in our lives, has often been considered a memory specific to humans. Tulving (1983) defined episodic memory as memory that “receives and stores information about temporally dated episodes or events and temporal-spatial relations between them.” Thus, episodic memory is the conscious recollection of past events and is linked to mentally travelling back in time in order to re-experience events (Tulving, 1983). The awareness that an event has happened to oneself can normally easily be assessed in humans. However, asking participants whether they ‘remember’ an event or merely ‘know’ that it occurred can be challenging (Yonelinas, Kroll, Dobbins, Lazzara, & Knight, 1998), as this judgement entirely relies on the subjective experience of the participant. Additionally, this judgment is prone to inaccuracy and is difficult to explain, especially to brain damaged populations whose subjective experience may not be typical. It is impossible to directly ask animals about their past experiences, because of the absence of language. Therefore, researchers have argued that episodic memory, as Tulving described it, is unique to humans (for discussion see Clayton, Bussey, Emery, & Dickinson, 2003; Suddendorf & Busby, 2003). Nonetheless, there are evolutionary advantages to episodic memory. Past experiences are essential for planning future actions and help us keep track of events, suggesting that other species may also benefit from this type of memory (Dudai &

Carruthers, 2005). As such Clayton and Dickinson (1998) have suggested examining 'episodic-like memory', which excludes the necessity of conscious inferences. Using Tulving's initial definition, Clayton and Dickinson described episodic-like memory as what happened where and when.

1.3.1. What-where-when memory in animals

Clayton and Dickinson (1998) were the first to demonstrate what-where-when memory in Western scrub jays. Using their natural food-storing habits the birds were taught that food could be claimed at short (4hours) and long intervals (124hours) from a constant location where the birds themselves had cached it. At short intervals both peanuts and worms could be retrieved fresh, but after a long delay only peanuts would be fresh and worms would decay. In the wild these birds favour worms and preferentially search for these, and it would therefore be of advantage to them to remember where and how long ago the food was stored. In a controlled experiment, the scrub jays were given multiple trials and they quickly learnt to cache peanuts when the delay was increased. In doing so scrub jays expressed memory for the food (what), its place (where), and when it was stored. However, one issue with this paradigm is that it is based on the natural food-storing habits of these birds, making it difficult to compare the findings to other laboratory animals, such as rats or mice.

In an attempt to demonstrate what-where-when memory in rats Babb & Crystal (2005) investigated the discrimination of configurations in rats using an eight-arm radial maze. Rats were trained on a forced-choice task, where they had to find a food pellet at each of the eight arms in the maze. Testing was divided into forced choice and free-choice phases, separated by retention intervals. Animals were required to discriminate between short (30min) and long (4hrs) retention intervals. After the 4hour delay the chocolate replenished, after the 30min delay the chocolate pellets did not replenish. Rats learnt to use the length of the delay in order

to determine whether the arm with the chocolate had been replenished and whether it was worth re-visiting. Once the chocolate pellets were paired with lithium chloride, rats decreased their number of visits to the chocolate containing arms. By not going back to the original chocolate location after the LiCl administration, rats demonstrated memory for what happened where and when. Although this provides some evidence of episodic-like memory in rats, studies using food as reward undermine the true nature of episodic memory. Episodic memory in humans does not involve any training; it can take place without rule learning (Zentall, 2006) and it is automatic (Tulving, 2002). One major criticism of Babb & Crystal's (2005) study is that rats could have developed alternative strategies based upon the time of day, as rats were tested in the forced choice phase in the morning and the free choice was tested in the morning or afternoon after the retention intervals (Hampton, Hampstead, & Murray, 2005). This strategy would involve a non-episodic solution to the task, as rats could use rules to revisit the location at different rates in the morning and afternoon. Rats could have timed different retention intervals rather than remembering the actual episode, which would suggest that memory for 'when' was not sufficiently demonstrated (Hampton et al., 2005; Roberts et al., 2008). One way of overcoming this issue is to maintain a constant time of day at the test phase, but to randomly mix short and long intervals (see Nasqshbandi et al., 2007).

Another way of testing episodic-like memory in animals was devised by Kart-Teke, De Souza, Silva, Huston and Dere (2006). Their study was based on the spontaneous recognition paradigm (see also: Ennaceur & Delacour, 1988), because this task does not require any training and is assessed through the animals' natural preference for novelty. Their task involved two sample phases and a test phase. The rats received 5min trials with a 50min inter-trial interval. At the first exposure rats were individually presented with four identical copies of an object (A1-4) in an open field. In the second exposure, an additional four

identical objects (B1-4) in different locations were presented. During the test phase animals encountered two copies of each item (A1/2, and B1/2), but only A1 and B1 were in the same place ('stationary old' and 'stationary recent'), and the other two copies (A2 and B2) had moved ('displaced old' and 'displaced new'). The animals showed differential exploration of the displaced objects, as they remembered the order of presentation by exploring the item they have seen the longest ago. This demonstrates that rats have an integrated episodic-like memory for the object (what), its location (where) and when it was seen.

However, tasks that use temporal aspects to assess episodic-like memory have been questioned. It has been argued that such a task can be solved using relative memory of 'how long ago' an event occurred based on strength of memory trace rather than specific memory for an event (Eacott & Easton, 2010; Roberts, 2008). If an animal can only remember if an event was more or less recent, but cannot remember the occasion on which it happened, then researchers are not assessing integrated episodic memory. For example, Babb & Crystal's (2005) study showed that rats can judge the availability of food, but they may not be using episodic-like memory to solve the task. The paradigm can also be solved on the basis of the strength of the memory trace for what was cached where. If an animal uses memory trace strength to define how long ago an object was seen, then the study by Kart-Teke et al. (2006) does not necessarily demonstrate integrated episodic memory.

Therefore, animals may not be capable of having a memory for an absolute point in time. This highlights the importance of defining what is meant by 'when'. Furthermore, humans have a bad sense for when events happened (Friedman, 2007) and 'when' can be used to refer to different types of temporal information (morning or afternoon; first or second time) (Eacott & Easton, 2010). These may not always be relevant to episodic memory (such as morning or afternoon), because episodic memories refer to precise events (Eacott & Easton, 2010; Iordanova, Good, & Honey, 2008). Therefore, it has been proposed that episodic memory can

be described in terms of what happened on an occasion and not at an explicit point by merely referring to the timing of contexts and events (Gaffan, 1994; Eacott & Gaffan, 2005; Eacott & Norman, 2004).

1.3.2. What-where-which memory in animals

Having established that defining a specific point in time by only using temporal references (such as when) is too restrictive, we need to explore alternatives to investigating episodic memory in animals. Using contextual cues to discriminate when something happened could be useful as it narrows the time frame and we benefit from multiple contextual cues to distinguish similar events (Eacott & Easton, 2010; Easton, Zinkivskay, Eacott, 2009). Eacott and colleagues have suggested a different definition of episodic-like memory, which is called ‘what-where-which’ (or object-location-context) memory. From their perspective, episodic memory is better described what happened where and ‘which’ defines an occasion.

The importance of such contextual occasion specifiers was demonstrated in a study by Gaffan (1994). In this study, performance of monkeys in an object discrimination task was enhanced by using different visuo-spatial contexts. In this paradigm pairs of objects were presented against different backgrounds and some objects were kept at a constant location, and other object pairs were associated with a different background in different locations. Gaffan (1994) argued that the ‘scene’ (i.e. the unique background) in this task helps to identify the occasion and facilitates the distinction of multiple similar events. This ‘scene memory’ is claimed to be the similar to episodic memory in humans (Gaffan, 1994). By using tasks of what-where-which animals have to remember what happened in this scene, without specifically being asked about temporal information of the event. It should be noted that ‘which’ can act as any kind of information which distinguishes one event from another (it

does not only refer to contexts or backgrounds), defining episodic memory as a type of memory for items in a specific environmental spatial configuration (Eacott & Easton, 2010).

Eacott et al. (for example: Eacott, Easton, & Zinkivskay, 2005; Eacott & Norman, 2004) have successfully carried out a series of experiments investigating episodic-like memory in rats. Using rats' natural preference for novel aspects in an environment Eacott & Norman (2004) devised a paradigm, which showed that rats could remember what object was seen in what specific spatial location and on which occasion (Figure 1.2). This task is based on Ennaceur & Delacour's (1988) spontaneous object recognition paradigm. In the first sample phase rats were shown two objects (A and B) in context 'X', followed by a second sample phase in which phase animals were placed in context 'Y' with the same objects (A and B), but they had switched locations. In the test phase rats were placed either in context 'X' or 'Y' with either two copies of A or B. By the time animals reached the test phase the context, location and objects were familiar, but the combination of object-location-context configuration was novel. Intact rats explored the novel aspects of the environment more than the familiar configuration for up to one hour, providing clear evidence of episodic-like memory. However, fornix lesioned rats were severely impaired on the object-location-context task, even after short delays. Interestingly, rats with these lesions were able to perform paradigms, which only required the subcomponents of the original task (object-context and object-location; see Figure 1.2) (Norman & Eacott, 2005). The difference in performance cannot only be attributed to difficulty, as a different group of rats with lesions outside of the hippocampal system were impaired on the object-context task, but unimpaired at object-location-context (Norman & Eacott, 2005). These results seem to suggest that memory processes underlying the what-where-which task differ from those required in what-which and what-where (see also Langston and Wood, 2010). Therefore, it has been argued that tasks

that can only be solved using recollection (i.e. episodic-like memory) are impaired by lesions within the hippocampal system, whereas lesions to the hippocampal system do not affect familiarity-based tasks. Despite this evidence, one cannot rule out the possibility that rats used familiarity-based mechanisms to distinguish novel from old objects, as all of the tasks were all conducted in an open field.

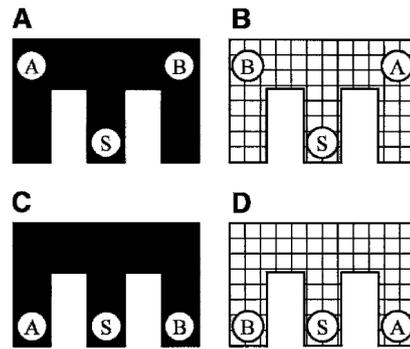
The OLC task provides useful evidence of episodic-like memory for occasions in rodents and insight into the neural mechanisms of recognition memory. The task does not require any training and is assessed using spontaneous behaviour, which reflects the true nature of human episodic memory.

1.4. Familiarity and recollection mechanisms in animals

One way to identify the contributions of familiarity and recollection on memory is the use of receiver-operating characteristics (ROC). Fortin et al. (2004) used this approach to assess recollection and familiarity process in rats. ROC for rats with no hippocampal damage reflected a typical familiarity and recollection components, which were similar to human ROC patterns (Yonelinas, 2001). The ROC of the hippocampal lesions however, showed familiarity processes only, supporting the hypothesis that the hippocampus is important for recollection. Additionally, Eacott, Easton and Zinkivskay (2005) used an E-shaped maze where objects were placed out-of-sight when a choice of novelty had to be made (Figure 1.6) in order to investigate recollection and familiarity based mechanisms in episodic-like memory. In this experiment animals were allowed to freely explore two objects in a specific context. Following that a different context was used and the objects switched locations. Next, a habituation phase followed and the animals were habituated to one of the objects. At test one of the contexts was used with the same objects in the same locations as they had occupied in that context previously. What is crucial about this procedure is that the objects

were not visible to the animal from the start arm. The turn behaviour was used as an indication whether the rat remembered where the object was and in which context. They have found that intact rats were able to seek out the novel object and perform above chance levels on this task, demonstrating episodic-like memory. Unlike in previous tasks (i.e. Eacott & Norman, 2004) the objects were not visible to the animal when it entered the E-maze, meaning that the task can only be solved using recollection. In another study Easton, Zinkivskay and Eacott (2009) investigated the role of the fornix on the E-maze task. It was found that lesioned animals were not able to determine the non-habituated object when the objects were not visible from the start. However, the same animals performed significantly above chance on the object recognition part of the task, suggesting that fornix lesions impair recollection, but not familiarity. The hippocampus seems to play an essential role in spatial memory (see also Mumby et al., 2002; Save et al., 1992) and Easton et al. (2009) have suggested that the fornix lesions impairment may be the result of a spatial memory deficit, but not necessarily a deficit of the integration of different components of episodic-like memory. Animals might have been impaired on the E-maze task, because they were unable to seek out the correct object location, but the location (where) is only one component that makes up episodic memory. However, when individual components of what, where and which were tested in an open field, animals with fornix lesions were able to perform tasks of recognition and object-location (Eacott & Norman, 2004; Langston & Wood, 2010)

Figure 1.6. Schematic representation of the E-maze apparatus by Eacott et al. (2005).



A. In the first sample phase rats freely explore two objects (A and B) in context X, which are visible from the start arm (S). **B.** In the second sample phase the two objects (A and B) swap locations and animals are given more time to explore in context Y. **C/D.** Same procedure as before, but objects are not seen from the start arm. In the habituation phase, which happens between the second sample and test animals are habituated to either object A or B (as shown in Eacott et al. 2005).

1.5. Role of context and interference in episodic memory

Context is an essential part of learning and memory in humans and animals and the hippocampus is critical for encoding information about contexts. One of the hippocampus's roles is to prevent interference by using contextual information to separate events. The ability to manipulate the context in spontaneous recognition tasks is important, especially when a continual trials apparatus is used. In an earlier experiment with human participants Godden & Baddeley (1975) showed that items on a word list were better remembered when participants were tested in the same context as learning took place, indicating that associating memories with the original context protects them from interference. Memories for an event that occurs in a specific context will cause the hippocampal context code to be re-expressed when the relevant context is revisited (Smith & Bulkin, 2014). The retrieval of the correct memory is promoted and interference from similar memories is minimised. Being able to encode contexts and distinguish between familiar and novel contexts is essential in everyday life, as this provides protection from interference. Therefore, context can be seen as a retrieval cue. When returning to a familiar context memories are more available and may be recalled more easily (but not necessarily automatically) (Smith & Bulkin, 2014). This allows us to separate

events from interfering memories. Interference is a potential problem in continual trial paradigms as well as in everyday life. Many classic cognitive psychology experiments have shown the importance of contextual information for preventing interference (for review see Smith & Bulkin, 2014). For example, Butterly, Petroccione, and Smith (2012) trained rats on two odour discrimination tasks and training either took place in the same context or in two different contexts. Interference was introduced by having the same odours on both tasks. Rats that learnt the two lists of odours in different contexts significantly experienced less interference and performed much better than rats that learnt the list in the same context and therefore, demonstrating the role of context in reducing interference. Many tasks that rely on the hippocampus are prone to interference, as subjects are required to respond to cues that have been encountered many times. Therefore, memory for the different events has to be separated so that one trial can be distinguished from the previous one - this is called pattern separation (Olton & Papas, 1979). Animals with hippocampal lesions can still form new memories and retrieve them, but they cannot associate them with the context. The hippocampus may not necessarily link the individual components that make up episodic memory, but instead the hippocampus may link memories with contexts (Smith & Bulkin, 2014).

If we want to understand the underlying neural mechanisms of SOR and episodic memory, researchers need to be clear about the features and the nature of the contexts being used (for review see Robertson et al., 2015). Manipulating the contextual information in experiments can vary to a great extent. The context of an environment can be changed by manipulating the auditory stimuli, odour cues, background colour, mood, or by spatial information (Murnane, Phelps, Malmberg, 1999). Distinguishing between these different types of contexts is essential when investigating the role of the hippocampus and its surrounding structures. For example, the what-where-which and what-where-when tasks both

use spontaneous recognition to assess memory and both tasks rely on contextual information. However, the nature of the context is different. In the what-where-which task as used by Eacott and colleagues the context is used to define an occasion using physical components, such as different walls and floors of an arena, whereas in what-where-when task the context was defined as a time point. It has been established that both tasks depend on the hippocampal system, but what-where-which (OLC) can only be solved using recollection like processes; What-where-when can be solved using recollection or familiarity (Easton et al., 2009). In mice with Alzheimer's disease pathology the what-where-which task is impaired at 6 months, but the what-where-when task in contrast remains unimpaired until 12 months, even when the MTL region is affected (Davis et al. 2013). This further demonstrates the distinction between different contexts and their effects on neural mechanisms, but might also reflect the ability to use trace strength as a cue as discussed above. Lesions to the hippocampus impair object-location-context memory in rats, but when immunotoxic lesions of the cholinergic input to the hippocampus are made rats show no impairment, indicating that the task demands are different. The reliance on cholinergic projections to the hippocampus (HPC) varies despite both tasks addressing what-where-which questions.

1.6. Neural mechanisms underlying OLC

Even though there is still much debate about the paradigms used to investigate episodic-like memory, their contribution allows us to consider the underlying neural systems of OLC tasks. The hippocampus of rats and other animals represents their environments, locations within those environments and their contents. More specifically, the effects of fornix lesions demonstrated that the hippocampal system plays an important role in learning and episodic memory. However, there are competing theories of the involvement of the hippocampus and its surrounding structures. Squire, Stark and Clark (2004) considered the hippocampus as a

part of a wider medial temporal lobe (MTL) system (including perirhinal and parahippocampal cortex), and the degree of impairment in memory tasks is proportionate to the amount of damage within the system. On the other hand, Aggleton and Brown (1999) proposed that the hippocampus is part of a wider system (including mammillary bodies and anterior thalamus), that is involved in recollection and episodic memory whilst other structures of the MTL support familiarity based memory. This theory argues that the degree of impairment is dependent on the exact location of the damage and the nature of the memory task. Despite this debate, both theories consider the hippocampus crucial for episodic memory and damage to the hippocampal system in humans causes a poor performance in tests of episodic memory (see Scoville & Milner, 1957; Squire & Zola, 1996). The classic patient HM had substantial impairments in memory and was unable to form new episodic memories. HM had severe damage to the hippocampus, but also surrounding structures were affected (Scoville & Milner, 1957; Milner, Corkin, & Teuber, 1968). Horel (1978) suggested that damage to white matter pathways within the temporal lobe was the cause of HM's amnesia. Bilateral damage to the temporal stem white matter and fornix lead to memory impairment in monkeys (for review see: Easton and Eacott, 2013), as they were unable to learn new strategies to remember items after the surgery. Large white matter lesions produced anterograde amnesia and interrupts memory for events. Investigations by Gaffan (1994) into scene learning in monkeys have provided evidence that damage to the fornix leads to impairments in episodic-like tasks. By using an object discrimination task in monkeys Gaffan (1994) demonstrated that when objects are shown in specific locations against unique backgrounds, the rate of learning of the animals increased significantly. The discrimination task required the presentation of object pairs in specific background scenes and the same object was always in the same position in the same background. In this task, contextual information of the background is essential to help the animal to discriminate between similar

events. Animals with fornix lesions were impaired when the discrimination was associated with a particular spatial location in a specific background scene. However, when the discrimination was only associated with the location or only to the scene, fornix lesions had only mild effects on performance. This ‘scene memory’ is claimed to be analogous to human episodic memory. The backgrounds (or scenes) help to identify the specific occasion and promote the separation of similar experiences. The system described by Aggleton and Brown (1999) is supported by the work on scene learning in monkeys. Additionally, the medial dorsal nucleus of the thalamus is said to be crucial for episodic like memory (Gaffan & Parker, 2000). The frontal cortex has also been shown to be necessary for scene learning (Browning, Easton, Buckley, & Gaffan, 2005). Moreover, an intact fornix is necessary for successful performance in object-location-context tasks in rats (see Eacott & Norman, 2004; Easton et al., 2009). Whereas the spatial component of episodic memory has been well examined, less is known about non-spatial aspects of episodic memory and associative memory. The medial entorhinal cortex (MEC) and the lateral entorhinal cortex (LEC) are two of the streams of input to the hippocampus, which provide place cells with information about locations of events (Anderson & Jeffrey, 2003; Leutgeb et al., 2005; Ainge et al., 2012). To address this lack in the literature, Wilson et al. (2013) examined c-fos expression of rats in an object-context (OC) task and found increased activation in the LEC. Following on from this, rats with lesions to the LEC were unable to perform the OC task, but showed intact object recognition and intact context recognition. The data suggest that object-context recognition requires the LEC and that contextual features are integrated with object identity. The spatial information comes via the MEC to form episodic memory in the hippocampus. Hence, the LEC may play a crucial role in binding together information about object, contexts and places (Wilson et al., 2013). In addition to the LEC, the prefrontal cortex is an important structure in associative recognition and in relaying information to the hippocampus. To find out to what

extent the connection between the PFC and LEC is involved in processing episodic memory in rats, Chao et al. (2016) used a disconnection procedure to test recognition memory. Rats that received lesions of the mPFC and LEC in the same hemisphere, showed intact episodic memory (in this study defined as what-where-when) and object recognition. When the lesions were placed in the opposite hemispheres, episodic memory and memory for object identity, location and context were impaired. However it is important to note, the disconnection did not impair the components of episodic memory (what-where-when) per se. This suggests that the mPFC and LEC are a critical part of a neural circuit that underlies episodic memory.

Overall, episodic-like tasks (such as the OLC task) are dependent on the hippocampal system, whereas tasks sharing similar features (such as object-context task) are not. These episodic-like tasks in animals can begin to enhance our understanding of complex neural networks involved in memory and trigger questions about the roles of neurotransmitters. Cholinergic projections from the basal forebrain are known to be necessary for scene learning in monkeys (Easton, Ridley, Baker, & Gaffan, 2002) and the impairment seen on the object-context-location task in Eacott and Norman (2004) could be explained by the interruption of an intact of cholinergic system, as cholinergic septohippocampal projections travel via the fornix (Bartu & Kurz, 1985).

1.6.1. Role of the cholinergic system in learning and memory

Acetylcholine (ACh) has long been implicated in memory and learning (e.g. Drachman, 1977), but the exact role of the neurotransmitter still needs to be established. The encoding and retrieval scheduling (ERS) framework describes how in novel environments hippocampal acetylcholine levels are increased which creates a situation in which encoding of novel information is prioritised and in familiar environments ACh levels are low (for review see Easton et al., 2012; Hasselmo 1999, Hasselmo, 2006). Following cholinergic manipulation

changes to neuronal representation of space in the hippocampal network can be observed and these underlie behavioural consequences in tests of memory. ACh projects throughout the central nervous system (Mesulam, Mufson, Wainer, & Levey, 1983; Mesulam, 2012). The basal forebrain cholinergic system can be divided up into four groups of cells: medial septum, vertical limb of the diagonal band of Broca, horizontal limb of the diagonal band of Broca, and nucleus basalis (Baxter, 2001). Cholinergic as well as non-cholinergic projections are interspersed in the basal forebrain and the proportion of cholinergic basal forebrain neurons vary (Baxter, 2001). The cholinergic projections from the basal forebrain to the hippocampus that are contained within the medial septum and vertical limb of the diagonal band (MS/VDB) have specifically been linked to memory (Easton, Ridley, Baker, & Gaffan, 2002; Hasselmo, 2006). Lesions of the basal forebrain in humans can lead to severe amnesia (Norlen & Olivecrona, 1953). However, the issue with lesion studies in humans is that the impairment is rarely restricted to one specific area of the brain and the role of ACh is therefore difficult to determine. Early experiments (Dunnett, Whishaw, Jones, & Bunch, 1987; Markowska, Wenk, & Olton, 1990; Page, Everitt, Robbins, Martson, Wilkinson, 1991; Wenk, Harrington, Tucker, Rance, & Walker, 1992) in rats and primates have used electrolytic lesions to damage the basal forebrain system, but such lesions damage other fibres of passage. Many studies examining the effects of hippocampal lesions in recognition memory have produced different results possibly because of differences in methodology and lesion size (see Ainge et al., 2006). On the other hand, injection of 192 IgG-Saporin into the basal forebrain produces selective damage to the cholinergic system, without damaging non-cholinergic neurons. Despite the finding of behavioural deficits following 192 IgG-Saporin lesions, several questions remain unanswered.

Alzheimer's disease (AD) involves the loss of cholinergic neurons in the early stages, which leads to memory impairments (Bartus & Dean, 2008). Patients show a change in cells

of the basal forebrain and a decreased level of acetylcholine (Whitehouse et al., 1982). Yet, researchers have challenged the degree to which loss of these neurons is responsible for memory impairments in Alzheimer's disease, because the disease causes many other pathological changes. If the cholinergic system is critical for memory then one would expect significant impairments from lesions of the cholinergic projections to the hippocampus. Lesions of the fornix and lesions of the medial septum impair many hippocampal-dependent tasks (see Kelsey & Landrey; Markowska, Olton, Murray, & Gaffan, 1989), but more specific lesions using an immunotoxin often fail to produce impairments in hippocampal-dependent memory tasks and would suggest that ACh is not required for an intact memory system (Baxter et al., 1996; Frick, Kim, & Baxter, 2004). The findings of different experiments are difficult to interpret and detailed consideration is beyond the scope of this thesis (for a review see Parent & Baxter, 2004). Briefly summarising results, it has been shown that cholinergic activity is correlated with memory performance and the recovery of acetylcholine after damage is sufficient to save memory (Parent & Baxter, 2004). Nonetheless, disruption of the cholinergic system only produces minimal impairments on some hippocampal-dependent memory tasks. Parent and Baxter (2004) have therefore concluded that the cholinergic neurons may be involved in some memory processes, but memories can be retrieved and formed without cholinergic projections. Instead, the neurotransmitter acetylcholine controls reactions to new information in the environment. It encourages learning of new material by promoting it over the retrieval of already existing representations; it increases exploration and long-term synaptic plasticity (for review see: Douchamps et al., 2013; Easton, Douchamps, Eacott, & Lever, 2012). High levels of acetylcholine in the hippocampus support encoding when rats are placed in a novel environment (Lever, Burton, & O'Keefe, 2006) and place fields in the subfields of the hippocampus, CA1 and CA3, are claimed to be particularly responsive to environmental changes (Lever, Wills, Cacucci, Burgess, & O'Keefe, 2002;

O'Keefe & Nadel, 1978). Importantly, rats with cholinergic depletions of the hippocampus exhibit different firing patterns in CA1 and CA3 to new testing environments (Ikonen et al., 2002).

Studies examining the effects of 192 IgG-Saporin lesions indicate that some hippocampal-dependent tasks are affected. In order to characterise the processes that require ACh, Janisiewicz, Jackson, Firoz, & Baxter, 2004 damaged the cholinergic input to the hippocampus and assessed its effects using a contextual-spatial condition discrimination task. In this task, rats were taught to respond to a location where the correct location was dependent on the context in which the stimulus was presented. The context was defined as the shape of the environment and distinct cues on the walls were used for orientation. The locations that had to be identified in each context changed from trial to trial. Animals with cholinergic lesions were impaired when contexts were presented as novel environments. When one of the contexts was shown to the animals before and they were highly familiar with it, the task was unaffected by the lesions. Rats faced a large amount of interference in this task and this may therefore be the basis of their impairment. In another study by Baxter et al. (1995) they tested rats' performance on the standard Morris water maze task. Lesions of the cholinergic projects did not impair the place task, but did impair the delayed-match-to-place version. There is a crucial difference between the two tasks. In the place task, the goal locations remained stable across multiple days, whereas in the delayed match to place (DMTP) task the goal location continually changed. In the DMTP task is more scope for interference from the previous trials and an intact ACh system would be of advantage. Easton, Fitchett, Eacott, & Baxter (2011) tested rats with selective lesions of basal forebrain neurons in the medial septum and vertical limb of the diagonal band (MS/vDB), which caused a cholinergic depletion of the hippocampus, on an object-location-context task. Animals with acetylcholine depletion showed no deficit in their innate preference for novel

items (i.e. what-where-which/OLC task). However, these rats were unable to perform a location-context task, which required an association of different places with different contexts (for description of the task refer to: Method in Study 1, Chapter 2). This finding suggests that a loss of cholinergic input to the hippocampus does not affect the memory for the association of objects, places and contexts, but medial septal neurons appear to play a role in spatial and contextual memory (Easton et al., 2011). The authors argued that the discrepancy in performance is not due to the tasks per se, but due to the (in) stability of locations. In the original what-where-which (object-location-context) task the locations of objects do not vary. In the exposure as well as in the test phase there is an item on the right and left, although the identity of the objects on the left and on the right may change. In contrast, in the where-which (location-context) task the locations which are occupied by objects changes between exposures and test, meaning there is no stability of object locations. Rats were unimpaired in the task in which locations remained constant, perhaps because place cell maps did not have to be updated (Easton et al., 2011). However, when locations continued to change between events, remapping became essential and rats with cholinergic lesions performed at chance (Easton et al., 2011), suggesting that animals do not encode a novel environment by constructing new place cell maps, but instead go back to an existing map (Easton et al., 2012). As the location-context task exhibits more differences between occasions the rat is required to quickly revise its place cell representation, and it is claimed that this process is interrupted by a damaged cholinergic system (Easton et al., 2011; Ikonen et al., 2002)

Lesions to the cholinergic input to the hippocampus may only impair memory tasks where locations are unstable, which is true of Baxter et al. (1995), Janisiewicz et al. (2004) and certainly Easton et al (2011). A recent study by Cai, Gibbs & Johnson (2012) supported this notion by highlighting the importance of the cholinergic input to the hippocampus (from the medial septum of the basal forebrain). Animals received either saline or 192 IgG-saporin

injections. Two weeks later rats were tested on a task of novel object recognition. Following another twenty-four hours, rats were tested for object location recognition. Cholinergic lesion had no significant effect on object recognition, but produced a significant impairment in the object-location task, demonstrating the importance of ACh in spatial processing (i.e. when an object moves location).

1.7. Episodic memory in humans

Episodic memory is the type of declarative memory, that requires the conscious recollection of past events (Tulving, 1983). Because the personal experience of previous events requires self-consciousness, it led people to believe that episodic memory is unique to humans. Tulving and Markowitsch (1998) also suggested that episodic memory requires the ability to mentally travel through time, which cannot be tested in animals. However, an operationalisation of episodic memory made it suitable for neuroscientific investigations in animals (Easton & Eacott, 2008). Developing behavioural tasks that can be used in animals has given us useful insight into the neural mechanisms of memory, but it is also of importance to further develop these tasks to study human memory. Recent studies have examined episodic memory in humans by adapting the what-where-when and what-where-which tasks (e.g.: Easton, Webster, & Eacott, 2012; Holland & Smulders, 2011) in order to validate their animal work. Traditional recognition tasks require participants to study a list of words or pictures and following a delay they are presented with a list of items, which contains novel and old information. Originally, it was thought that when the participant recognised an item on the list, they remembered the item and therefore demonstrated recollection. However, this is not always the case and does not reflect the true nature of memory as people may recognise something as familiar, but do not recollect any details about the event.

1.7.1. What-where-which in humans

Episodic memory has been extensively studied in rodents, but it is equally important to devise tasks that test episodic memory in humans for comparison. Holland and Smulders (2011) designed a what-where-when task for a human, which is based on the idea of food hoarding in birds (see Clayton & Dickinson, 1988). Participants were asked to hide two items on two separate occasions. They were then tested for their memory of what was hidden where and when. Additionally, participants were asked to complete another episodic memory test, which included (unexpected) questions about the occasion when they were asked to hide the items. One group of participants was asked to memorise the information. The other group of participants did not know that they would be tested on an episodic memory task. Both groups reported to use time travel as a strategy to recall what they did where and when. It was also found that participants remembered locations from the first session better than from the second one, which is inconsistent with decay theories. There are several possible explanations for this finding. First, information from Day 1 could have interfered with retrieval of information from Day 2. Second, information from the first day had more time to consolidate. Third, information from Day 1 could have been reinforced on Day 2 in order to avoid placing the items in the same location. Finally, it could have been possible that on Day 1 participants used the more obvious (salient) hiding places and remembered these for that reason. Even though this experiment does not assess participants' subjective experience, this task shows that humans use recollection-like mechanisms to solve this 'real-world' episodic memory task. In contrast, Easton et al. (2012) investigated the performance of human participants on an episodic memory task, which resembled the episodic-like memory task in rats. In this task participants were asked to look at two sequentially-presented PowerPoint slides that consisted of symbols in several locations on different backgrounds. Their memory for the objects (what), the location of the symbol (where) and the first/second slide (when), or the slide background (which) was tested. Unlike in Holland and Smulders (2011) study, participants

were asked to make a subjective experience judgment. They were asked to make a 'remember', 'know', or 'guess' judgement about their response. It was shown that object recognition questions could be answered correctly by using recollection or familiarity, but the what-where-which question could only be answered correctly using recollection. What-where-when question could be solved using recollection as well as familiarity, which is in contrast with the prediction from animal research. Many studies have claimed that what-where-when memory is episodic in nature and relies on recollection (for example Babb & Crystal, 2005). Therefore, it has been suggested that what-where-when tasks can be solved using non-episodic mechanisms, such as trace strength (Roberts et al., 2008).

Taken together the evidence suggests that what-where-which is a valid way to test episodic memory in humans and seems to be a more robust than relying on temporal aspects of what-where-when paradigm. It will be of interest to use virtual reality to investigate human's memory for past experience, as this resembles the real world more. Technological advances and the need for repeatability across human experiments have led to an increase in the use of virtual environment experiments. This methodology allows us to explore everyday tasks of finding our way around an environment or remembering personally experienced events.

1.8. Virtual environment – role of event boundaries

The view of episodic memory as reliant on 'scene memory' as discussed above has proved useful. Nonetheless, in everyday life, we experience a continuous stream of inputs. When remembering our past, we recall events that we separate in time and space. As we navigate our way through an environment we continuously receive sensory input. Previous research has provided much evidence for a role of spatial boundaries in the separation of episodic memories. For example, when participants are asked to watch a video, they

automatically segment it into events and most of the participants are in agreement on the location of those event boundaries (Newtson, 1973). The segmentation of events in to space and time emphasizes the spatiotemporal nature of human episodic memory (O'Keefe & Nadel, 1978) and short-term memory is affected by event boundaries (Swallow, Zacks, & Abrams, 2009). When watching video clips of a series of events, which included objects, subjects were better at remembering objects when they were still watching the same event. People also read more slowly when they noticed a spatial shift in the story (Zwaan, Langston, & Graesser, 1995). One possible explanation for this is that the spatial shift leads to a mental update of the situation and a new situation model needs to be created. However, it is not uncommon for the memory or information load to be unaffected by a spatial shift (Radvansky & Copeland, 2006), which could be the result of unknown space and the changes are too difficult to monitor. When Radvansky and Copeland (2006) investigated the ability of humans to remember information about objects as they moved through a virtual environment, they used a situation model (see Johnson-Laird, 1983) to argue that mental representations have continuously be monitored and adapted and that kind of cognitive processing is interrupted by spatial shifts. In a series of experiments, Radvansky and Copeland (2006) had participants move through an environment, where they had to pick up an object, move to the next room, set the object down, pick up a new object and move to the next room. People were probed at various points throughout the experiment. They were presented with an image of the object and had to identify whether it is the object they are currently carrying or the object they had just set down. People only had to remember two objects at any given time and it was found that after a spatial shift, participants responded more accurately and faster when the object probed for was currently being carried in the virtual environment. This means that participants actively interact with the situation and monitor the spatial changes. However, there was some ambiguity as to what was driving this effect. The following experiment

assessed the spatial affect more closely and asked participants to walk through a doorway to another room. Essentially, the person walked through the door to the other room and could either be probed halfway through a room or just after the door. In this case, the earlier effect was replicated, but it was also shown that moving through a doorway hindered retrieval. Moving from one room to another might have made the information less available. Radvansky and Copeland call this the 'location updating effect', as moving to a new room caused the event model to be updated and this compromised memory performance. The event horizon model by Radvansky et al. (2011) expands on this idea of event segmentation, which is common when people move from one room to another and update new event models (see also Kuryb & Zacks, 2008). In a 'real world' experiment, Radvansky et al. (2011) tested if the location updating effect found in their previous studies was due to the updating of event models (such as the number of rooms) or the encoding (such as the context of the rooms). When participants were asked to move through actual rooms, it was found that the memory for objects after a shift was poorer. However, memory could not be re-instated by re-visiting a previous context (i.e. room) which confirmed that the effect was not simply a result of contextual cuing. Again, the doorways served as an event boundary, which reduced the availability of information.

Although a lot of the research has looked at short-term episodic memory, it is not known how segmentation of events contributes to long-term episodic memory. Therefore, Horner, Bisby, Wang, Bogus, and Burgess (2016) were interested in how we create discrete episodic memories from continuous input. They adopted the protocol by Radvansky and Copeland (2006) to explore event boundaries in long-term memory within a specific event. Participants were asked to navigate through rooms in a virtual environment and were presented with two objects in each room. Following the encoding phase, subjects performed several memory tasks, such as answering questions about the spatial and temporal aspects of

the configurations. Commonly participants were required to respond to the following two questions after being presented with an object: ‘which object came next?’ or ‘which object came immediately before?’ The results have shown that a spatial boundary does affect long-term memory for the order of objects. When two objects were presented in the same room, temporal memory was more accurate than when the two objects were presented in different rooms. In other words, long-term memory was disrupted when two objects are separated by a spatial boundary and the presence of a boundary at encoding impairs our ability to remember this information later.

1.9. Conclusions

Spontaneous object recognition and episodic-like memory tasks in rodents have significantly contributed to our understanding of the neural mechanisms of memory. The definition of episodic-like memory has undergone many changes and the methodology of the tasks has often been questioned (Babb & Crystal, 2005; Clayton & Dickinson, 1988; Clayton, Bussey, & Dickinson, 2003; Eacott & Norman, 2004; Ennaceur, 2010; Kart-Teke et al., 2006). However, these tasks are easy to administer and do not require any kind of training or reinforcements. The improved design of a continual trials apparatus has the potential to provide a valuable complement to the study of memory. Carrying out SOR tasks that involve context changes and running them closer together in time are essential requirements for lesions and pharmacological studies.

Studies support the view that perirhinal cortex is important for object recognition memory and the hippocampus for episodic memory (Aggleton & Brown, 1999; O’Keefe & Nadel, 1978; Langston & Wood, 2010). More specifically, loss of cholinergic input to the hippocampus is associated with episodic memory impairments (Bartus, 2000). However, the extent to which cholinergic deficits account for impairments in Alzheimer’s disease remains

unclear. Some selective lesion studies have failed to produce clear results, as no substantial impairment on many tests of memory in animals was found (Voytko et al., 1994; Baxter & Gallagher, 1997). It is difficult to translate experiments in animals to humans, as some of the memory tasks may not capture the true nature of episodic memory. Therefore, more relevant investigations of the role of cholinergic neurons in episodic memory are necessary. As such, Easton et al. (2011) have demonstrated that selective removal of cholinergic hippocampal input does not impair episodic-like memory in rodents, but it does impair the ability to associate contexts with places. This clearly shows that episodic memory is more than the sum of its parts (Eacott & Easton, 2010) and more research is necessary to determine the exact role of neurotransmitters in memory.

When testing episodic-like memory in rodents, researchers have argued to use contextual features of an environment to define an occasion, rather than using temporal order. Context is an essential component of learning in animals and humans. Using contextual information to discriminate events is more useful as it narrows the time frame and we benefit from multiple contextual cues to distinguish similar experiences (Eacott & Easton, 2010; Easton, Zinkivskay, Eacott, 2009). The role of the hippocampus is to prevent interference by using contextual information to separate events. Manipulating the context in spontaneous recognition tasks is important, especially when a continual trials apparatus is used. In order to understand the underlying neural mechanisms of SOR and episodic memory, we need to be clear about the features and the nature of the contexts being used (Robertson et al., 2015), as the definition varies from experiment to experiment.

SOR tasks have widely been used in memory research and continue to be critical for clinical research. The contribution of animal studies to our understanding of human memory should not be underestimated. Adapting the well-controlled behavioural tasks in rodents to assess human memory will allow us to minimise or even replace animals' studies.

The uses of multiple trials in animal studies have led us to investigate how we separate events from each other. When remembering the past, we recall events that we separate in time and space. As we navigate our way through the world we receive sensory input, which means there is scope for significant interference through having to distinguish between consecutive experiences. It remains to be determined how event segmentation contributes to episodic memory.

1.10. Aim & hypotheses

The overall aim of this thesis was to explore episodic memory and its demands on hippocampal function by using different methodological and practical approaches in rats and humans. All animal experiments were performed in accordance with the U.K. Animals Scientific Procedures Act (1986) and associated guidelines and ethical approval was received before any procedures were carried out.

Firstly, the aim of this thesis was to develop various tasks of episodic memory for use with rodents to investigate its demands on the hippocampus. Chapter 2 aimed to replicate the object-location-context and the location-context task in Easton et al.'s (2011) study. Given that the original task of episodic memory (what-where-which or object-location-context) in rats is not impaired by cholinergic lesions of the basal forebrain, but a context-place memory task is impaired it was important to clarify the particular role of the cholinergic system in variants of episodic learning. This was achieved by using an open field where animals encounter locations within contexts that are not constant across and within trials (see also: Baxter et al., 1995; Janisiewicz et al., 2004). The aim of this experiment was to replicate the finding that intact rats can successfully carry out different versions of object-location-context and location-context tasks. Animals were tested on four tasks of episodic memory: stable OLC, unstable OLC, stable LC and unstable LC. These tasks will provide us with an opportunity in the future to test animals with the same lesions as in Easton et al.'s (2011)

study to see if the previously found difference in performance is due to the task or to the stability of locations. Based on rats' innate preference for novel items it is predicted that animals will spend more time exploring a novel item configuration compared to a familiar one.

Chapter 3 explored a slightly different approach to testing episodic memory in rats, because running only one trial per day (as in Chapter 2) is very time consuming and a large number of rats is needed to maintain statistical power. Furthermore, encountering two different tasks in one day seemed to impair the rat's performance on episodic memory tasks. Therefore, experiment 2 addressed an alternative approach asking whether episodic-like memory tasks can be tested in a new continuous trial apparatus. Developing tasks of episodic memory in animals, which can be run closer together in time, is important to enhancing our understanding of the neural processes involved. It was hypothesised that fewer animals ($n = 6$) would be sufficient to test episodic memory in a continual trials apparatus. Given that all the animals perform a greater number of trials, the noise in the data is reduced but does increase the chances of proactive interference. Yet, it was hypothesised that rats would significantly explore the novel object configuration and remain interested in the task.

Building on the methodological work in the first two experimental chapters, the second aim (Chapter 4) of this thesis was to investigate the role of medial septal cholinergic neurons in episodic and spatial/contextual memory. Based on a study by Easton, Fitchett, Eacott, & Baxter (2011), I also tested rats with selective lesions of basal forebrain neurons in the medial septum and vertical limb of the diagonal band (MS/vDB), which caused a cholinergic depletion of the hippocampus, using the new continual trials apparatus. In Easton et al.'s (2011) study animals with acetylcholine depletion showed no deficit in their innate preference for novel items (i.e. what-where-which task). However, these rats were unable to

perform a where-which task, which required an association of different places with different contexts. Experiment 3 sought to build on the results of experiment 2 to determine how effectively episodic memory can be tested in a multiple trial apparatus in intact as well as lesion animals. The study aimed to demonstrate the reliability of the dissociation within the hippocampus based on cholinergic function within the hippocampus, and to verify the new apparatus as assessing episodic-like memory in the same manner as earlier studies, improving the reliability of the task and having a significant 3Rs benefit.

The final aim of the thesis was to investigate how animals and humans separate events and how they use contextual information for segmentation in episodic memory. A series of behavioural experiments (Chapter 5 – 8) were carried out. While running multiple trials has lots of advantages, there is a risk of increased interference and investigating how trials might be segmented into events is highly relevant. Experiment 4 aimed to measure memory performance on the original episodic-like (what-where-which/OLC) memory task without the presence of some objects at test. A modified version of this task allowed me to assess the memory for location of objects in unstable conditions within given contexts across multiple events. Based on previous research and my own observations it was hypothesised that animals would use recollection-like processes in this preference paradigm and can successfully recall history of events. In Experiment 5 it was of interest whether multiple sample and test phases can be conducted without disrupting memory. Two different protocols for the object-context-location tasks were developed in order to see if preference for novel configurations could be maintained. It was investigated if rats maintain preference for novel OLC configurations over multiple sample and test phases and if context made a difference to memory performance. Experiment 6 tested rats tested on the LC task in which contexts were not only defined in terms of its physical properties, but also in terms of flavours. Depending

on the group, either each trial or exposure phase was defined by flavoured pellets. The aim of this experiment was to investigate how animals separate the trials (i.e. events) in the LC task, as this task requires object change on every exposure. Events are therefore less distinguishable than in the OLC task that only changes objects after each trial. It was hypothesised that flavour can be used to define an event and create boundaries, and this would help animals to discriminate between similar novel and familiar context-place configurations.

Animal models can serve as an important starting point and the work in previous chapters successfully demonstrated different ways of testing rodents' behaviour in variations of the OLC task. These experiments were well controlled and aimed at reducing animal numbers used in memory research. However, improving the translation between human and rodent models provide researchers with an opportunity to further reduce the number of animals used. In humans, memory tasks are often carried out very differently as participants are able to express themselves verbally. Therefore, it is now of interest whether the findings of Chapter 7 are comparable to humans. In humans, the basic paradigm for recollection in memory involves asking people to make a judgement about the nature of their memories. Radvansky and Copeland (2006) demonstrated a detailed assessment of the influence of changing events on memory, which formed the basis of the last chapter. Using human participants, I tested their memory of objects and their experience associated with it. The aim of this last study was to investigate the ability of people to retrieve information about objects as they move through a virtual environment and link it to previous animal studies.

Chapter 2

Study 1: Memory for Objects, Locations and Contexts in the Open Field

2.1. Introduction

Spontaneous object recognition (SOR) tasks have widely been used in memory research by using an animal's innate preference for novelty to assess their memory (Ennaceur and Delacour, 1988). By showing a greater exploration of the novel object configuration, animals demonstrate that they can remember what they had seen previously. These SOR tasks have become very popular, but it is crucial to consider more complex tasks that do not only look at recognition memory for objects. More recently these SOR tasks have also been used to explore episodic memory (Eacott & Norman, 2004). Many experiments have concentrated on episodic memory function, by investigating the what, where and which components of a memory. Rats show an innate tendency to explore novel configurations in an environment and their exploration time is measured in order to distinguish their change in investigation of the novel and the familiar configurations. Developing these kinds of animal models of episodic memory is important to enhancing our understanding of the neural processes involved.

The episodic (object-location-context or OLC) task of Eacott and Norman (2004) requires animals to identify objects in locations in particular contexts (refer to Chapter 1 for details). In addition, other types of contextual tasks have been used to demonstrate dissociations in function within memory systems. For example, Easton, Fitchett, Eacott, & Baxter (2011) tested rats with selective lesions of basal forebrain neurons in the medial septum and vertical limb of the diagonal band (MS/vDB), which caused a cholinergic depletion of the hippocampus, on the standard one-trial a day episodic OLC task. Animals with acetylcholine depletion of the hippocampus showed no deficit in performance on this

task of episodic memory. However, these rats were unable to perform a ‘where-which’ (location-context or LC) task, which required an association of different places with different contexts. The encoding and retrieval scheduling (ERS) framework describes how in novel environments hippocampal acetylcholine levels are increased which creates a situation in which encoding of novel information is prioritised and in familiar environments ACh levels are low. Following cholinergic manipulation changes to neuronal representation of space in the hippocampal network can be observed and these underlie behavioural consequences in tests of memory. Place fields in the subfields of the hippocampus (CA1 and CA3) are sensitive to changes to the context and rats with lesions of the cholinergic input to this area show a different pattern of remapping (Anderson & Jeffery, 2003; Ikonen et al., 2002; Kentros et al, 1998; Leutgeb et al., 2005; Leutgeb et al., 2004; Lee, Rao, Knierim, 2004). Place cells show initial changes in a new environment but then revert to the map of the familiar environment – this has been interpreted as a breakdown of pattern completion mechanism. This breakdown of the remapping mechanism could underlie the behavioural dissociation seen following cholinergic lesions of the medial septum in Easton et al. (2011). Rats would be unimpaired in tasks in which the same locations remain consistent across contexts as no remapping is necessary, but where different locations are relevant in different contexts, remapping is crucial and failure to do so will result in behavioural disruption. Based on this finding it is important to clarify the particular role of the cholinergic system in variants of episodic learning. The effects of task differences (and especially the role of location consistency) need to be investigated more closely to test this proposal. This can be achieved by using an open field where animals encounter objects in locations within contexts that are not constant across and within trials (see also: Baxter et al., 1995; Janisiewicz et al., 2004). I developed secondary behavioural tasks which will allow me to further test the hypothesis that place cell remapping causes a specific problem for the LC task as the location

of objects are unstable. In the OLC task the location in which objects appear remain constant across events.

One aim of this experiment was to extend on previous findings that intact rats can successfully carry out different versions of what-where-which (OLC) and where-which tasks (LC). Another aim was to develop new tasks to be able to test the hypothesis of place cell remapping in episodic and spatial memory in later experiments. Animals were tested on four tasks of episodic memory: stable OLC, unstable OLC, stable LC and unstable LC. Furthermore, I intended to speed up the testing procedure by interleaving tasks such that animals were exposed to two tasks per day (morning and afternoon). These tasks will provide me with an opportunity to test animals with the same lesions as in Easton et al.'s (2011) study to see if the previously found difference in performance is due to the task or to the stability of locations. Based on rats' innate preference for novel items it was predicted that animals would spend more time exploring a novel configuration compared to a familiar one.

2.2. Method and Materials

2.2.1 Subjects

Thirty-two experimentally naïve male Lister hooded rats supplied by Harlan were housed in groups of four in rooms maintained on a 12h light/dark cycle. Behavioural testing was carried out in a separate room during the light phase. Rats had free access to food and water throughout the study. Animals were divided into two experimental groups. Group 1 encountered the stable OLC and the unstable LC task (as in Easton et al., 2011). Group 2 encountered the unstable OLC and the stable LC task. Within the groups animals were counterbalanced, meaning that eight rats in group 1 encountered the stable OLC followed by the unstable LC and the other eight encountered it vice versa. Eight of the animals in group 2 encountered the unstable OLC and then the stable LC and the other eight the other way

around. All rats experienced two test sessions per day (one in the morning and one in the afternoon which each lasted 15minutes) and were tested four days per week.

2.2.2. Apparatus and objects

All testing took place in a 1m² open field with 48cm high walls. The features of the arena could be changed by inserting wall and floor panels. Context 'X' consisted of grey walls and wire mesh overlaid on the floor and context 'Y' consisted of white walls and a grey floor. Duplicate copies of objects made of plastic or ceramic that varied in their shape, color and height were used (Figure 2.1). A camera was positioned above the arena to record animals' exploratory behaviour for analysis. The open field and the stimuli were cleaned using disinfectant wipes. Exploration was taken when the animal was at a distance of less than 1cm of the object and actively exploring it (i.e. sniffing at or touching it). Actions such as using the item as support during rearing or sitting on the object were not considered exploratory behaviour. Rats were placed in the center of the apparatus at the start of the sample and the test phase. The running order, the testing contexts, the novel object and placement of the novel object were counterbalanced within and between animals.

Figure 2.1. Examples of objects used in Study1.



Objects were counterbalanced across all trials and were not repeated within a testing session. They varied in their shape, size and texture.

2.2.3. Habituation

Each animal was handled daily for three days prior to habituation. Rats were habituated to moving between rooms (cage covers were used to minimise stress), the testing room, the open field, the objects and contexts. Behavioural testing took place in a separate room under dim white light and white noise in the background to cover environmental noise. The procedure resembled Easton et al.'s (2011) study as far as possible. Prior to the start of testing, animals received four habituation sessions. On day one, rats were exposed to context 'X' in pairs for 15 minutes. On day two, this was repeated with the open field configured as context 'Y'. On day three, rats were individually placed in the open field and given 5 min of free exploration in one of the contexts. They were then placed in a holding cage for 2 min while the arena's features were changed. Animals were returned to the open field for another 5 min to explore the alternative context. On the last day of habituation, the procedure of day three was repeated but there was a single object in the middle of the open field. This object was used again for the stable LC task, because I wanted animals to be highly familiar with

this object. To prevent scent marks, different copies of each object were used during the sample and test phases.

2.2.4. Stable object-location-context (stable OLC)

In the object-location-context (see Eacott & Norman, 2004, Figure 2.2.A) animals experienced two exposure phases in which objects were in the same location, but in two different contexts. In the first sample phase in context 'X', object A was on the left and object B on the right; in the second sample phase in context 'Y', object A was on the right and object B on the left. In the test phase, animals saw two copies of either object A or B in either context 'X' or 'Y'. In this example, if the test phase showed two copies of A in context X, rats would spend more time exploring the right copy of object A, because its location is mismatched with the context. In both exposure phases and the test phase animals were given 2 min of exploration. Between phases, rats were placed in an empty holding cage, while the arena was changed.

2.2.5. Unstable location-context (unstable LC)

In the location-context task, rats received two exposure phases in which they saw two identical copies of an object (A and B) in different places and in different contexts (X and Y). The task is defined as unstable, because objects' locations changes between sample and test (Figure 2.2.B). As this task is independent of the object's identity and reflects the novelty of place-context configurations, distinct objects were used in each phase. For example, in the first exposure phase animals saw two copies of object A in the 9 o'clock and 12 o'clock position in context 'X'. In the second exposure phase animals saw two copies of object B in the 12 and 3 o'clock position in context 'Y'. In the test phase, rats encountered two copies of object C at the 9 and 3 o'clock position in either context. If the test phase was configured as

context 'X', the right copy of C would be explored more. This is because no item was encountered in the previous exposure phase on the right in context 'X'.

2.2.6. Unstable object-location context (unstable OLC)

This task is the unstable version of the object-location-context task. The task is defined as unstable because the locations of items are different at each stage (Figure 2.2.C). In the first exposure phase animals encountered three objects (A, B, C) in context 'X' in. In the second phase, object A and B swapped locations and object C was in a new place in context 'Y'. Object A had been encountered previously in contexts 'X' and 'Y' and filled all of the places that were used in the test phase. However, in this example, the configuration of A in context 'X' in the 12 o'clock position was a novel combination of object, place and context and therefore would be explored more.

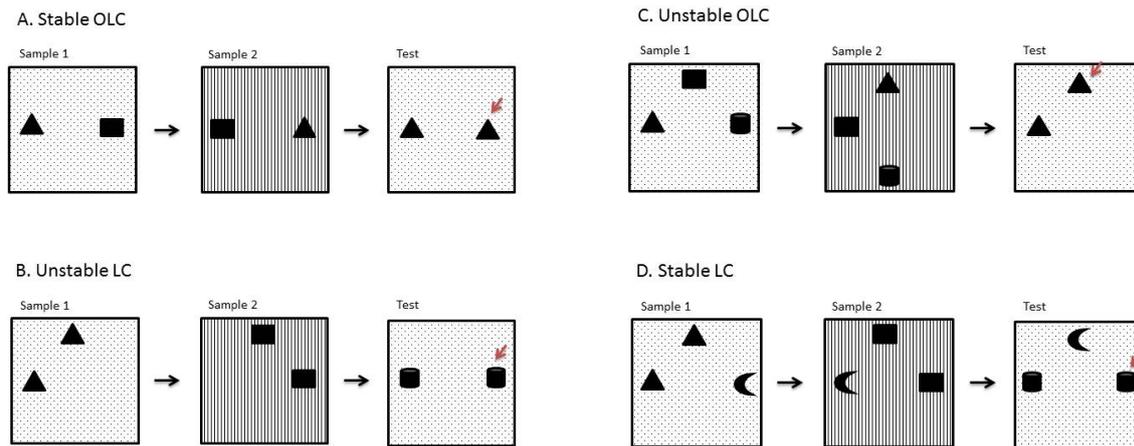
2.2.6. Stable location-context (stable LC)

Just as in the unstable LC task new objects are used on each exposure and at test because it is the position of the object that is of interest (Figure 2.2.D). In the first exposure phase animals saw two copies of object B in context 'X'. In the second exposure phase animals encountered two copies of object C in context 'Y'. In the test phase animals encountered two copies of object D in either context 'X' or 'Y'. One object (A) is presented on each occasion, and has also been encountered during habituation. Over time, object A would fill every location in the arena and it would be less explored because of its high familiarity. Consequently, both copies of object D would be explored over object A. Of the two objects D, the one which is in the 3 o'clock location would be explored preferentially, as this position was occupied by a familiar item before.

2.2.7. Data analysis

The discrimination ratio (D2; Ennaceur and Delacour, 1988) was calculated. When two objects were present at test, D2 was calculated using the following formula: $(\text{novel exploration time} - \text{familiar exploration time}) / \text{total exploration}$. When three objects were at test the following formula was used: $(\text{novel} - (\text{familiar 1} + \text{familiar 2})) / \text{total exploration}$. On this measure D2 scores ranged from -1 to +1. With -1 indicating greater exploration of the familiar object, zero indicating no difference in exploration and higher values indicating greater exploration of the novel object. One-sample t-tests (one-tailed) were used to compare the animals' performance to chance. Exploration times (in sec) were analysed for all animals across all four tasks. Following the calculation of the discrimination ratio and exploration, as a basic measure to verify whether the task was carried out successfully (i.e. animals determined the novel environmental configuration), it was used to analyse the rodents' behaviour in the open field. Given that these tasks rely on the rat's spontaneous behaviour, which can vary depending on various factors (time of day, mood, noise, experimenter), it was crucial to analyse variables such as the effect of test order, the performance on the very first trial of each task and the bias measures. Furthermore, G*Power (Erdfelder, Faul, & Buchner, 1996) was used as a power analysis program for statistical tests. This programme runs widely and can be used with different computers (Windows XP, Windows Vista, and Mac OS X 10.4).

Figure 2.2. Schematic representation of the four behavioural tasks used in Study 1.



A. Stable object-location-context (what-where-which). **B.** Unstable location-context (where-which). **C.** Unstable object-location-context. **D.** Stable location-context. Red arrow shows the novel object in each configuration. Animals encountered one trial per day, which consisted of two sample phases and one test phase.

2.4. Results

2.4.1. Discrimination measures

The primary interest was the performance on the object-location-context and context-location memory tasks. One-tailed t-tests were used for comparing performance on each task, because it was expected that rats preferentially explore the novel object. Exploration times were taken by each animal and D2 scores were calculated as a measure of preference. D2s represent the discrimination ratio. One rat was excluded from the analysis, because it failed to explore the objects in the sample phases.

2.4.2. Stable OLC

Sixteen rats received four trials of the object-location-context task. To determine whether the animals' preference was above chance, one sample t-tests on D2 scores were carried out. D2 scores (mean D2 = 0.04, SD = 0.18) were not above chance $t(15) = 1.048$, one tailed $p = 0.16$, which means that rats did not discriminate between novel and familiar objects (Figure 2.3). A post hoc power analysis was conducted with the program G*Power 3 * (Faul,

Erdfelder, Lang &, Buchner, 2007) in order to obtain the statistical power for this task. The effect size was 0.239 (which is rather small) with a calculated power of 0.228 for sample sizes of sixteen subjects.

2.4.3. Unstable LC

The same 16 rats were also unable to show a clear preference for novel over familiar items in the unstable location-context task rats, meaning that Easton et al.'s (2011) were not replicated. D2s (mean D2 = 0.03, SD = 0.19) were not significantly above chance $t(15) = 0.829$, one tailed $p = 0.21$ (Figure 2.3). A post hoc power analysis was conducted with the program G*Power 3 (Erdfelder, Faul, & Buchner, 1996) was used as a power analysis program for statistical tests) in order to obtain the statistical power for this task. The effect size was 0.15 (very small) and a calculated power of 0.149 for sixteen animals.

2.4.4. Unstable OLC

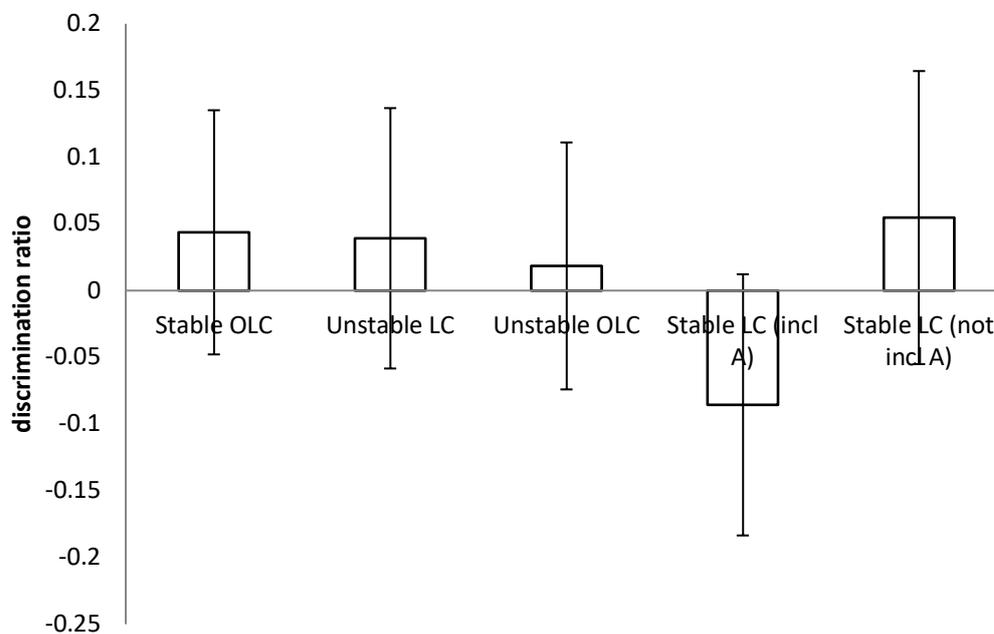
Another group of rats encountered the unstable object-location-context task. Animals' preference for novel objects over familiar items as indicated by the D2 score (mean D2 = 0.018, SD = 0.23) was not significant $t(15) = 0.319$, one tailed $p = 0.377$ (Figure 2.3). A post hoc power analysis was conducted with the program G*Power 3 in order to obtain the statistical power for this task. The effect size was 0.08 (considered very small) and a calculated power of 0.09 for a size of sixteen animals.

2.4.5. Stable LC

The same rats that encountered four trials of the unstable OLC performed the stable location-context task. One object (object A) was presented in each exposure and test phase. As a result of this, the object should be highly familiar and explored less at test. D2 scores for

each trial were calculated including but also excluding the exploration times of object A. Animals spent on average 5.90 seconds (SD = 4.42) exploring object A, whereas the novel configuration was explored for 21.63 sec (SD = 11.66) and the familiar for 20.23 sec (SD = 12.60). Based on this it was found that average D2s (mean D2 = 0.05) excluding object A were not significantly above chance, $t(15) = 0.872$, $p = 0.199$. However, when exploration of object A was included in the analysis, D2s (mean D2 = 0.09) were close to significance $t(15) = 1.638$, $p = 0.061$ (Figure 2.3). A post hoc power analysis and the effect size was 0.04, which is small, and a calculated power of 0.089.

Figure 2.3. Discrimination ratio (D2) in all four tasks of episodic memory.



The discrimination ratio shows the average D2s for the stable OLC, unstable LC, unstable OLC and stable LC tasks. None of the tasks were significantly above chance. Error bars represent the SEM.

2.4.6. Test order

To determine whether the running order of tasks made a difference to the spontaneous exploratory behaviour a repeated measure ANOVA (group x task) was carried out.

Eight animals in Group 1 did the OLC task in the mornings followed by the LC task in the afternoons. The other eight animals encountered the LC task first and then the OLC. Average D2s showed no difference in performance in the OLC ($M = 0.044$, $SD = 0.167$) task and in the LC task ($M = 0.039$, $SD = 0.189$) and an ANOVA of task (OLC, LC) by task order (morning, afternoon) showed no significant main effect of task order $F(1,14) = 0.005$, $p = 0.945$ and no significant interaction $F(1,14) = 1.838$, $p = 0.197$.

Rats 1-8 in Group 2 did the unstable OLC task in the mornings followed by the stable LC task in the afternoons. Rats 9-16 performed the stable LC task first and then the unstable OLC. There was no significant difference between the tasks $F(1,14) = 0.011$, $p = 0.473$. D2 scores showed difference in the unstable OLC ($M = 0.018$, $SD = 0.231$) and the stable LC ($M = 0.055$, $SD = 0.251$) task. When exploration of object A was taken into account, the test order analysis was marginally significant $F(1,14) = 0.087$, $p = 0.057$.

2.4.7. Separate analysis of D2 scores

Given that none of the D2 scores in the four tasks were significantly above chance, lacked statistical power and effect size, we were concerned that exposing animals to two different tasks in one day influenced their performance. Therefore, a separate analysis of D2 scores was carried out. In this analysis only the trial which was encountered first within a day by the animal was taken into consideration. Rats that encountered the stable OLC task first within a day, showed a tendency to discriminate between the novel and the familiar object configuration. T-test revealed that the performance was marginally significantly above chance $t(7) = 1.599$, $p = 0.07$. The other group of rats that did the unstable LC task first each day did not show a preference for the novel object, as their performance not significantly different from chance $t(7) = 1.077$, $p = 0.16$. The third group of animals encountering the unstable OLC task showed a preference for the new configuration of object-location-context t

(7) = 1.599, $p = 0.07$. The fourth group of rats' performances also differed from chance, when the exploration of object A was not included $t(7) = 1.779$, $p = 0.06$. However, when the exploration of A was taken into account, animals only performed at chance $t(7) = -0.063$, $p = 0.48$. This suggests that there was no effect of order, but the number of tasks that are run within a day affect performance.

2.4.7. Bias measures

To see if exploration preferences have been influenced by a bias for exploring objects in a certain position, bias measures were calculated. This score was calculated by taking the sum of exploration times for objects on the left minus the sum of exploration times of objects on the right, which was then divided by the total exploration. This gives a left-right-bias score which varies between +1 and -1. A score of +1 indicates a bias for objects on the left and a score of -1 bias for objects on the right. When three objects were at test, bias scores were compared as left/right, right/top and left/top. An analysis of the scores showed that animals did not have a significant preference for objects on either side in the open field in any of the tasks. Stable OLC $t(15) = 1.514$, $p = 0.151$; Unstable LC $t(15) = 1.868$, $p = 0.08$; Unstable OLC $t(15) = -0.571$, $p = 0.576$; Stable LC Left-right: $t(15) = -1.896$, $p = 0.077$; Left-top: $t(15) = -1.874$, $p = 0.081$; Top-right: $t(15) = -0.016$, $p = 0.987$

2.5. Discussion

Study 1 was designed to investigate an extension to the standard procedure to the object-location-context task developed by Eacott & Norman (2004) and the location-context task created by Easton et al. (2011), with the aim to speed up the testing procedure by exposing animals to two tasks per day (morning and afternoon). However, this experiment failed to replicate previous findings in which rats demonstrated reliable object discrimination

in these tasks (e.g.: Easton et al, 2011) when only one task is carried out per day. Alternative versions of these tasks, which differ in the stability of locations across events, were also tested. However, this part of the study also failed to show reliable object recognition in the unstable OLC and stable LC tasks and showed very low effect sizes and statistical power.

D2 scores of individual rats in the stable OLC task ranged from -0.2 to 0.4, but the effect seems to be driven by a very small number of animals. The average D2 score was only 0.04, which is lower than the discrimination ratio reported in previous studies (Eacott & Norman, 2004; Ennaceur & Delacour, 1988). Therefore, no significant exploration of a novel object-location-context configuration was found. Individual average D2 scores in the unstable LC ranged from -0.3 to 0.3, giving an average of 0.04. In comparison to performance of control rats in Easton et al.'s (2011) study D2 scores were very low in this task, leading to insignificant results. Thus Easton et al.'s (2011) finding where sham-operated rats displayed discrimination scores above chance was not replicated. The unstable OLC task is similar to the stable OLC task as novel object has been encountered previously, but at test it is placed in a novel combination of object, location and context. Rats did not show episodic-like memory in this task. The individual average D2 score ranged from -0.2 to 0.6. As with the unstable LC task new objects were used at exposure and at test, because the stable LC task is not about the object identity. One object was presented on each occasion, making that object very familiar. As it was expected the repeated exposure of this object led to animals spending less time exploring this object compared to the novel and other familiar object. On average animals spent only 6 seconds exploring object A, whereas the novel object configuration was explored for about 22 seconds. However, the findings of this study were not significant. Analysis of the exploration times for objects position on the left, right and top of the open field revealed that animals did not show a preference for exploring objects in a certain location. Bias measures in the stable OLC, unstable OLC, unstable LC, and stable LC were

not significant, implying that any preferential exploration of objects was not due to a position bias.

Whilst other groups have reported data in which multiple SOR tasks have been run in a single day (Gutoreva et al., 2015), in this experiment I showed that two tasks run with one trial each per day was not successful in showing recognition of either location-context memory or object-location-context memory. This is surprising given Gutovera's study included assessment of object-location-context memory. Based on the finding that D2 scores in the four tasks were not significant, I was concerned that exposing animals to two different tasks in one day influenced their performance. One potential difference between Gutorevas' data and my own is that in their study of the development of memory, animals are typically only tested once on each task, whilst in the current study animals are tested on the same two tasks repeatedly over several days. In order to assess the potentially damaging effect of interference in running repeated trials, I explored the performance on only the first task, and in this case animals showed a non-significant trend to prefer the novel object in the object-location-context task, though not the location-context task. Although one trial with so few animals is unlikely to (due to lack of power), in itself, provide comparison data to that of Gutoreva et al. (2015), it is suggestive that the problem in replication through this method comes from repeated use of similar tasks, alternating within a day.

Encountering two different tasks in one day seems to impair a rat's performance on these tasks. Interfering memories of similar events only a few hours before in another context may be the underlying cause. Nevertheless, it might still be possible to collect more data in a shorter amount of time without decreasing statistical significance or increasing animal numbers. Ameen-Ali et al. (2012) have developed a standard object recognition procedure which assesses the animals' memory through the use of continual trials. They successfully demonstrated that recognition memory can be tested in a continual trials apparatus which

allows for multiple trials within a session. However, Ameen-Ali et al.'s (2012) approach has not been used to assess episodic-like memory. If episodic-like memory can be assessed in a multiple trials apparatus, then this would provide an alternative way to collect substantial amounts of data from each animal in a short amount of time. Nevertheless, it might be that multiple exposures to different events produce a large amount of interference, making the tasks impossible in this kind of environment. Study 2 in Chapter 3 will address whether the same OLC and LC tasks can be tested in a new continuous trials apparatus.

Chapter 3

Study 2: Object-Location-Context Memory in a Continuous Trials Apparatus

3.1. Introduction

More recently, spontaneous recognition tasks have also been used to explore episodic memory (Eacott & Norman, 2004). Tulving (1983) defined episodic memory as memory that “receives and stores information about temporally dated episodes or events and temporal-spatial relations between them.” Thus, episodic memory is the conscious recollection of past events (Tulving, 1983). In contrast to Tulving’s definition it has been proposed that episodic memory can be described in terms of what happened on a particular occasion and not at an point defined in terms of its timing (Gaffan, 1994; Eacott & Gaffan, 2005; Eacott & Norman, 2004). This operationalisation of episodic memory makes it suitable for neuroscientific investigations in animals (Easton & Eacott, 2008). As explained in Chapter 1, SOR and episodic memory tasks take advantage of rodents’ innate preference for novel configurations of an environment. Rats have a tendency to spend more time exploring a novel item than a familiar one.

However, whilst the simplicity of administering these SOR tasks has allowed for their widespread use in memory research in rodents, there are some issues associated with them. In a standard spontaneous recognition tasks (of any sort) the animal completes one trial a day, which means that multiple sessions have to be run and data accumulation is very slow and subject to significant behavioural noise through day to day variations. Furthermore, it has been argued that behaviour of rodents can be heavily influenced through stress of handling, because the animal is taken in and out of the apparatus (Hurst and West, 2010).

Previous paradigms, such as the bow-tie maze and the rotating E-maze, have been shown to be useful in investigating spontaneous recognition in rodents, but apparatuses have their disadvantages. The bow-tie maze developed by Albasser et al. (2010) measures novelty discrimination by combining features of a spontaneous object recognition task with a delayed nonmatching-to-sample task. In this task animals are placed in one side of the maze containing an object A. The animal is then allowed to move to the other compartment which contains the familiar object A and unfamiliar object B. In the next phase animal shuttles back to other side which shows object B (which is now familiar) and a novel object C. This procedure is repeated for a certain number of trials in one session. The main disadvantage of this paradigm is that it is not comparable to others, which makes the comparison of data between different studies difficult. This apparatus only allows testing spontaneous object recognition; one cannot assess preferences for spatial locations and contexts. Animals shuttle from one compartment to another, which means that they essentially see a reflection of what they have seen before. It is not possible to assess whether animals are using allocentric or egocentric strategies to seek out the novel item.

Ameen-Ali et al. (2012) adopted a new paradigm that further develops a task designed by Albasser et al. (2010). The main difference being that animals do not shuttle between two testing areas, instead there is one testing and one holding area. This paradigm allows for multiple trials per session, and measures exploration preference by determining how much time an animal spends with a novel and familiar object. The animals move to the testing area and see two copies of object A. After a short delay in the holding area, animals shuttle back and now see object A (familiar) and a novel object B. After two minutes the animal goes back to the holding area. When the animal then shuttles back to the arena object B is now familiar and object C is novel. Again, this process is repeated for a certain number of trials within one

session. This approach was successfully used in three typical spontaneous recognition tasks, object recognition (OR), object-location (OL) and object-context (OC). Ameen-Ali et al.'s (2012) apparatus was designed to allow four contexts to be put in place for the OC trials, through a rotating mechanism whilst the animal waited in the holding area. However, whilst Ameen-Ali demonstrated that the animals could demonstrate OC memory, the design of the apparatus was not ideal, as it was very heavy and the context change produced some noise when contexts were rotated into place which may have itself induced stress and produced the type of behavioural noise the apparatus was initially designed to remove.

Tasks that involve context changes are essential for understanding the neural basis of memory. The episodic (OLC) task of Eacott and Norman (2004) requires animals to identify objects in locations in particular contexts. In addition, other types of contextual task have been used to demonstrate dissociations in function within memory systems. For example, Easton, Fitchett, Eacott, & Baxter (2011) tested rats with selective lesions of basal forebrain neurons in the medial septum and vertical limb of the diagonal band (MS/vDB), which caused a cholinergic depletion of the hippocampus, on the standard one-trial a day episodic OLC task. Animals with acetylcholine depletion of the hippocampus showed no deficit in performance on this task of episodic memory. However, these rats were unable to perform a LC task, which required an association of different places with different contexts. Therefore, it is important to be able to overcome the problems of standard one-trial a day spontaneous recognition tasks in a way that allows testing of tasks requiring context changes so that mechanisms such as that explored by Easton et al. (2011) can be understood. As the apparatus of Ameen-Ali et al. (2012) does not appear suitable for tasks involving context changes, I approached the problem through an alternative approach. I developed a new

continual trials apparatus which consist of a testing area and a holding area where the animal is placed at the start of testing and returns to after the completion of a phase (see Figure 1.5).

When critical lesion groups are being tested an appropriate timeframe is essential and there would be a distinct advantage to running trials closer together in time. Therefore, study 2 addressed a different approach, asking if the object-location-context and location-context tasks can be tested in the new continuous trials apparatus with multiple trials per day in a single task.

3.2. Method and Materials

3.2.1. Subjects

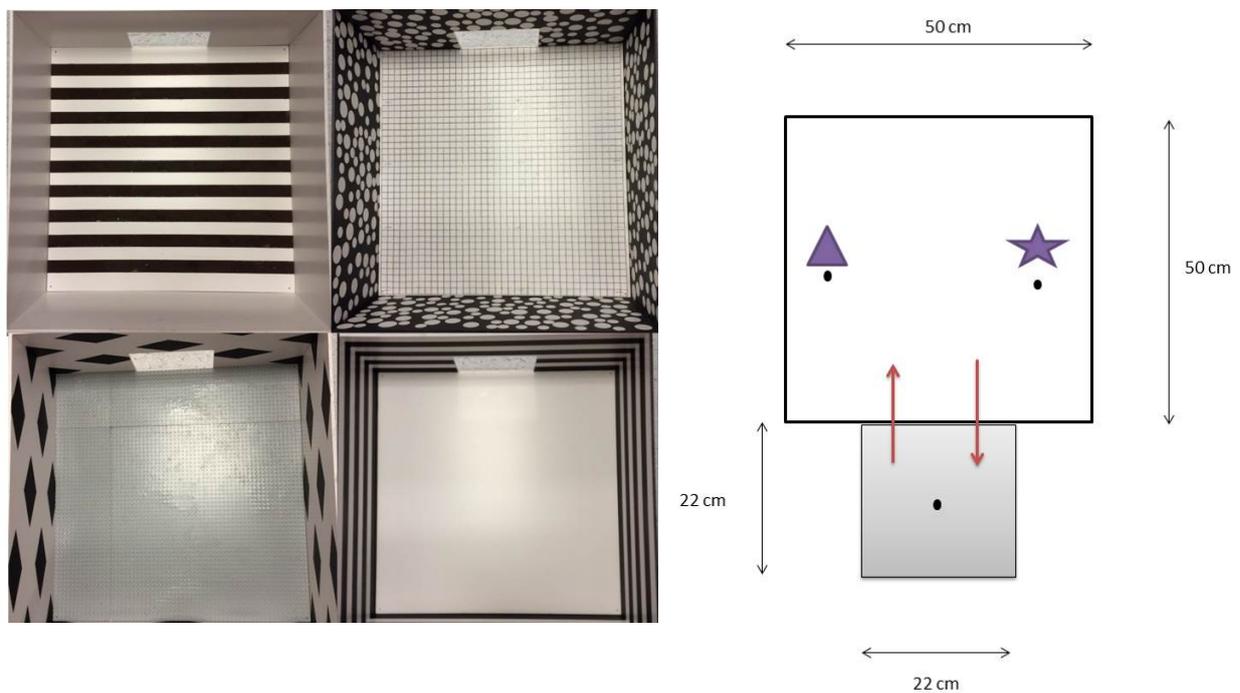
Six naïve Lister hooded rats supplied by Harlan were used in study 2. They were housed in groups of three in rooms maintained on a 12hr light/dark cycle. Testing was only carried out during the light phase and water was available ad libitum throughout the study. During habituation animals were fed a restricted diet to ensure they were 85% of their free-feeding body weight of age matched controls. Due to weight issues, it was decided to take animals off food restriction after the habitation phase. All rats had free access to food during the four weeks of behavioural testing. Animals started testing when they were eight weeks old.

3.2.2. Apparatus and objects

The animals were tested in a square shaped open field and a holding area. The apparatus was 50cm² with the walls' height at 20 cm (Figure 3.1). The holding area measured 22x22x20cm (l x w x h). The features of the arena could be changed by inserting four different contexts. A door divided the testing area from the holding area, which could be opened by the experimenter. The four contexts were as follows: context 1 – horizontal stripes & white walls, context 2 – grey Lego floor and white walls with black diamond shapes, context 3 – wire mesh floor and white dot pattern walls, context 4 – white floor and

horizontal stripes on walls (Figure 3.1). Duplicate copies of objects made of plastic or ceramic that varied in their shape, colour and height were used. Objects were never repeated across different sessions for an animal. The running order, the contexts at sample and test, the novel object and placement of the novel object were counterbalanced within and between animals. The open field and the stimuli were cleaned using disinfectant wipes. Animals were recorded throughout the training and testing. The camera was positioned above the arena to record the animals' exploratory behaviour for analysis.

Figure 3.1. Shape and dimensions (in cm) of the continual trials apparatus.



The white area represents the testing area where the animal encounters the objects. This area can be changed by inserting different contexts. The grey area is the holding area where the animal starts a trial, returns to between phases and finishes a session. The two compartments are separated by a door which is controlled by the experimenter. The black circles represent the food pellets and the red arrows show the movement of the animal.

3.2.3. Habituation and pre-training

Each animal was handled daily for three days prior to habituation. Rats were habituated to moving between rooms (cage covers were used to minimise stress), the testing room, the open field, the objects and contexts. Behavioural testing took place in a separate room under dim white light and white noise in the background to cover environmental noise. Pre-training

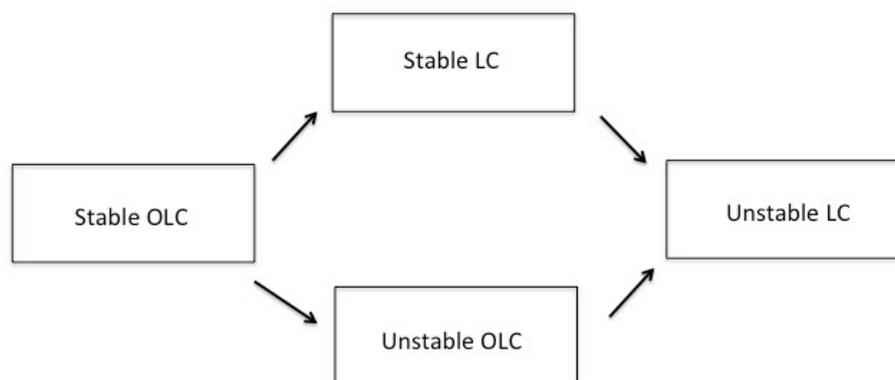
involved four phases aimed to habituate the animals to the environment which lasted 8 days. Phase 1 involved placing the animals in threes into the apparatus for 30 minutes in each context. This allowed them to explore the open field freely. In phase 2 animals were placed singly into the apparatus and were given 15 minutes of exploration in each context. For phase 3 the goal was to train the animals to shuttle between the two areas of the apparatus: the testing area and the holding area. This phase consisted of four sessions (one for each context) and involved placing pellets (20 mg, Purified Diet; BioServ) on the floor and using the doors to control the animal's movement. In phase 4 an object (object A) was introduced and baited with pellets. The object was placed in the middle of the open field in each context and animals were given 10 minutes to explore.

3.2.4. Test protocol

Animals were given a single test session for all tasks, which lasted two hours (Figure 3.2). A testing session consisted of 12 trials. Normally, two rats were tested between 8am and 12pm and another two rats between 1pm and 5pm. It was ensured that each rat was tested at the same time of day for each task. For example and depending on counterbalancing for the task order, if rat 1 was tested on the stable OLC task on a Monday morning in week 1, rat 1 was then tested again on the unstable OLC in week 2 on a Monday morning. All rats had at least one week in between testing the four tasks to avoid interference. At the start of each session, the animal was placed in the holding area. The door would then open to allow the animal to move to the testing area. In both exposure phases and the test phase animals were given 2 min of exploration. Between phases rats were in the holding area while the arena was changed. Objects on each trial were baited with a food pellet to encourage exploration, but these pellets were not used as rewards. Exploration was taken when the animal was at a distance of 1cm of the object and actively exploring it (i.e. sniffing at or touching it). Actions

such as sitting on the objects or using the item as support during rearing were not considered exploratory behaviour. The duration of exploration was measured off-line on a key pad on the computer. The running order, testing contexts, the novel object and placement of the novel object were counterbalanced. The criterion for ending a session was if the animal failed to shuttle between the two areas after three minutes. The data of that animal would not be included in the analysis.

Figure 3.2. Representation of test order.



All animals encountered the stable object-location-context task first. Animals were then split in to two groups. Group one encountered the stable location-context followed by the unstable location-context. Group two encountered the unstable object-location-context task followed by the unstable location-context task.

3.2.5. Stable object-location-context (stable OLC)

The new continual trial apparatus was more closely modelled on the open field, which was used for a one-trial a day what-where-which (OLC) testing. The apparatus consisted of a testing and a holding area. A door allowed the experimenter to control the movement of the animal between the two compartments. As the holding cage was attached to the open field arena, animals were trained to shuttle using pellets. After two minutes of exploration the animal was allowed to shuttle back in to the holding area and the context of the arena was changed. This process was repeated until 12 trials were completed. As in study 1 (Chapter 2)

animals experienced two exposure phases in which objects were in the same location, but in two different contexts. For further details on the task, refer to study 1 in Chapter 2.

3.2.6. Unstable location-context (unstable LC)

The continuous trials procedure is the same as for the OLC task. Rats receive two exposure phases in which they see two identical copies of an object (A and B) in different places and in different contexts (X and Y). For details on the task, refer to study 1 in Chapter 2.

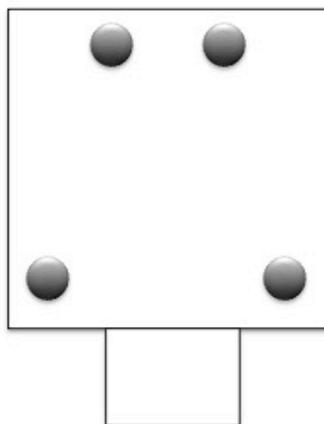
3.2.7. Stable location-context (stable LC)

For details on the task, refer to study 1 in Chapter 2.

3.2.8. Unstable object-location-context (unstable OLC)

This task was carried out as previously described in experiment 1. However, the design of the new testing apparatus did not allow a 6 o'clock position. Therefore, objects were placed in slightly different locations (see Figure 3.3).

Figure 3.3. Drawing of the continual trial apparatus with the objects' locations in the unstable OLC task.



Circles represent the possible locations of the objects. Locations varied to the previous Chapter 2, due to the size of the apparatus and objects. Three of these locations were occupied at the sample phase and two at test.

3.3. Data Analysis

As in the previous experiment (see section 2.2.7 in Chapter 2) D2 scores were calculated. D2 is the discrimination ratio and calculated by dividing the D1 score and the total exploration time. The cumulative D1 score is the sum of the exploration times devoted to the novel objects across the trials minus the sum of the exploration of the familiar objects; the cumulative exploration times are the sum of the total exploration across all trials (see Albasser et al., 2010). Therefore, the cumulative D2 represents an updated score based on the cumulative D1 and cumulative exploration. One-sample t-tests were used to determine if the performance of the animals was above chance. Exploration times (in sec) were analysed for all animals across all tasks. Following the calculation of the discrimination ratio and exploration, as a basic measure to verify whether the task was carried out successfully (i.e. animals determined the novel environmental configuration), it was used to analyse the rodents' behaviour in the continuous trials apparatus. Given that this was a new way of testing episodic and spatial memory in rats and similar trials were repeated multiple times over two hours tasks, the rat's spontaneous behaviour was further analysed. The effect on the discrimination ratio and exploration time by using four different contexts, running 12 trials in one day, and increasing the chance of proactive interference were investigated using repeated measure analyses. Furthermore, exploration times were compared across all four tasks to ensure that memory performance was not affected by low or high interest (i.e. exploration) in specific objects configurations. Exploratory behaviour in the sample phases was observed while scoring test phases to ensure reliability, but there did not seem to be any issue in relation to the reliability of the behaviour of rats (see also Barker et al., 2007; Ozawa et al., 2011)

3.4. Results

Two animals were not included in the data analysis after the first week of testing. One animal was not tested because of weight issues and the other animal failed to shuttle before 12 trials were completed. Therefore, the analysis of the unstable OLC, stable LC and unstable LC is based on the remaining four animals.

3.4.1. Stable OLC

3.4.1.1. Average D2 & cumulative D2

Six rats received 12 trials of the object-location-context task. To determine if the performance of the animals was above chance, one sample t-tests were carried out (one-tailed) and D2 scores were compared against zero. Average D2 scores were significantly above chance, showing a clear discrimination between objects $t(5) = 8.73$, $p < 0.001$. The mean D2 score was 0.2. In addition, the cumulative D2 score over the 12 trials showed a similar result. Animals significantly discriminated between the novel and familiar object configuration $t(5) = 4.576$, $p = 0.003$. The cumulative score was 0.26 (SD = 0.03) (see Figure 3.4.A).

A post hoc power analysis was conducted with the program G*Power 3 (Erdfelder et al., 1996) in order to obtain the statistical power for this task. The effect size was 9.29 and a calculated power of 1.0, which is very high given the much smaller group sized compared to Study 1. Furthermore, Norman & Eacott's (2005) study showed an effect size of 2.38 and a power of 0.99 with eleven subjects, suggesting that the current multiple trial apparatus produces data which is comparable, but with fewer animals. As another comparison, an object-location task by Langston & Wood (2010) employed a sample size of 12 subjects, which gave an effect size of 1.99 and statistical power of 0.99.

3.4.1.2. Investigation of discrimination performance over 12 trials

The following analyses investigate the use of multiple trials as well as different contexts in the new apparatus. Using repeated trials and configurations may have changed an animal's performance throughout the tasks and caused interference.

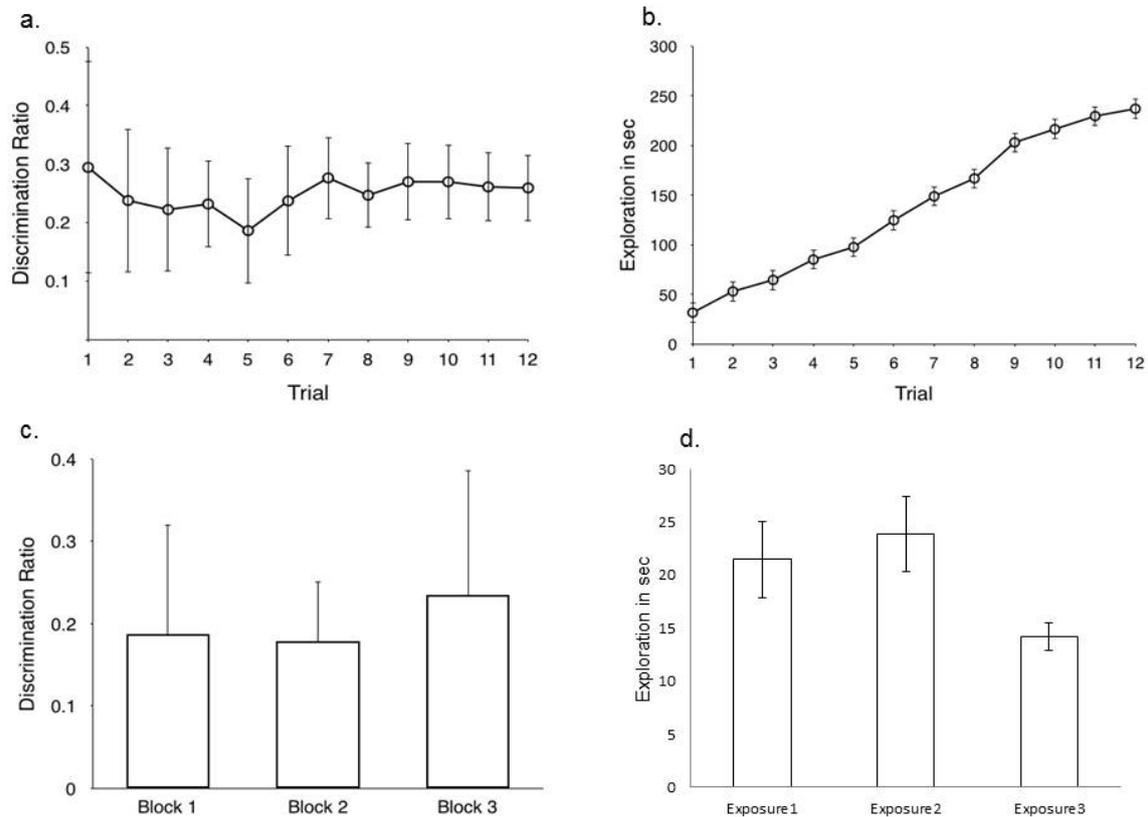
One session consisted of 12 trials, which means that the animal saw each of the four contexts three times at test. To see if animals performed better on the last exposure of the same context due to a habituation effect, or whether they performed worse on the last exposure due to interference, a repeated-measures ANOVA (context block x discrimination ratio) was carried out. D2 scores for each animal were separated into three blocks, consisting of four trials of different context combinations. A mean D2 was calculated for each block using the individual D2 scores within that block. No habituation effect was found $F(2,10) = 0.079$, $p = 0.924$. If anything, the performance declined towards the end, meaning that the performance on the first exposure (mean D2 = 0.23) was better than the performance on the third exposure (mean D2 = 0.17).

Given the trend towards a lower discrimination ratio at the last exposure of a context and as each session involved 12 trials there is a possibility of increased proactive interference towards the end. This was tested by comparing the D2 scores of Trial 1 and 2 (low interference) with the D2 scores of trial 11 and 12 (high interference). Using a paired t-test it was found that there was no significant difference between the first and last trials, suggesting that there was no build-up of interference $t(5) = -0.153$, $p = 0.884$.

In order to see if performance changed over a testing session (regardless of context), the D2 scores for each rat were separated into three blocks of four trials. For each animal, an average D2 score was calculated within that block. Using a repeated-measures ANOVA (block x discrimination ratio) no effect of block was found $F(2,10) = 0.309$, $p = 0.741$, indicating that the performance remained stable throughout the task (Figure 3.4.C)

In conclusion in the stable object-location-context task, no evidence of interference was found.

Figure 3.4. Stable OLC – Discriminations ratios and exploration times (sec).



A. Cumulative D2 scores for object-location-context across 12 trials. **B.** Cumulative exploration for object-location-context task was calculated as the sum of the total exploration across the total number of trials **C.** Average D2 for each rat across 3 blocks. **D.** Effect of multiple exposure to the same context. See also Table 3.1 for comparison.

3.4.1.3. Investigation of effect of contexts

There were six possible context combinations, meaning that within a session of 12 trials each combination was encountered twice at the sample phase. To find out if there was a difference between the two exposures, a two-way repeated measures ANOVA with exposure and context as factors was run. If memories from previous sample phases interfered with performance, a poorer performance on the second exposure of the same context combination

could be expected. No effect of context combination and no significant interaction between combination of context and exposure were found. However, there was a significant effect of exposure. The D2 score on the second exposure (mean D2= 0.33) was significantly better than the D2 score of the first exposure (mean D2 = 0.07) $F(1, 25) = 33.674, p = 0.002$.

To see if a preference for one of the contexts affected performance during testing, a one-way ANOVA was carried out. The D2 scores did not differ significantly between contexts $F(2,23) = 1.396, p = 0.273$. Nevertheless, the discrimination ratio was the lowest in context 4 (mean D2 = 0.09) and the highest in context 2 (mean D2 = 0.25).

3.4.1.4. Investigation of exploration times

As discussed before, within a session of 12 trials each combination of contexts was encountered twice at sample. To see if the total exploration times at test changed when the same contexts were encountered twice at sample another paired t-test was carried out. There was no significant difference between the exploration times at test when the same contexts combinations were seen at sample $t(5) = -.0487, p = 0.647$.

The animal saw each of the four contexts three times at test and to see if exploration times change over the three exposures, a repeated-measures ANOVA on D2s (context block x discrimination ratio) was done. Animals could show a decreased exploration time over a session for two reasons: either they lose interest in the task or they become more habituated. An analysis of the exploration times has shown that there was a trend towards higher exploration times on the second exposure (24 sec) and lower exploration times on the third exposure (14 sec) $F(2,10) = 3.953, p = 0.054$. Furthermore, the exploration times were divided into three blocks of four trials (as it was done with the D2 scores) and an average exploration time was calculated for each block. There was no effect of block, exploration times remained stable $F(2,10) = 0.833, p = 0.463$ (Figure 3.4.C/D)

In conclusion, there is no evidence of changing exploration times in the stable object-location-context task.

3.4.2. Unstable LC

3.4.2.1. Average D2 & cumulative D2

Average and cumulative D2 scores were calculated. In the location-context task animals also showed a clear preference for the object in the novel configuration. Average D2 scores ranged from 0.1 to 0.4, giving an average of 0.3. Animals preferentially explored the novel object over the familiar object configuration $t(3) = 7.828$, $p = 0.002$. The cumulative D2 score over the 12 trials showed a significant discrimination between objects $t(3) = 5.501$, $p = 0.006$. The cumulative D2 score was 0.3 (SD = 0.068) (Figure 3.5.A).

A post hoc power analysis was conducted with the program G*Power 3 (Erdfelder et al., 1996) in order to obtain the statistical power for this task. The effect size was 4.41 and a calculated power of 0.99, which is very high compared to the unstable LC task which was carried out in Study 1.

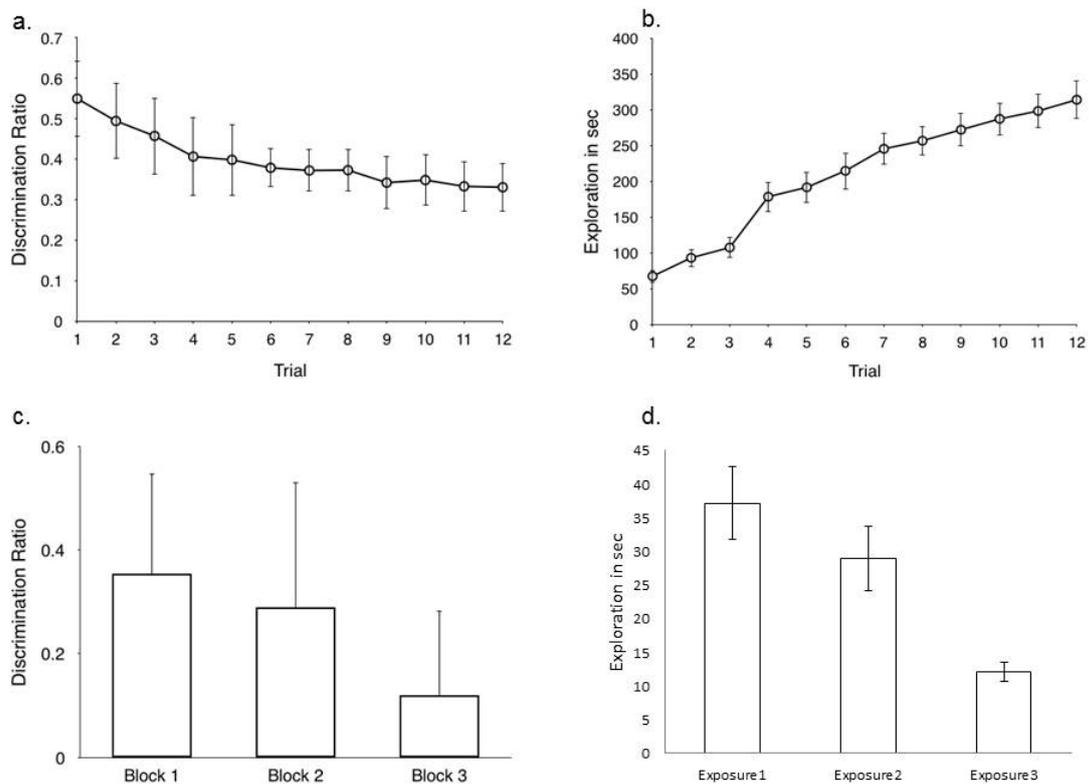
3.4.2.2. Investigation of discrimination performance over 12 trials

An animal saw each context three times at test. To find out if animals performed better on the last exposure of the same context due to a habituation effect or worse because of interference, a repeated-measures ANOVA (context block x discrimination ratio) was carried out. D2 scores for each animal were separated into three exposure blocks of four and a mean D2 was calculated for each. Unlike in the stable OLC task, there was a significant effect of exposure $F(2,6) = 7.273$, $p = 0.025$. Discrimination on this task decreased, as the mean D2 of the first exposure was 0.37, the second 0.29 and the third exposure block had an average of only 0.11. This indicates that interference took place and the following analysis will look at this more closely.

Because each session involved 12 trials, there is a possibility of a build-up of interference towards the later trials, which was already seen in the previous analysis. Therefore, the D2 scores of Trial 1 and 2 and the D2 scores of Trial 11 and 12 were compared using a paired t-test. The results showed that there was possibility that interference from previous memories have diminished performance towards the end of a session $t(3) = 3.119$, $p = 0.052$. The first trials had an average D2 of 0.45, whereas the last trials had an average of 0.08.

Because the performances of the first trials were different from the last trials, the D2 scores for each rat were separated into three blocks of four trials. For each animal, an average D2 score was calculated within that block (regardless of context combinations). Interestingly, using a repeated-measures ANOVA (block x discrimination ratio) no effect of block was found $F(2,6) = 1.499$, $p = 0.296$, suggesting that over the total session there was no difference in performance. Nevertheless, considering the proactive interference analysis, it should not be surprising that the first block (trials 1-4) had the highest D2 score (mean = 0.36), whereas the last block (9-12) had the lowest D2 score (mean = 0.12) (Figure 3.5.C)

Figure 3.5. Unstable LC – Discrimination ratios and exploration times (sec)



Vertical bars show the standard error of the mean. **A.** Cumulative D2 scores for location-context across 12 trials. **B.** Cumulative exploration for location-context task was calculated as the sum of the total exploration across the total number of trials. **C.** Average D2 for each rat across 3 blocks. **D.** Effect of multiple exposure to the same context. See also Table 3.1 for comparison.

3.4.2.3. Investigation of effect of contexts

There were six possible context combinations and within a 12-trial session all combinations were encountered twice at sample. A two-way repeated-measures ANOVA (context combination x exposure x discrimination ratio) has shown that there was no significant effect of context combination and no effect of exposure. However, discrimination performance was worse on the second encounter of the same context combination (mean D2 = 0.16) than on the first encounter (mean D2 = 0.33). There was no interaction between exposure and context combination.

During habituation, it was noted that some contexts (i.e. context with sand stripes) elicited more anxiety from the rats than others a one-way ANOVA was carried out to see if there was

a difference in performance depending on the context. The D2 scores did not differ significantly between contexts $F(3,12) = 1.589$, $p = 0.243$, but the discrimination ratio was lowest in context 2 (grey Lego floor and white walls with black dots).

3.4.2.4. Investigation of exploration times

Because a session consisted of 12 trials each combination of context was encountered twice. To see if the total exploration times decrease when the same contexts are encountered more than once, another paired t-test was carried out. There was a significant difference between the exploration times on the first and second encounter $t(3) = 7.723$, $p = 0.005$. Animals spent less time exploring objects on the second exposure (14 sec) than the first exposure (38 sec).

Each animal saw each context three times at test and an analysis of the exploration times has shown that there was significantly less exploration on the third exposure (12 sec) than on the first (37 sec) $F(2,6) = 8.271$, $p = 0.019$. When the exploration times were divided into three blocks, regardless of context, there was also a significant decrease in exploration $F(2,6) = 25.173$, $p = 0.001$. Block 1 had an average exploration of 45 sec, block 2 20 sec and block 3 had 14 secs (Figure 3.5.C/D). In conclusion, there is evidence of changing exploration times throughout the session.

3.4.3. Unstable OLC

3.4.3.1. Average D2 & cumulative D2

Animals significantly differentiated between the novel objects and the familiar environmental configuration $t(3) = 4.38$, $p = 0.01$. Compared to the other tasks, the D2 scores had a wider range from -0.03 to 0.23, with an average D2 score of 0.1. The cumulative score showed a slightly different result, but animals still differentiated between the two

configurations $t(4) = 2.345$, $p = 0.05$. The cumulative D2 was 0.15 (SD = 0.03) (Figure 3.6.A).

A post hoc power analysis was conducted with the program G*Power 3 (Erdfelder et al., 1996) in order to obtain the statistical power. The effect size was found to be 5.00, which is medium, and the calculated power was 0.98.

3.4.3.2. Investigation of discrimination performance over 12 trials and interference

Given that each context is encountered three times at test, there is a possibility of a habituation effect and that the animal might do better on the last exposure because it is getting used to the context. Alternatively, similar memories from previous trials could cause interference and cause a worse performance. Unlike the stable OLC task animals showed a better performance on the second exposure (mean D2 = 0.21) than on the first (mean D2 = 0.18) and the third (mean D2 = 0.08) exposure. Nevertheless, a repeated-measures ANOVA (context block x discrimination ratio) showed that there was no difference between the D2 scores $F(2,6) = 0.323$, $p = 0.736$.

As with the previous tasks, due to the use of multiple trials there is a possibility of increased proactive interference. This was tested by comparing the D2 scores of Trial 1 and 2 with the D2 scores of Trial 11 and 12. Using a paired t-test it was found that there was no significant difference between the first and last trials $t(3) = 0.247$, $p = 0.821$.

D2 scores for each rat were separated into three blocks of four trials, but the discrimination performance of rats over a testing session did not change. Although block 3 did show a lower D2 score (mean = 0.09) than block 1 (mean = 0.17) and block 2 (mean = 0.16), a repeated-measures ANOVA (block x discrimination ratio) has shown that there was no effect of block $F(2,6) = 0.143$, $p = 0.870$. Discrimination was stable throughout the session (Figure 3.6.C)

In conclusion, even though this task showed lower D2s than others and there is a trend of interference, this did not seem to affect the overall discrimination performance.

3.4.3.3. Investigation of effect of contexts

There were six possible context combinations, meaning that within a session of 12 trials each combination was encountered twice. To see if performance was worse on the second exposure of the same context combination a two-way repeated-measures ANOVA (context combination x exposure x discrimination ratio) was carried out. There was no main effect of exposure and no interaction between combination and exposure. However, looking at the D2 scores, the second (mean = 0.06) encounter of the same context combination was found to be much lower than the first (mean = 0.23). There was a main effect of context combination $F(5,15) = 9.376, p = 0.028$.

Animals performed equally well in all four contexts $F(3,12) = 2.502, p = 0.109$, but performance in context 1 (horizontal stripes on floor and white walls) was particularly low (0.01).

3.4.3.4. Investigation of exploration times

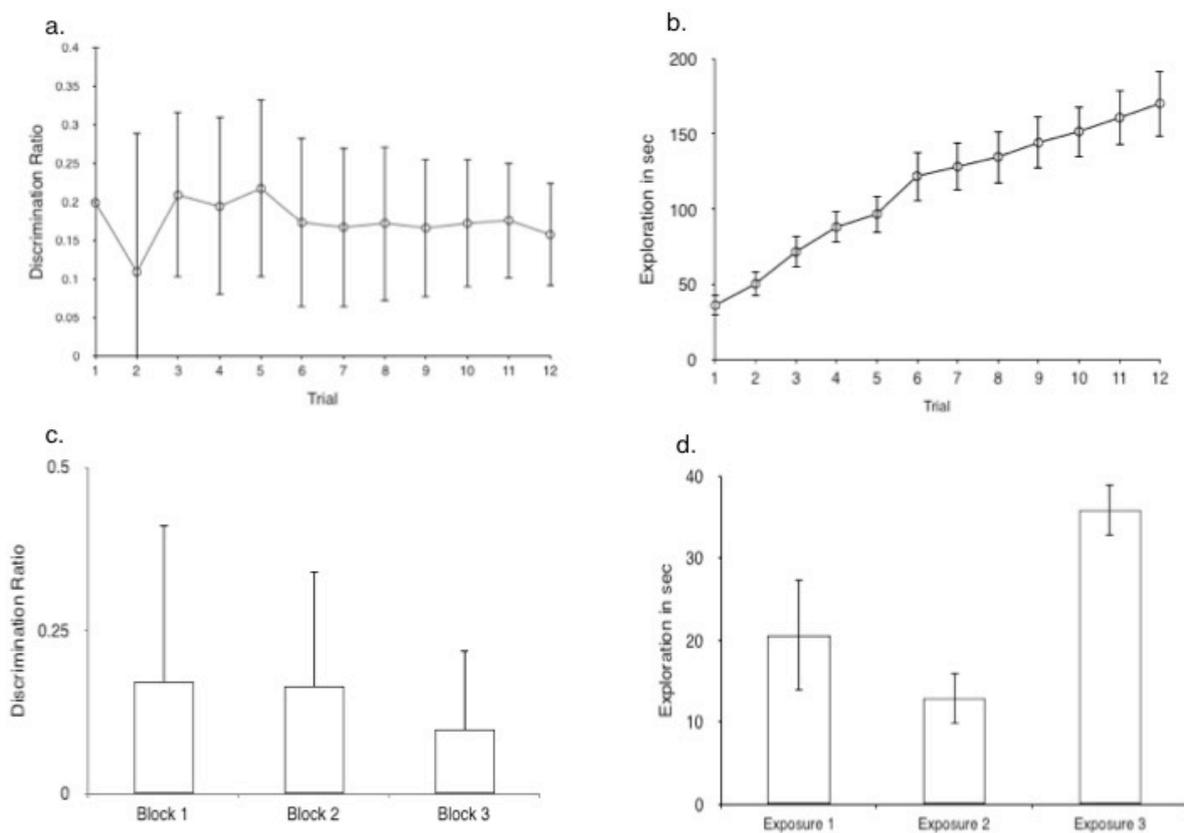
A session consisted of 12 trials and all combinations of contexts were encountered twice. To see if the total exploration times decrease when the same contexts are encountered more than once, a paired t-test was carried out. When the same context combination was seen later in a session the exploration times decrease (from 16 secs to 13 secs). This difference was significant $t(3) = 4.819, p = 0.017$.

An animal saw each context three times at test. An analysis of the exploration times has shown that these times significantly decrease $F(2,6) = 11.395, p = 0.009$. Exploration times decrease from 21 secs on the first exposure to 13 secs on the second exposure to 9 secs on the

third exposure. A repeated-measures ANOVA has also shown that, without taking specific context exposures into account, exploration times decrease significantly throughout the session $F(2,6) = 51.370, p < 0.001$ (Figure 3.6.C/D)

In conclusion, the exploration times in this task decrease significantly over the session, which is interesting as this task involves three objects (not only two as the previous two tasks) and animals were expected to explore more in this task, as it contains more objects.

Figure 3.6. Unstable OLC – Discrimination ratios and exploration times (sec).



Vertical bars show the standard error of the mean. **A.** Cumulative D2 scores for location-context across 12 trials. **B.** Cumulative exploration for location-context task was calculated as the sum of the total exploration across the total number of trials. **C.** Average D2 for each rat across 3 blocks. **D.** Effect of multiple exposures to the same context. See also Table 3.1 for comparison.

3.4.4. Stable LC

Scores were calculated including but also excluding the exploration of object A. The average time spent exploring object A was lower than the average exploration of the novel object and the familiar object. Given that the exploration times of A are very low (average exploration time of 2.5 sec) and this object is only used to fill a position it does not add any further noise to the data. Therefore, it was decided that the following analysis excludes object A (unless otherwise stated).

3.4.4.1. Average D2 & cumulative D2

Animals significantly discriminated between location-context configurations when the exploration of object A is not included $t(3) = 4.185, p = 0.013$. However, this is not quite the case when object A is taken into consideration $t(3) = 2.245, p = 0.06$. The mean D2 in this task was 0.2, with a range of 0.1 to 0.3. The cumulative D2 score over the 12 trials showed a similar result. Animals significantly discriminated between the novel and familiar object when the exploration of object A is taken into account $t(3) = 6.331, p = 0.004$, and also when the exploration of A is ignored $t(3) = 8.378, p = 0.002$. The cumulative D2 scores were 0.14 and 0.26, respectively (Figure 3.7.A).

A post hoc power analysis was conducted with the program G*Power 3 (Erdfelder et al., 1996) and the effect size was shown to be 8.66 and with a calculated power of 1.0, which is very good in terms of power.

3.4.4.2. Investigation of discrimination performance over 12 trials and interference

The animal saw each of the four contexts three times at test. To see if animals performed better or worse on the last exposure of the same context due to a habituation or interference effect, a repeated-measures ANOVA (context block x discrimination ratio) was carried out. D2 scores for each rat were separated into three blocks of exposure and a mean D2 was

calculated for each block. Animals did not perform better on the last exposure than on the first or second exposure of the same context $F(2,6) = 0.587$, $p = 0.585$, suggesting no interference.

A within-session build-up of interference could develop towards the end and this was tested by comparing the D2 scores of Trial 1 and 2 and the D2 scores of Trial 11 and 12. A paired t-test has shown that there was difference in ability to differentiate novel from familiar objects $t(3) = 0.902$, $p = 0.434$.

Performance of animals did not change over a testing session. When D2 scores for each rat were divided into three blocks, the average D2 of each block has shown that discrimination performance remained relatively stable throughout the 12 trials $F(2,6) = 3.712$, $p = 0.089$. Animals performed equally well in the first (mean = 0.3) and third (0.25) block, but showed a lower D2 in the second block (0.07) (Figure 3.7.C)

Overall, there is little evidence of interference in this task and discrimination remained stable.

3.4.4.3. Investigation of effect of contexts

There were six possible context combinations, meaning that within a session of 12 trials each combination was encountered twice at the sample phase. To find out if there was a difference between the two exposures a two-way repeated measures ANOVA (context combination x exposure x discrimination ratio) was run. There was no main effect of context combination, no effect of exposure and also no interaction between the two variables.

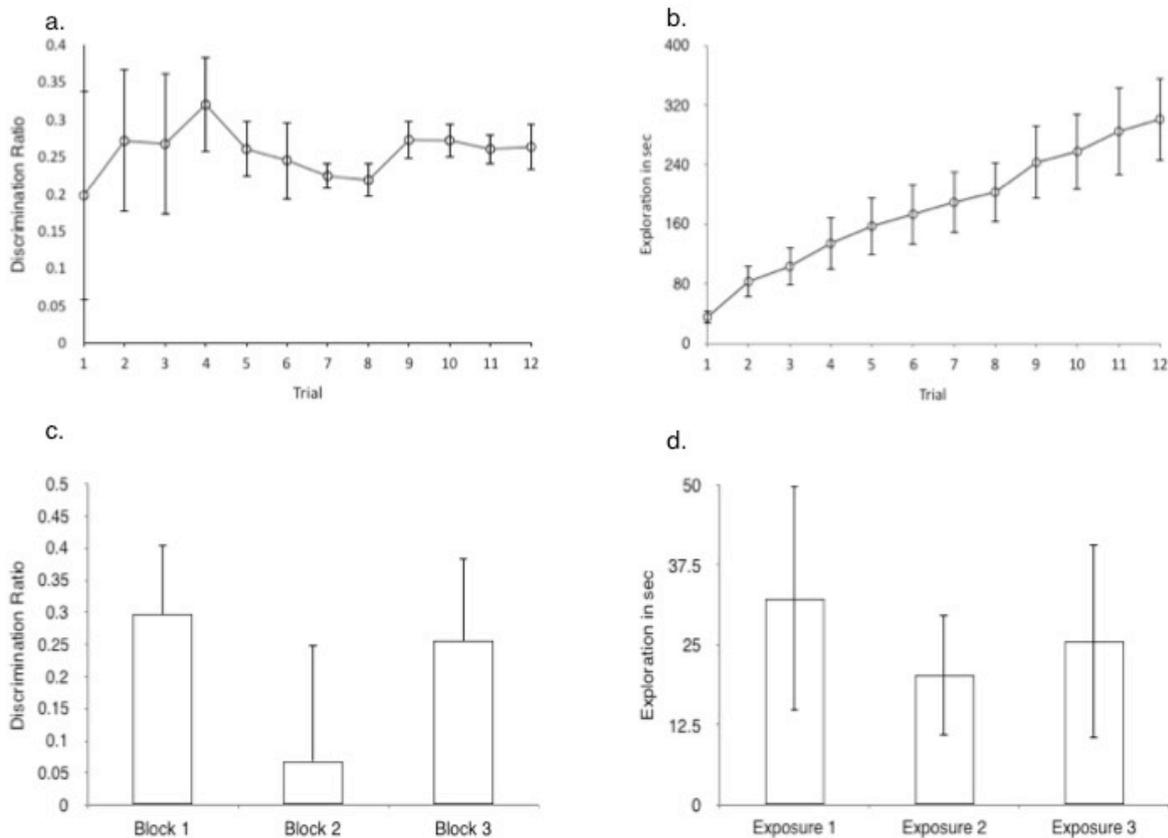
Animals performed well in all four contexts $F(3,12) = 1.028$, $p = 0.415$. Context 4 had the lowest D2 score (mean = 0.12) and context 3 the highest (0.28).

3.4.4.4. Investigation of total exploration times

The six combinations of contexts were encountered twice at sample. To test the hypothesis if the total exploration times decrease when the same contexts are encountered more than once, a paired t-test was carried out. Even though animals explored the objects less on the second exposure (20sec) than on the first encounter (30sec), this difference was not significant $t(3) = 1.929, p = 0.149$.

Unlike the unstable tasks, exploration times did not decrease over a session when animals see the same context repeatedly at test $F(2,6) = 1.033, p = 0.411$. The same result was found when exploration times were divided into blocks and average exploration time was calculated $F(2,6) = 2.683, p = 0.147$, suggesting that animals remain interested in the task (Figure 3.7.C/D).

Figure 3.7. Stable LC – Discrimination ratios and exploration times (sec).



Vertical bars show the standard error of the mean. **A.** Cumulative D2 scores for location-context across 12 trials. **B.** Cumulative exploration for location-context task was calculated as the sum of the total exploration across the

total number of trials. **C.** Average D2 for each rat across 3 blocks. **D.** Effect of multiple exposures to the same context. See also Table 3.1 for comparison.

Table 3.1. Summary table of the results in Study 2 across the four tasks.

| | Cumulative D2 | Cumulative Exploration (sec) | Exposure to contexts in blocks (1-3) (sec) | | | Average D2 across blocks (1-3) | | |
|---------------------|---------------|------------------------------|--|---------------|----------------|--------------------------------|----------------|---------------|
| | | | 1 | 2 | 3 | 1 | 2 | 3 |
| Stable OLC | 0.26 (0.03) | 236.9 (25.6) | 21.5 (3.1) | 23.4 (2.9) | 14.2 (1.1) | 0.19 (0.1) | 0.18 (0.07) | 0.24 (0.2) |
| Unstable LC | 0.37 (0.06) | 314.1 (26.9) | 37.2 (5.4) | 28.9 (4.7) | 12.1 (1.4) | 0.35 (0.2) | 0.29 (0.2) | 0.12 (0.2) |
| Unstable OLC | 0.15 (0.06) | 170.2 (21.97) | 20.6 (6.8) | 12.9 (3.1) | 35.9 (3.1) | 0.17 (0.2) | 0.16 (0.2) | 0.09 (0.1) |
| Stable LC | 0.26 (0.03) | 300.8 (55.96) | 32.2 (17.5) | 20.2 (9.5) | 25.6 (15.1) | 0.29 (0.1) | 0.07 (0.2) | 0.26 (0.1) |

This table summarises the main findings of the effects across the stable OLC, stable LC, unstable OLC and unstable LC in Study 2. Numbers in brackets represent SEM.

3.4.5. Comparison of tasks and effect of test order

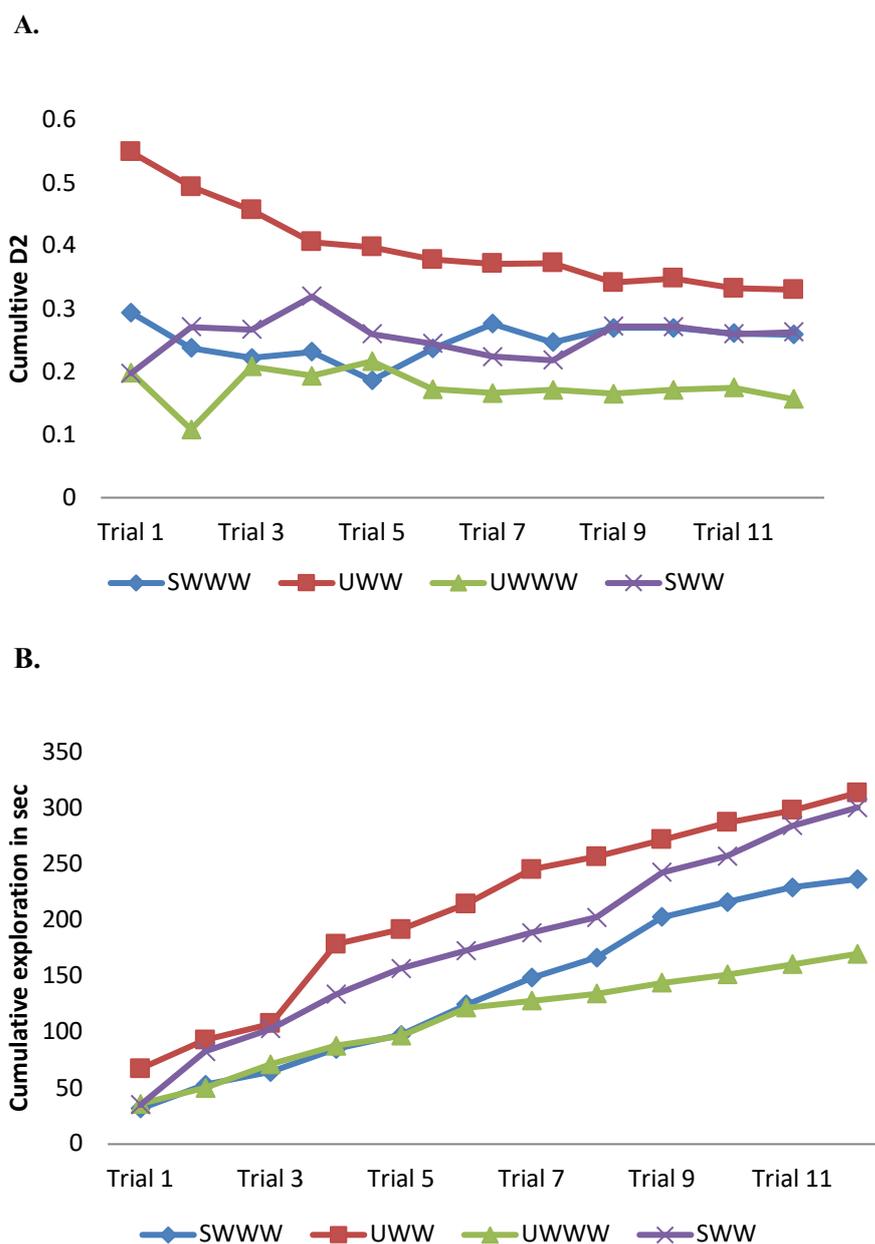
The previous analysis only looked at performance by task (for summary see Table 3.1 and Figure 3.8). The purpose of the following analysis is to compare the performance between the four tasks. Given the uneven number of animals in the tasks (six in the stable OLC and only four in the remaining tasks) make an analysis challenging and any findings can only be seen as a trend. Figure 3.8 shows that the unstable LC task has the highest exploration and the unstable OLC has the lowest exploration of all tasks. The exploration in the unstable LC task is expected to be higher, because the objects keep changing locations. However, the low exploration in the unstable OLC task is surprising. Animals should be exploring more, given that there are more objects in the arena.

There is a clear, but non-significant, difference between the performance in the unstable LC and the unstable OLC task $t(4) = -2.757, p = 0.07$ and in exploration $t(4) = -2.603, p = 0.08$.

Overall, Figure 3.8 shows that the performance in the memory tasks mirror the exploration of the novel configurations.

To determine if the running order of tasks made a difference to the spontaneous exploratory behaviour of animals, a larger number of animals in the groups would have been required. Two animals were dropped after the first week and only one animal encountered the unstable OLC task first. Three animals encountered the stable LC task first. Based on the low number of animals no firm conclusion can be made about the effects of test order.

Figure 3.8. Cumulative D2 and exploration times (in sec) of all four tasks for comparison.



A. Graph shows the cumulative D2, which are very high over 12 trials. **B.** Graph shows exploration (in sec) of the novel environmental configuration over 12 trials. Performance mirrors exploration differences by task.

Based on these findings (for summary see Table 3.1) it can be hypothesized that there is a relation between exploration times and D2 scores. When a rat sees a context three times at test in the **stable OLC** task, the D2 score declined over the course of a session, but it was not significant. However, when performance was divided into blocks performance was slightly better towards the end of a session. Total, novel and familiar exploration times remained mainly stable (only a minor decrease could be observed). This could be an indication that D2 scores and exploration times are related. When the performance does not significantly change over a session, the exploration times of both items (novel and familiar) remain stable.

In the **unstable LC** task, the D2 score declined over time when the same context was seen three times. When performance was divided into blocks performance also decreased. Total exploration and exploration of novel objects decreased over time, whereas familiar exploration remained stable.

This means that when D2 scores decreased, the exploration of novel items also decreased. But the familiar exploration remained stable. The decrease in performance could be due to a decrease in exploration times of the novel object.

In the **unstable OLC** task, the D2 score also declined towards the end when the same context was encountered three times at test, but it was not significant. Block performance was not significant, but D2 scores decreased. Total exploration and exploration times of novel and familiar items decreased significantly.

This shows that when the D2 score decrease, the exploration times of both objects also decrease.

The **stable LC** task is different from the others, because the lowest D2 score was found on the second exposure of the same context and so was the block performance. The exploration times did not change significantly, but familiar times decreased slightly. Novel exploration times were lowest in the second block. The relation between D2 and exploration is different in this task, but also similar. When D2 is low, the novel exploration is also low.

3.5. Discussion

The present study arose from the need to devise a more reliable open field apparatus to test episodic memory in rodents. The goal was to assess this new method which allows for multiple trials per session. This new testing apparatus reduces the amount of handling, as the animal does not have to be taken in and out of the open field after every sample and test phase. As a consequence, the animal is less stressed and provides more reliable object discrimination. Much of the variance that is found in normal recognition tasks is also reduced and the statistical power is much higher than in the previous chapter. When the statistical power of the current tasks was compared to for example Norman & Eacott (2005) and Langston & Wood (2010), the power much higher and fewer animals were used to achieve it. High levels of discrimination were found in all four tasks of episodic-like and context-place memory. Animals were rewarded for running back and forth between the two areas, because the objects were baited. However, these rewards do not influence the discrimination performance because all objects (familiar and novel) had a food pellet next to it. Despite the fact that animals were on free feeding during the entire time of testing, they were motivated to explore the objects and shuttle between the testing and holding area. This demonstrates the wider potential of this new testing apparatus.

The open field provided high levels of performance in the stable and unstable versions of the episodic-like memory tasks. This shows the robustness of this testing procedure. Clear exploration differences between novel and familiar object configurations were evident. In the stable OLC task average D2 scores ranged from 0.1 to 0.3. Furthermore, the cumulative D2 scores were clearly within the range of scores reported in previous studies (e.g. Eacott & Norman - D2 of 0.4; Dix & Aggleton - D2 of 0.2). The D2 score in Eacott & Norman is relatively high, but a minor procedure change can account for the poorer performance in the current study. An interval of 1 min between phases was used, whereas Eacott and Norman used 2 min. The shorter gap between exposures may increase the difficulty of the task, because phases become less distinguishable. The memory load in a continuous trials apparatus is higher than in the original open field. Interference has been defined as the 'confusion between memories between similar events, arising from the perceptual similarity of events' (Gaffan, 1994). Because of the use of multiple trials there is a risk of proactive interference. Proactive interference occurs when the subject has to reject recently learnt items that are no longer relevant to the current situation (Atkins et al., 2011). Therefore, later trials may be more demanding than earlier trials, because rats bring earlier memories to the new trial. Some studies, like Albasser et al. (2010), have found a build-up of proactive interference, because previously stored associations interfere with new associations. In the current apparatus, the use of multiple trials within a session did not seem to affect discrimination performance of animals. When an animal is exposed to the same context multiple times, there is a chance that animals' performance decreases, because of interference of similar memories from other test phases. An investigation of exploration times has shown that even though total exploration, novel and familiar exploration times decrease this does not affect performance. The animals remain interested in the task, because the novelty performance on the last trials was still good.

Whereas I was unable to replicate the findings of Easton et al.'s (2011) study of the unstable LC task in the original open field, the new apparatus demonstrates that intact rats are indeed able to discriminate between novel and familiar location-context configurations. The D2 scores in this task ranged from 0.1 to 0.4, which is very promising. Unlike the stable OLC task, a decrease in D2 scores was found when the animal saw one context multiple times. The reason for this decline can be one of two things: either the animal becomes more habituated to the environment, or the animal is faced with increased interference from previous test trials. A trivial build-up of proactive interference was found and earlier memories may have influenced discrimination towards the end of a session. Nevertheless, no significant change in D2s was found, suggesting that performance remains stable throughout a session.

The unstable OLC D2 scores ranged from -0.03 to 0.2. Whereas this discrimination ratio is lower than the D2 score of the stable OLC task (or any of the other tasks), it should be taken into account that this task may be more difficult. Objects are not in a constant location, animals do not know the placement of the object, and animals go from three objects in the sample phases to two objects in the test phase.

It was also observed that animals had a tendency to visit the location of an object when it was no longer physically present. Using Figure 3.3 as an example, if object C was in the bottom right position in context Y at sample phase 1 and at bottom left in context X in sample phase 2, it was observed that animals would briefly explore the bottom left location in context X at test. This suggests that in one task we can assess object-location-context memory and location-context memory at the same time, which will be followed up in Chapter 5. Rats can clearly discriminate between the four contexts and remember where an object was placed previously, without it being physically present. However, it could be argued that this

tendency to explore the old location of C is driven by the odour of the object remaining at its previous location.

The discrimination of objects in the stable LC task was significantly above chance, when the exploration of the continuous object (A) was not taken into account. This object is mainly used to fill a place to make the task stable. Over the course of the testing session this object becomes highly familiar and the object itself and the location it occupies is explored less. The average D2 scores ranged from 0.1 to 0.3 and animals showed reliable object discrimination in all trials. Compared to other tasks no interference affected discrimination and the exploration times also stayed constant.

Even though this new apparatus offers many advantages over the original open field, it does have some drawbacks. Because of the use of multiple trials there is a risk of proactive interference. It has been argued that later trials may be more demanding than earlier trials. Despite of declining exploration times, performance on the final trials was still effective. An investigation into the discrimination changes over time has shown that there is no clear evidence that performance drastically changed across sessions. There was no indication that a build-up of proactive interference influenced the performance of animals. Results may have declined towards the end of a block of trials, but animals still performed well throughout the whole testing session. The scores show that novelty performance is still good towards the final trials, because scores continue to increase (see Albasser et al., 2010). There was also no indication that performance may have been better towards the end of a testing session, which was found in Ameen-Ali et al.'s (2012) study.

One major disadvantage of the current set-up of the apparatus is the small size of the holding area. When the holding area was designed, it was not taken into account that animals might develop a fear of the restricted amount of space. Three animals that were bigger than the others did not shuttle as reliably towards the end of the four weeks of testing. One animal

failed to shuttle on consecutive trials in the unstable what-where-which task, which is why this animal was dropped. Therefore, the size of the holding area will be adjusted to make sure that larger animals still feel comfortable in this compartment. Performance during the pre-training phase may be an indication of how well an animal is habituated to the apparatus. In some cases, further habituation may be required. However, this particular animal did not show any signs that it was not well habituated. Hence it is argued that the chances of a failure to shuttle in this apparatus will be very low once the size of the holding area is increased.

The present study sought to determine whether rodents can demonstrate episodic-like memory in a new testing apparatus. Overall the data obtained from this continuous trial apparatus is comparable to other studies. Due to the nature of the apparatus the number of animals used in the present study was also reduced, without compromising statistical power. From the data, it is clear that the improved design of the open field has the potential to provide valuable complement to the study of memory in rodents. Being able to offer such a paradigm which works in four different tasks of episodic-like memory has wider potential. Because of its small size, the simplicity and rapid data accumulation it could also be used for transgenic mice and pharmaceutical companies. This continual trial apparatus allows for tasks of episodic-like memory, which was not possible in the rotating E-maze (Ameen-Ali et al., 2012 unpublished data). Although their design of the apparatus includes different contexts and allows carrying out object-context tasks, it does not allow for different versions of object-location-context and location-context. Based on these findings the study has presented a useful novel testing apparatus to investigate the neural mechanism of memory and learning.

Study 2 shows that it is possible to collect reliable data in the continual trials apparatus for tasks that involve changes in context. Whilst Ameen-Ali et al. (2012) had found

context change to affect performance on their memory tasks; here the adaptation of the apparatus to reduce the impact of context change on the animal has allowed both episodic object-location-context recognition and location-context recognition to be performed. As with Ameen-Ali's study, the continual trials approach has the advantage of reducing the number of animals needed, with the location-context task reaching significance with only four animals being tested. This new testing apparatus reduces the amount of handling, as the animal does not have to be taken in and out of the open field after every sample and test phase. Consequently, the animal is less stressed and provides more reliable object discrimination (Ameen-Ali et al. 2012).

It is worthy of note that the memory load in this continuous trial apparatus is higher than in the original open field task, given that animals are required to remember and distinguish a number of similar events concurrently within a 2-3 hour time window. Because of the use of continual trials there is a risk of proactive interference, when the subject has to reject recently learnt items that are no longer relevant to the current situation (Atkins et al., 2011). Therefore, later trials may be more demanding than earlier trials, because rats bring earlier memories (with similar objects and within the same contexts) to the current trial. Some studies using a similar approach of consecutive trials on spontaneous recognition tasks, for example Albasser et al. (2010), have found a build-up of proactive interference, because previously stored associations interfere with new associations. In the current apparatus, the use of multiple trials within a session did not significantly affect the discrimination performance of animals in the OLC task.

In all tasks, object exploration remained constant throughout the session, showing that objects remained motivated to explore the objects throughout, and that this varied little as the session progressed. However, after multiple sessions performance showed some evidence of

reducing, and this was also apparent after multiple repeats of the same contexts. The evidence would suggest that whilst the current protocol can be used to assess memory in these tasks, longer sessions with additional trials would likely have worse performance at the end of a session as a result of increasing proactive interference. As this interference can begin to be seen after only three repetitions within each context, then a key component of the success of the current paradigm is the inclusion of multiple contexts. Although only two contexts are used within a trial, a number of contexts were used across the session such that no two consecutive trials used the same two contexts. This reduces the amount of interference between consecutive trials by using different background contexts to separate the events from one another, in the way proposed by Gaffan in his scene memory task in monkeys (Gaffan, 1994).

Overall, the data obtained from the continuous trial approach in Experiment 2 is comparable to other studies using this same approach in other SOR tasks (Ameen-Ali et al., 2012 & Ameen-Ali et al., 2015). The change to a continual trials approach produces less variability in the behaviour on these SOR tasks (due most likely to reduced stress from handling and reduced natural sensitivity of a one trial a day approach) (Ameen-Ali et al, 2012). As for other SOR tasks using this approach, this reduction in variability of behaviour leads to a reduction in the number of animals used, without compromising statistical power. A recent Web of Science search found 857 research publications in the five-year period to 2015 drawn from 31 subject areas which include the terms “spontaneous object recognition” or “novel object recognition” with the terms ‘rat’ or ‘mouse’. A sample of the papers led to a conservative estimate of each publication including two groups of animals, each with an N of 12. Using these estimates, I approximate over 20,000 animals published using SOR tasks over this 5-year period. With a potential 40% reduction in animal use (Ameen-Ali et al, 2012)

adopting this approach would result in over 1,500 fewer animals each year published using these tasks (not including the potential to reduce additional animals in non-published studies).

I present a novel task involving the discrimination of object, place and context, and also discrimination of place and context. Like human episodic memory, memory in this task is assessed without motivation. These tasks in combination with this apparatus provide an effective and also simple way of testing episodic-like memory in rats. This apparatus can now be used to investigate the role of acetylcholine in memory. The next experiment (Chapter 4) will look at the effects of lesions of the cholinergic projections to the hippocampus. By using this continuous trial apparatus, the number of animals used in the following surgical procedure can be reduced. There is no reason to believe that running multiple trials within a day in the same animal diminishes discrimination performance. When performance of animal is compared between the four tasks, it is evident that the same animal can be used multiple times without hindering discrimination and exploration measures. Based on these results it can be concluded that rats distinguish one trial from another and are able to form distinct episodic memories of individual trials.

The improved design of the open field has the potential to provide a valuable complement to the study of memory in rodents. Being able to offer such a paradigm which works in tasks of episodic-like memory has wider potential. Carrying out spontaneous recognition tasks that involve context changes are essential for understanding the neural basis of memory. This continuous trials apparatus allows for tasks of episodic-like memory, which was not possible in the rotating E-maze (Ameen-Ali et al., 2012 unpublished data). Although their design of the apparatus includes different contexts and allows carrying out object-location tasks, it does not allow for different versions of object-location-context and location-context. This new apparatus extends on Ameen-Ali et al.'s (2012) findings and demonstrates

that rats distinguish one trial from another and are able to form distinct episodic memories of individual trials. The same animal can be used multiple times without hindering discrimination and exploration measures. Because of its small size, the simplicity and rapid data accumulation it could also be used for transgenic mice and pharmaceutical companies. Based on these findings the study has presented a useful novel testing apparatus to investigate the neural mechanism of memory and learning.

Chapter 4

Study 3: Behavioural Effects of Basal Forebrain Cholinergic Lesions on a Model of Episodic Memory

4.1. Introduction

Acetylcholine (ACh) has long been implicated in memory and learning (Drachman, 1977). For example a deficiency of cholinergic neurons is related to memory impairments in patients with Alzheimer's disease (Bartus, 2000) and these patients show a change in cells of the basal forebrain and a decreased level of acetylcholine (Whitehouse et al., 1982). However, researches have questioned the previous findings of the cholinergic system and its exact role still has to be established (e.g. Baxter & Chiba, 1999; Easton & Parker, 2003). As mentioned previously in Chapter 1 the basal forebrain cholinergic system can be divided up into four groups of cells: medial septum, vertical limb of the diagonal band of Broca, horizontal limb of the diagonal band of Broca, and nucleus basalis (Baxter, 2001). Over the last few years, there have been different models of the role of acetylcholine which have helped to understand the neural mechanisms involved. The 'encoding versus retrieval scheduling' (ERS) framework is one of the theories, and aimed to explain how the hippocampus separates encoding from retrieval (see Hasselmo, 2006). In a study by Douchamps, Jeewajee, Blundell, Burgess, & Lever (2013) the idea of the ERS framework was applied to investigate the hippocampal ACh's reaction to environmental novelty in rodents. Testing the hypotheses of the ERS framework and ACh involvement, Douchamps et al (2013), recorded from pyramidal cells in the CA1 in rats, which were freely moving around in an open field and they were either injected with scopolamine or saline. They made several discoveries: When a rat was placed in an environment which they never encountered acetylcholine was found to be high and encoding was prioritized; CA1 pyramidal cells also fired at a later phase of the theta rhythm than when the context in which they were in was

familiar ('later-theta-phase-in-novelty effect'); and the extent of place cell remapping was reduced. Additionally, using the ERS framework as a model it has been shown in other studies that the neurotransmitter acetylcholine influences reactions to new information in the environment (for detailed review see: Easton, Douchamps, Eacott, & Lever, 2012), which is why levels of hippocampal ACh are possibly increased when rats are placed in a novel environment and low levels of hippocampal ACh increase interference and promote the recollection of irrelevant memories during encoding (Bentley, Driver, & Dolan, 2011; Micheau & Marighetto, 2011; Lever, Burton, & O'Keefe, 2006). A detailed review of the evidence is beyond the scope of this chapter but for further analyses see papers by Hasselmo and colleagues (Hasselmo & Schnell, 1994; Hasselmo, Wyble & Wallenstein 1996; Meeter et al., 2004). More specifically, place fields in the subfields of the hippocampus (namely CA1 and CA3) are said to be specifically responsive to alterations in the surroundings of a rat (Ikonen, McMahan, Gallagher, Eichenbaum, and Tanila, 2002; Lever, Wills, Cacucci, Burgess, & O'Keefe, 2002; O'Keefe, 1979), suggesting that cholinergic inputs to the HPC are selectively involved in spatial processing. In other words, hippocampal acetylcholine plays a role in memory flexibility of representations. When Ikonen et al. (2002) analysed immunotoxic lesions of the cholinergic input to the hippocampus, firing patterns of hippocampal place cells varied. Cholinergic depletion did not affect the stability of place fields in a familiar environment, but affected place representation in CA1 and CA3 in a novel environment. Place cells in lesioned animals did not change their firing pattern, which demonstrates a loss of flexibility across unstable environments. Interruption to the cholinergic input and a decrease in ACh to the hippocampus, might explain behavioural data where rodents' response to an unstable and changing testing situation resulted in spatial learning impairment (Bizon, Han, Hudan, & Gallagher, 2003). Additionally, this can also explain the

effects of cholinergic drugs (such as donepezil) and how they can improve some deficits in memory flexibility seen in aged mice (Marighetto et al. 2000, 2008).

Being able to separate memories with overlap in content is important for episodic memory in animals and humans. Memories have lots of overlapping features, such as the same people in them, different things happen close together in time or the same location. Time is one cue that can be used to separate memories, but it is difficult to observe the effect of this type of memory has in animals (Eacott & Easton, 2010; Easton & Eacott, 2008). Temporal information can take many forms and may not be truly episodic in nature (Davis, Easton, Eacott, & Gigg, 2013; Eacott, Webster, & Easton, 2012). Using a specific point in time to assess memory may be too restrictive, which is why another way of testing episodic memory in animals was explored. Eacott and Norman (2004) proposed an alternative to what-where-when. By using an approach of what was encountered, where and on which occasion (what-where-which) we can use contextual cues to discriminate events (Eacott & Easton, 2010; Robertson, Eacott, & Easton, 2015). Even though there is still much debate about the paradigms used to investigate the involvement of the cholinergic system in episodic-like memory, their contribution allows us to consider the underlying neural systems of what-where-which (OLC) tasks. Effects of fornix lesions demonstrated that the hippocampal system plays an important role in learning and episodic memory (Eacott & Norman, 2004; Easton et al., 2009). Episodic-like tasks (such as the object-location-context task) are dependent on the hippocampal system, whereas tasks sharing similar features (such as an object-context task) are not. These episodic-like tasks enhance our understanding of neural networks involved in memory and the roles of neurotransmitters. Cholinergic projections from the basal forebrain have been shown to be necessary for scene learning in monkeys (Easton, Ridley, Baker, & Gaffan, 2002). In contrast to the findings in monkeys, Easton,

Fitchett, Eacott, & Baxter (2011) tested rats with selective lesions of basal forebrain neurons in the medial septum and vertical limb of the diagonal band (MS/vDB). The removal of the cholinergic input of the hippocampus had no effect on the what-where-which task (OLC), which tested the rat's innate preference for novel objects. Hence their memory for what object was seen where and in what context remained intact. However, the same rats were unable to perform a 'where-which' task, which required the rat to remember a location which had previously not been filled with an object in a specific context. This finding suggests that a loss of cholinergic input to the hippocampus does not affect the memory for the association of objects, places and contexts, but medial septal neurons play an essential role in spatial and contextual memory (Easton et al., 2011). This dissociation in performance might not be caused by a difference in task demands, but by the (in) stability of locations in the environment. In the object-location-context task the objects' locations do not vary. Every time the animals enter the testing arena for either an exposure or test phase there are always objects on the left and right. On the other hand, in the location-context task objects change locations between exposures and test, meaning there is no stability of object location. When the animal moves from one compartment to another the object is the same but the location of it becomes less predictable. These results have been interpreted in terms of the importance of cholinergic neurons in pattern completion (Easton et al., 2012). In the OLC task confusion is limited as there are always objects on the left and right, whereas in the LC task confusion arises from the continuous change of locations. This leads to an inability to discriminate highly similar events from each other. In terms of place cells, the OLC task may be unimpaired, because place cell maps did not have to be updated. However, when locations continued to change between events, remapping became essential and rats with cholinergic lesions performed at chance. Instead, rodents do not encode a new environment by developing new place cell maps, but instead go back to an existing map. As the LC task

shows more differences between occasions the rat is required to quickly revise its place cell representation, and this process is interrupted by a damaged cholinergic system (Easton et al., 2011; Ikonen et al., 2002). This impairment in location-context memory (where-which) has been seen in other studies with similar cholinergic lesions of the hippocampus using learned (rather than spontaneous behaviour) tasks (Janisiewicz, Jackson, Firoz, & Baxter, 2004).

The current study uses the new continual trials apparatus (see Chapter 3) to investigate the effects of increased interference between trials on rats with cholinergic lesions to the medial septum. Running 12 consecutive trials (within 2 hours) leads to lots of overlap which may increase interference between trials. It is more challenging to keep events separate in memory when they run consecutively in only a few contexts. One might expect pattern separation to be more important in the new continual trials approach than in the previous one trial a day approach (Chapter 2). It is predicted that a reduction in pattern separation as a result of lesions of the MS/vDB cholinergic cells, including cholinergic projections to the hippocampus, will impair episodic memory in the continual trials apparatus where the same task was unimpaired in a one-trial a day version.

4.2. Materials and Method

4.2.1. Subjects

Ten male Lister hooded rats which were supplied by Harlan (200-220 g upon arrival) were housed in groups of three to four in rooms maintained on a 12hr light/dark cycle (light on from 7am to 7pm). Testing occurred during the light phase. During habituation rats were food restricted to 90% of their own free-feeding body weight. Animals started testing when they were 12 weeks old. Each animal was handled daily for three days prior to the surgery and handled again for two days before habituation started. It should be noted that sham and

lesioned animals were not separated after the surgery; instead they were housed together in their cages. All experiments were performed in accordance with the U.K. Animals Scientific Procedures Act (1986) and associated guidelines.

4.2.2. Surgery

Each rat was assigned to one of the two groups: Sham (n=4) and MS/vDB lesions (n=6). Surgeries for both groups were identical and surgical procedures to produce selective lesions of cholinergic neurons in the MS/vDB followed those previously described (Baxter et al., 1995). Anaesthesia and stereotaxic coordinates were adjusted accordingly. Rats were placed in an induction chamber charged with 4% isoflurane in 100% oxygen. They were then placed in the stereotaxic frame (Kopf Instruments, Tujunga, CA) with the head level between bregma and lambda. Isoflurane gas was delivered through a face mask attached to the stereotaxic frame. The skin was shaved and cleaned and a midline incision was made to expose the skull. Two holes were drilled in the skull at the coordinates AP +0.45mm, ML +/- 0.6mm. The 23-gauge needle of a Hamilton syringe was introduced through one of the holes and lowered to a depth of DV -7.8mm. 0.3 µl of either 192 IgG-saporin (0.15 µg/ul, Advanged Targeting Systems, San Diego, CA) or sterile phosphate-buffered saline (Dulbecco's Phosphate Buffered Saline, Sigma-Aldrich, UK) was injected over a 6min time period using a microinjection pump. The needle was left in place for 6min after the injection. The needle was then raised to DV -6.2mm and another injection was made of 0.2µl of either saline or toxin at the same rate and the needle was left in place for 4min. This was done once on each side, meaning that a total of 1µl saline or toxin in each rat was used. When the injections were complete, the skin was closed and the rat was placed in a recovery box. All animals received 0.1ml of baytril as an antibiotics (pre-op), 0.6ml of buprenorphine (0.015 mg/ml) s.c. for analgesia and 5ml of saline and glucose solution after the surgery. Rats were

returned to their home cages once they had regained normal posture and behaviour. Behavioural testing began 14 days following surgery. One rat had two holes drilled at the same position as the other animals, but was not injected with saline or toxin because of problems with the Microdrive. This left three rats as shams and six rats with MS/vDB lesions.

4.2.3. Apparatus and objects

The animals were tested in the same square shaped open field and a holding area as used in Chapter 3 (Study 2). The features of the arena could be changed by inserting four different contexts. For further details see Figure 3.1 in Chapter 3.

As previously stated (Chapter 3.2.2) duplicate copies of objects were used. Objects were never repeated across different sessions for an animal. The open field and the stimuli were cleaned using disinfectant wipes (Azowipes, Vernoon-Carus Limited, Lancashire, UK). Animals were recorded throughout the training and testing and the camera was positioned above the arena to record the animals' exploratory behaviour for analysis.

4.2.4. Pre-training and habituation

Each rat was handled daily for three days prior to habituation. Rats were habituated to moving between rooms, the testing room, the open field, the objects and contexts. Behavioural testing took place in a separate room under dim white light (25 W) and white noise in the background to cover environmental noise. As previously explained in Chapter 3, pre-training involved four phases, which aimed to habituate the animals to the environment. This lasted 8 days.

4.2.5. Test protocol

Animals were given a single test session for all tasks, which lasted two hours. As previously stated, a testing session consisted of 12 trials. Normally, rats were tested between 8am and 4pm and it was ensured that each rat was tested at the same time of day for each task. I.e. if a rat was tested 8-10am on the stable OLC task in week 1, it was made sure that the same rat encountered the unstable LC task in week 2 from 8 to 10am. Hence, all rats had at least one week in between testing the tasks to avoid interference and control for lesion effects. The door would open to allow the animal to move to the testing area. In both exposure phases and the test phase animals were given 2 min of exploration. Between phases rats were in the holding area while the arena was changed. Objects on each trial were baited with a food pellet to encourage exploration, but these pellets were not used as rewards. Exploration was taken when the animal was at a distance of 1cm of the object and actively exploring it (i.e. sniffing at or touching it). Actions such as sitting on the objects or using the item as support during rearing were not considered exploratory behaviour. The duration of exploration was measured off-line by holding down a key pad on the computer. The testing contexts, the novel object and placement of the novel object were counterbalanced.

4.2.6. Object-location-context (OLC)

This is the standard what-where-which task. The set-up of the task was the same as in Chapter 3.

4.2.7. Location-context (LC)

This is the where-which task from Easton et al. (2011). For details on the task, refer to Chapter 3.

4.2.8. Histology

At the end of the experiment each rat was deeply anesthetized with barbiturates and transcardially perfused with 100ml saline followed by 500ml 4% paraformaldehyde in phosphate-buffered saline. Brains were postfixed in 4% paraformaldehyde and then transferred to 30% sucrose in phosphate-buffered-saline (PBS). Once the brains had sunk, the tissues were processed and cut into coronal sections on a freezing microtome at 45µm thickness to map the cholinergic innervation. The cortex, hippocampus and striatum were processed for immunohistochemistry for choline acetyltransferase to verify the selectivity of the 192 IgG-Saporin and to provide a qualitative assessment (see also Baxter et al., 1995). Characterisation of the lesions is shown in Figure 4.1.

The detailed protocol for the choline acetyltransferase IHC was as follows:

- 1** Wash 3 x 5 mins PBS

- 2** Rabbit serum 60minutes

- 3** Primary antibody (Goat monoclonal anti ChAT(1:400)) for 24 hours

- 4** Wash 3 x 5 mins PBS

- 5** Secondary antibody (Rabbit anti-Goat) for 90mins

- 6** Wash 3 x 5 mins PBS

- 7** ABC (avidin-biotin-peroxidase) solution for 45-60mins

- 8** Wash 1 x 5mins in PBS

- 9** Wash 2 x 5mins in Tris

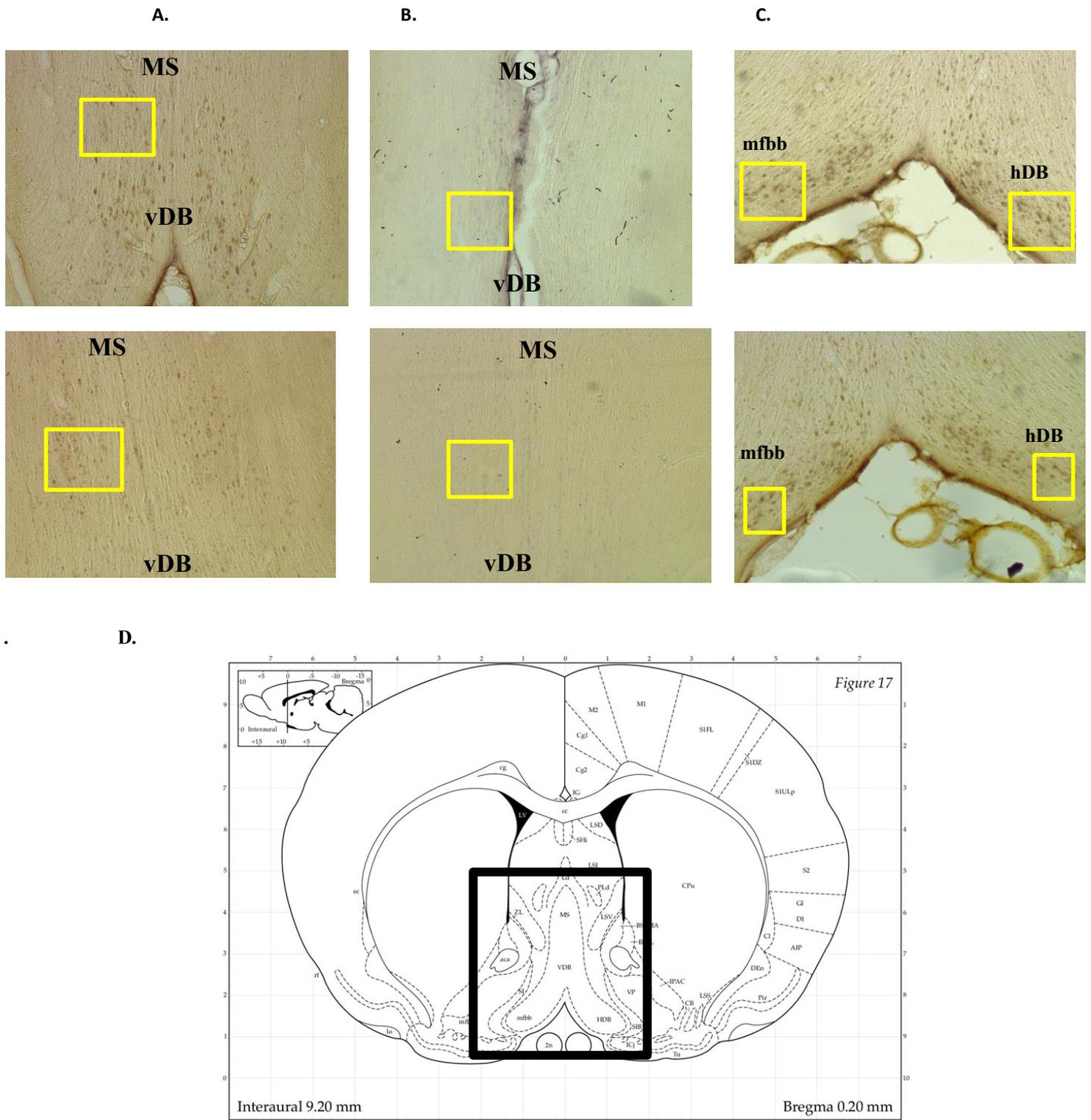
- 10** Enhanced DAB for 8 minutes

- 11** 0.3% peroxide for 8 minutes

- 12** Wash 2 x 5mins in Tris

- 13** Was 1 x 5mins in PBS

Figure 4.1. Examples of immunostained sections from the medial septum in animals from Group Sham and animals from Group MS/vDB.



Animals from Group Sham (**A**) showed dark stained cells throughout the medial septum and in the highlighted areas whilst these same cells are almost entirely absent at the same level in animals from Group MS/vDB (**B**). **C**. Shows the horizontal limb of the diagonal band and the middle forebrain bundle with high density of cholinergic cells in shams and lesions. **D**. Shows a coronal section of the MS/vDB, hDB, and the mfb, which was used to localise the cholinergic neurons (taken from: Paxinos & Watson, 2007)

4.3. Results

4.3.1. Histology

Regions of interest within the cholinergic sections were identified with reference to the rat brain anatomy atlas by Paxinos and Watson (2007), using a light microscope at 5x-40x magnification with photographs taken at 10x under consistent light conditions. Multiple photographs were taken and images were processed using Leica. For the region of the medial septum at least four photographs were taken and cholinergic neurons were identified by taking a mean colour scale of each image and identifying pixels that were darker than the average. To accurately count the number of cholinergic cells in the medial septum, the positions of regions of interests were highlighted using Leica. The slides were viewed at 40x magnification and all cholinergic cells within the boundaries of the defined MS/vDB on both sides of the brain were plotted and directly counted. Meaning cholinergic cells were counted manually by the main experimenter (and later on by an independent person who was blind to the conditions) and particularly dark stained cells were taken into consideration using one section per rat. The hDB served as control, as this region should not have been affected by the cholinergic lesions. The aim was to compare the relative number of cholinergic neurons in sham and lesioned rats, and therefore stereological correction factors were not used. Sections from animals in Groups Sham and MS/vDB are shown in Figure 4.1.

Cell counts were taken for those cells stained for choline acetyltransferase in both target regions (MS and vDB bilaterally) and non-target regions (hDB) for each rat and the mean section count was used for further analysis. Group MS/vDB showed a significant reduction in cells in the MS (mean Group Sham = 50.8; mean Group MS/vDB = 28.3; $t(8) = -5.614$, $p = 0.01$) and the vDB (mean Group Sham = 55; mean Group MS/vDB = 28; $t(8) = -2.467$, $p = 0.038$) but no reduction in cell count in hDB (mean Group Sham = 49; mean Group MS/vDB = 51.2; $t(8) = 0.254$, $p = 0.806$).

4.3.2. Behavioural measures

Memory was measured through a cumulative and average discrimination ratio (D2) (for details see section 2.2.7 and 3.3). For this experiment it was especially important to calculate both measures to detect any differences the statistics. First the average D2 can be derived by calculating the D2 for each individual trial and then averaging these to achieve a single D2 for the overall performance. In contrast, the cumulative D2 can be calculated by summing the total exploration of novel and familiar configurations over all 12 trials and then calculating a single discrimination ratio from these total explorations. Whilst average D2s (which give equal weighting to each trial) are typical in one-trial a day tasks, cumulative D2s (which give less significance to trials with small amounts of exploration) have been used when multiple trials are run in a single session (Albasser et al., 2010; Ameen-Ali, Eacott, & Easton, 2012). I typically report both measures here to allow the maximum comparison. After running one-sample t-tests (one-tailed) to compare the animals' performance to chance, exploration times (in sec) were analysed for all animals across the two tasks. Determining whether lesioned and control animals were capable of identifying the novel environmental configuration in the OLC task, was crucial for support the hypothesis outlined in the introduction of this chapter. Hence following the calculation of the average and cumulative discrimination ratio and exploration, other variables were taken in to account. To ensure that lesioned animals did not show a different spontaneous exploratory behaviour to sham rats, statistical tests were run. Average D2s and exploration times were compared between the two groups of rats in both tasks to test for the hypothesised differences due to cholinergic lesion effects and to highlight unexpected behaviour of lesioned rats. Hence, variables such as proactive interference (due to the nature of multiple trials), change in interest in the task and context repetition were analysed. Exploratory behaviour in the sample phases was observed while scoring test phases to ensure reliability, but there did not seem to be any issue despite

possible effects on motivation because of the location of the lesions (for summary of results across tasks see Table 4.1).

4.3.3. Object-location-context (OLC) task

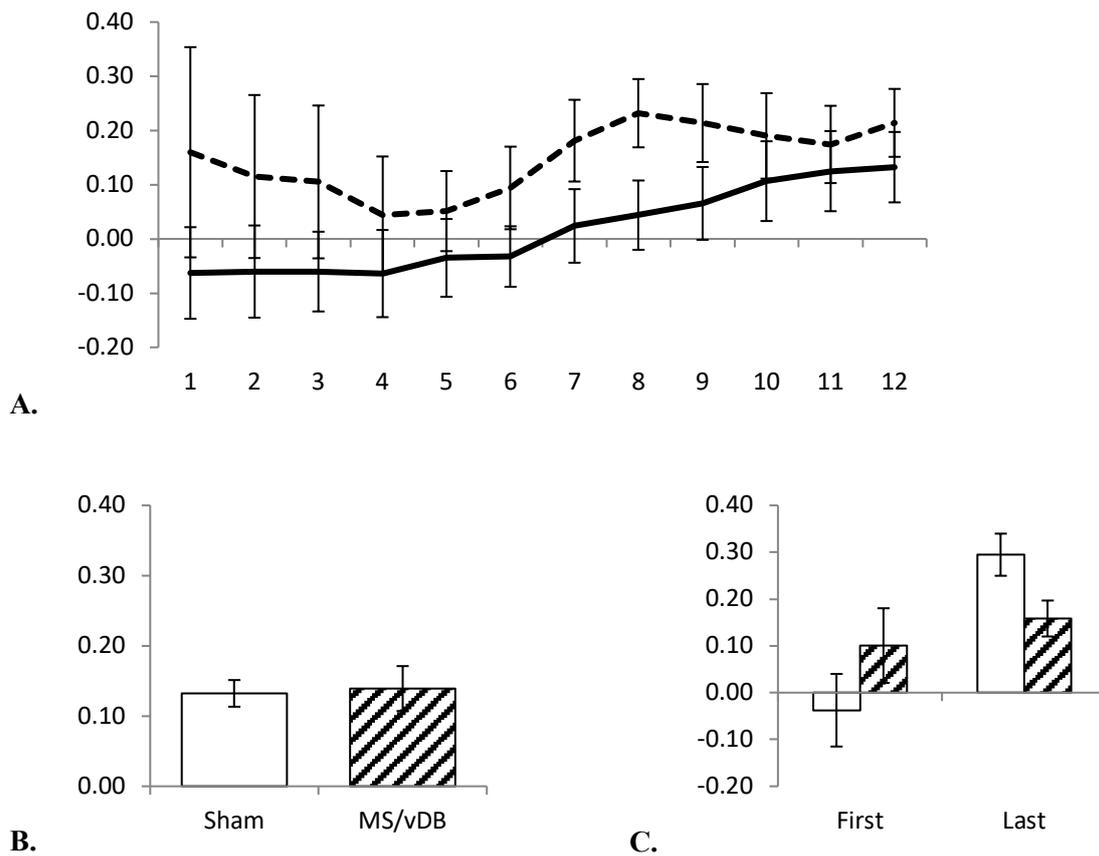
Both group Sham and group MS/vDB performed above chance on this task, showing evidence of episodic-like memory, see Figure 4.2.A/B. For both groups, the average D2 scores were significantly above chance (Group Sham mean = 0.13; $t(3) = 6.947$, $p = 0.003$; Group MS/vDB mean = 0.14; $t(5) = 4.372$, $p = 0.004$). The groups did not significantly differ from one another $t(8) < 1$.

For cumulative D2s Group Sham approached significance (mean = 0.133; $t(3) = 2.110$, $p = 0.06$) and Group MS/vDB were significantly above chance (mean = 0.214; $t(5) = 3.297$, $p = 0.01$). There was no significant difference between the groups $t(8) < 1$.

A post hoc power analysis was conducted with the program G*Power 3 (Erdfelder et al., 1996) to calculate the statistical power for this task and the difference between groups. The effect size was 0.44 (medium effect) and the calculated power was 0.15

In order to understand the effects of interference resulting from continual trials a repeated-measures ANOVA was carried out to compare the averaged D2 from the first block of two trials (where interference was lowest) with the averaged D2 from the final block of two trials (where interference was greatest) for each group, see Figure 4.2.C. The ANOVA showed no main effect of group $F(1,8) < 1$ or of block $F(1,8) = 3.10$, $p = 0.116$ and no interaction between the two $F(1,8) = 1.54$, $p = 0.25$. In addition, the final block of two trials was itself significantly above chance for both Group Sham (mean = 0.29; $t(3) = 3.27$, $p = 0.03$) and Group MS/vDB (mean = 0.16; $t(5) = 4.127$, $p = 0.005$).

Figure 4.2. OLC task after the first experience.



All error bars represent the standard error of the mean (SEM).

A. Cumulative D2 over 12 trials. Black line shows performance of Group Sham. The dotted line represents performance of Group MS/vDB.

B. Average D2 for Group Sham and Group MS/vDB over 12 trials. Both groups performed significantly better than chance and were not different from each other.

C. Performance of Group Sham (white bar) and Group MS/vDB (hatched bar) in the first and last two trials to examine effects of interference. There were no significant differences between the groups.

To see if performance changed over a testing session, D2s were separated into three blocks of four trials. Using the average D2 a 2 (group) x 3 (block) repeated ANOVA showed that there was no effect of Group $F(1, 3) = 1.666, p = 0.287$, but there was a main effect of block $F(2, 6) = 8.118, p = 0.02$. No interaction was found. There was no significant difference between Group Sham and MS/vDB ($p = 0.287$). The following block difference is therefore not driven by either group. A significant difference between block 1 and 3 was found ($p = 0.007$). The difference between block 1 and 2 was near significance ($p = 0.058$).

One session consisted of 12 trials, which means that the animal saw each of the four contexts three times at test. To see if exploration times changed over the three exposures, a repeated-measures 2 (group) x 3 (block) ANOVA was carried out. Neither a significant effect of block was found ($F(2,6) = 0.823, p = 0.48$), nor a significant group of group ($F(1,3) = 6.239, p = 0.088$) was found. There was no interaction $F(2,6) = 1.079, p = 0.398$

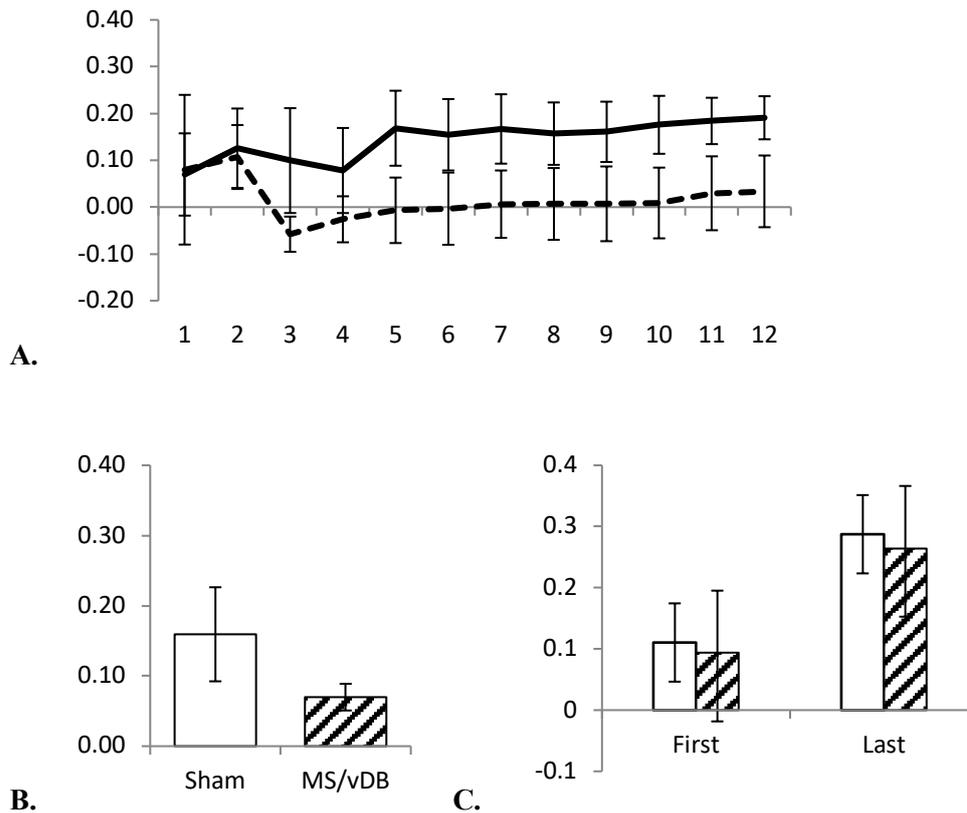
4.3.4. Location-context (LC) task

For cumulative D2 scores Group Sham was significantly above chance (mean=0.19, $t(3) = 2.489, p = 0.045$) but Group MS/vDB were not (mean = 0.03, $t(3) < 1$) showing a difference in performance on this task between the two groups (see Figure 4.3A/B). For the averaged D2s both Group Sham (mean = 0.16, $t(3) = 2.361, p = 0.05$) and Group MS/vDB (mean = 0.07, $t(3) = 3.634, p = 0.008$) were above chance, and on neither measure, was the performance of each group significantly different from one another (averaged D2s $t(8) = 1.529, p = 0.17$; cumulative D2s $t(8) = 1.88, p = 0.1$).

As in previous experiments, a post hoc power analysis was conducted using G*Power 3 (Erdfelder et al., 1996) to get the statistical power of the task and the difference between groups. The effect size was 0.9 (high effect) and the calculated power was 0.38.

In order to understand the effects of interference resulting from continual trials a repeated-measures ANOVA was carried out to compare the averaged D2 from the first block of two trials with the averaged D2 from the final block of two trials for each group, see Figure 4.3.C. The ANOVA showed no effect of block $F(1,3) = 0.023, p = 0.889$, but there was an effect of group $F(1,3) = 40.376, p = 0.008$. No interaction between the two was found ($p = 0.985$).

Figure 4.3. Performance in the LC task.



All error bars represent the SEM.

A. Cumulative D2 over the 12 trials, showing a difference between Group Sham and Group MS/vDB. Black line represents Group Sham, dotted line represents Group MS/vDB. Performance was significantly above chance for Group Sham, but not for Group MS/vDB

B. Average D2 for each group over 12 trials. Both groups performed significantly better than chance and were not different from each other.

C. Performance of Group Sham (white bar) and Group MS/vDB (hatched bar) in the first and last two trials to examine effects of interference. There was a significant difference between the groups.

Again, to see if performance changed over a testing session, the D2s were separated into three blocks of four trials. An average D2 was calculated within that block. A 2 (group) x 3 (block) repeated-measures ANOVA showed that there was no effect of Group $F(1,3) = 0.011$, $p = 0.924$ and no effect of block $F(2,6) = 0.195$, $p = 0.828$. No interaction was found. $F(2,6) = 3.450$, $p = 0.101$.

Given that all the animals saw each of the four contexts three times at test, a repeated-measures ANOVA was carried out for both groups of animals. A significant effect of block was found ($F(2,6) = 5.365, p = 0.046$), with the exploration times in each context decreasing throughout a session. There was no significant effect of group ($F(1,3) = 2.011, p = 0.251$), but a significant interaction ($F(2,6) = 8.597, p = 0.016$) was evident.

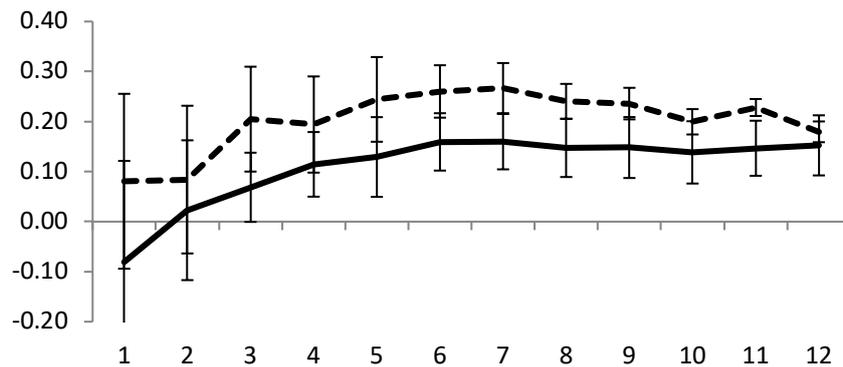
4.3.5. Repeat of OLC

To ensure that the LC result does not reflect a developing lesion over time, the animals were retested on the OLC task. Group Sham were significantly above chance on both D2 measures (averaged D2 mean=0.14, $t(3) = 5.737, p = 0.005$; cumulative D2 mean=0.15, $t(3) = 7.377, p = 0.003$). Group MS/vDB were also significantly above chance on both D2 measures (averaged D2 mean=0.12, $t(5) = 3.57, p = 0.008$; cumulative D2 mean = 0.18, $t(5) = 2.985, p = 0.016$), see Figure 4.4.A/B. The groups did not significantly differ from each other on either measure ($t(8) < 1$ for both D2 measures).

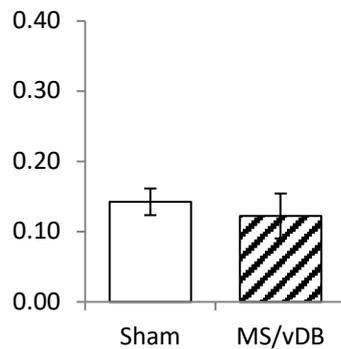
In order to understand the effects of interference resulting from continual trials a repeated-measures ANOVA was carried out to compare the averaged D2 from the first block of two trials with the averaged D2 from the final block of two trials for each group, see Figure 4.4.C. The ANOVA showed no effect of block $F(1,3) = 0.181, p = 0.699$, no effect of group $F(1,3) = 0.006, p = 0.941$ and no interaction $F(1,3) = 1.790, p = 0.273$.

Figure 4.4. OLC after second experience.

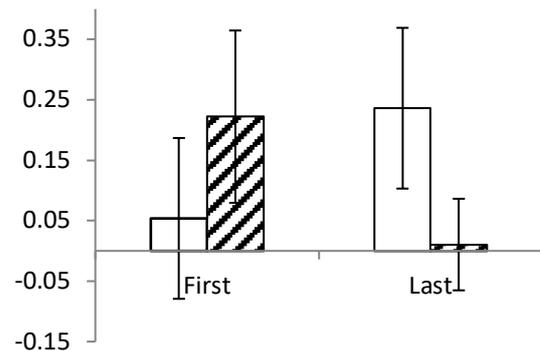
A.



B.



C.



All error bars represent the SEM.

A. Cumulative D2 over 12 trials. Black line represents the performance of Group Sham, dotted line represents the performance of Group MS/vDB. Performance was significantly above chance for both groups.

B. Average D2 for each group over 12 trials. Both groups performed significantly better than chance, and were not significantly different from each other.

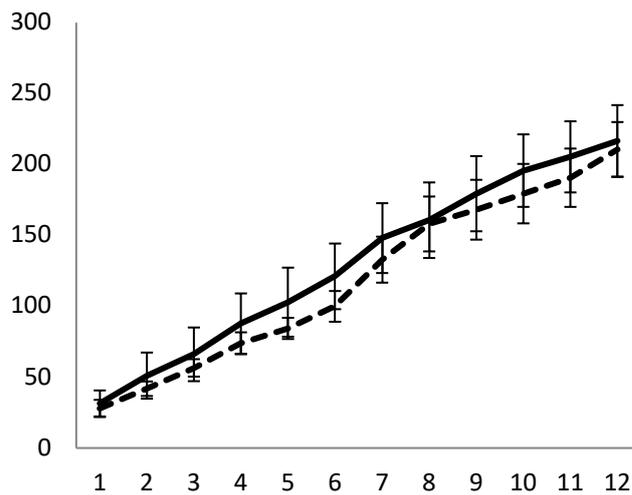
C. Performance of Group Sham (white bar) and Group MS/vDB (hatched bar) in the first and last two trials to examine effects of interference. There was no difference between the groups.

To see if performance changed over a testing session, individual D2 scores were separated into three blocks of four trials. An average D2 score was calculated within that block. A 2 (group) x 3 (block) repeated-measures ANOVA showed that there was no effect of block $F(2,6) = 0.135$, $p = 0.877$. However, there was an effect of group $F(1,3) = 11.821$, $p = 0.041$, with the MS/vDB (mean = 0.189) performing better than group Sham (mean = 0.14). No interaction was found $F(2,6) = 1.640$, $p = 0.270$.

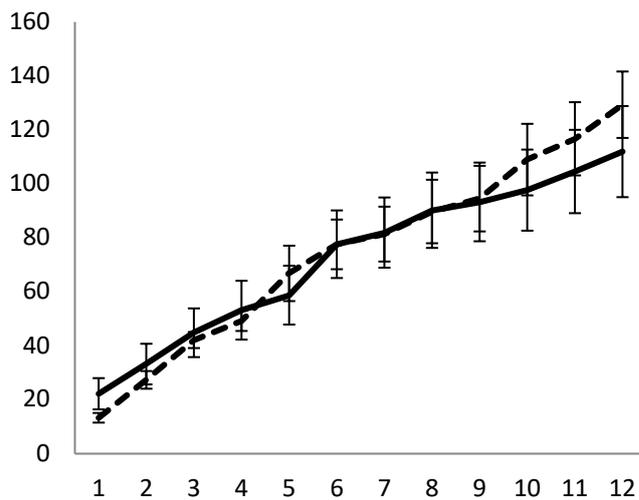
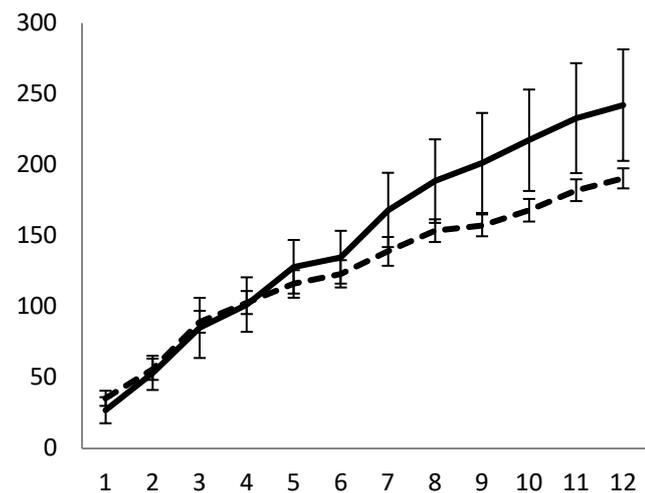
As previously, to see if exploration times change over the three exposures to each context, a repeated 2x3 ANOVA was done. Neither a main effect of block ($F(2,6) = 1.412, p = 0.314$), nor a main effect of group ($F(1,3) = 1.003, p = 0.390$) was evident. However, there was a significant interaction $F(2,6) = 9.680, p = 0.013$, suggesting that there was a different in the pattern of exploration.

Figure 4.5. Cumulative exploration (in sec) over 12 trials

A.



B.



C.

A. First experience of OLC. Graph shows the cumulative exploration of Group Sham (black) and Group MS/vDB. (dotted) Animals showed a significant change in exploration times over a testing session.
B. Exploration in LC. Both groups showed a decrease in their exploration times. However, this was only near significance
C. Second experience of OLC. Graph shows the cumulative exploration of Group Sham (black) and Group MS/vDB (dotted). Both groups showed a significant change in exploration.

Table 4.1. Summary table of the results in Study 3 across the two tasks in lesioned and sham animals.

| | Average D2 | | Cumulative D2 | | Proactive interference (D2) | | | | Cumulative exploration (sec) | |
|-------------|----------------|----------------|----------------|----------------|-----------------------------|----------------|--------------------|----------------|------------------------------|-----------------|
| | <u>Sham</u> | <u>MS/vDB</u> | <u>Sham</u> | <u>MS/vDB</u> | <u>First Trials</u> | | <u>Last Trials</u> | | <u>Sham</u> | <u>MS/vDB</u> |
| sOLC | 0.13 (0.08) | 0.14 (0.03) | 0.13 (0.06) | 0.21 (0.06) | <u>Sham</u> | <u>MS/vDB</u> | <u>Sham</u> | <u>MS/vDB</u> | 216.6 (25.2) | 210.6 (19.4) |
| | | | | | -0.03 (0.16) | 0.10 (0.08) | 0.29 (0.09) | 0.16 (0.04) | | |
| uLC | <u>Sham</u> | <u>MS/vDB</u> | <u>Sham</u> | <u>MS/vDB</u> | <u>First Trials</u> | | <u>Last Trials</u> | | <u>Sham</u> | <u>MS/vDB</u> |
| | 0.16 (0.06) | 0.07 (0.02) | 0.19 (0.07) | 0.03 (0.04) | <u>Sham</u> | <u>MS/vDB</u> | <u>Sham</u> | <u>MS/vDB</u> | 242.2 (39.2) | 190.5 (7.1) |
| | | | | | 0.11 (0.06) | 0.09 (0.10) | 0.29 (0.07) | 0.26 (0.11) | | |

This table summarises the main findings of the effects across the stable OLC and unstable LC in Study 3. Numbers in brackets represent SEM.

4.5. Discussion

Animals with immunotoxic lesions of cholinergic neurons in the MS/VDB were tested on tasks of episodic-like and context-place memory (see Table 4.1 for summary). The current study replicated the earlier findings of Easton et al. (2011), which used a one-trial day approach to investigate the effects of cholinergic lesions on memory. By using continual trials, it was found that depletion of the cholinergic input to the hippocampus did not impair episodic-like memory, but did impair spatial-contextual memory.

Continual trials were analysed using cumulative D2s, which was calculated from the total exploration of novel and familiar items over all trials in one testing session (see Ameen-Ali et al., 2012). This statistical analysis is based on work of Albasser et al. (2010) who used cumulative (or updated) scores to assess spontaneous recognition memory of rats in a bow-tie maze. Advantages of using the cumulative D2 over the average D2 are that all 12 trials happen within the same day over a time span of two hours and animals are handled less

between phases which means that the condition between trials are more consistent. However, cumulative D2 is very sensitive to interference and habituation. Given that many trials are run in a short period of time, an animal's exploration may drop because they get confused or habituated to the procedure. The drawback to this is that lower exploration times towards the end of a testing session will reduce the weighting of the overall performance. Therefore, using the average D2 is beneficial when it is important that every trial has the same weighting. Individual variations in performance are balanced out when trials are run over many different days. Nevertheless, the average D2 may not give appropriate weighting when exploration times vary in different situations. If exploration is low then the average D2 will treat these trials in the same way as if the exploration was high. Hence studies in this thesis report the cumulative and average D2 to develop a better understanding of the data.

The OLC task requires an intact fornix (Eacott & Norman, 2004) and hippocampus (Langston & Wood, 2010). Since cholinergic projections travel predominantly via the fornix it could have been possible that this input is crucial for episodic-like memory. However, the results showed that the object-location-context task was not affected by cholinergic lesions of the MS/vDB, suggesting that many memory functions of the hippocampus survive the loss of cholinergic input. It should be kept in mind that the memory load in a continuous trials apparatus is higher than in the original open field. The current task may have introduced more interference because multiple trials were run and on each trial two contexts were used. This means that over 12 trials, the four contexts were repeated three times in the test phase and caused much overlap. On each trial, there are two different contexts used and only five contexts are available overall. In every block of three trials at least two contexts will be introduced. Therefore, later trials may be more demanding than earlier trials, because rats bring earlier memories to the new trial. In this apparatus, the context is being used to identify the occasion and there will be more interference. Some studies (such as Albasser et al., 2010)

have found a build-up of proactive interference, because previously stored associations interfere with new associations. Theories of cholinergic function propose that the cholinergic system is necessary to allow separation between recollections of similar events (see Hasselmo, 2006), but there was no evidence of this in the current study. The lack of impairment was also not caused by ineffective lesions, build-up of lesions, or recovery function, as animals were tested again on the OLC task after the LC task. Intact episodic-like memory was shown before and after the impaired LC. To investigate if performance changed over a testing session, average D2 scores were separated into blocks. Shams did not show an effect of block, even though they did perform better towards the end of a testing session. On the other hand, MS/vDB did show a significant effect of block. This could be an indication that animals need a few trials to get accustomed to the maze and the testing procedure.

Whereas there was no impairment in the OLC task, impairment was found in the LC task, which supports Easton et al.'s (2011) findings. However, an impairment was only seen when the data were analysed using the cumulative D2s and not the average D2. This suggests that interference from multiple trials in this task did not make it more difficult for the lesioned animals than the object-context-location task. Cholinergic input to the hippocampus is not required to overcome additional interference. Hence, the nature of the spatial information presented may be crucial. Previous work on the depletion of the cholinergic input to the hippocampus was interpreted in relation to pattern completion (see Easton et al., 2011). In the beginning, it was hypothesised that a reduction in pattern separation as a result of reduced ACh in the hippocampus would produce impairment in the episodic memory (OLC) task. However, this study showed that a continuous trials approach had no impact on the effect of cholinergic depletion of the medial septum. Pattern separation models of acetylcholine function can still explain some deficits (such as the deficit in the location-context task), but not for all types of memories. In OLC objects are always on the left and the right when the

animal enters the arena, where in LC the location of items changes. In OLC animals will find objects in fixed positions, but in LC when objects are in a location where they were not expected it leads to confusion and impaired memory. Impairment in the LC task is less clear in the continual trials task than it was in the one-trial a day task. In the continual trials approach the same three locations are filled over the course of a trial, and every trial reuses the same three locations. It is possible that animals build up an expectation of where objects are going to be in the OLC as well as in the LC task. Having consecutive trials allows animals to learn the three locations in the LC task and become less confused. Performance may still be impaired, but not as much as when filled locations of objects are harder to predict. Unlike the OLC task when the average D2 were separated into three blocks, no effect was found. Shams performed well throughout a session. Group MS/vDB was not expected to show any change in their discrimination and they did in fact not show an effect of block, suggesting that animals habituated well to the procedure and apparatus.

In conclusion, the continual trials version of an episodic memory task is unimpaired by cholinergic lesions of the medial septum. However, the continual trial version of a location-context task is impaired in the same animals. These results replicate the effects of lesions on one-trial a day versions of the same tasks. Increased interference or overlap between events has no effect on the rodents' behaviour. This chapter replicated an earlier finding by Easton et al. (2011) in an apparatus where multiple trials are run in a single session, meaning a significant (approx. 50%) reduction in animal numbers. The findings demonstrate the reliability of the dissociation within the hippocampus based on cholinergic function within the hippocampus, and verifies the new apparatus as assessing episodic-like memory in the same manner as earlier studies, improving the reliability of the task and having a significant 3Rs benefit.

Chapter 5

Study 4: Recollection-like Processes in an Alternative Version of the Object-Location-Context Task

5.1. Introduction

Novel object and location recognition tasks are simple behavioural tests to assess rodents' innate exploratory behaviour. Their preference for novelty is expressed by greater exploration of the novel configuration. As detailed in the introduction (Chapter 1), recollection and familiarity have been dissociated as two distinct memory processes. For example, you might recognize a person as familiar but you are unable to recollect who the person is or where you have met them before. Recollection on the other hand brings back details of this experience.

One problem with the SOR tasks is that it is difficult to differentiate between recognition based on familiarity or recognition based on recollection. It has been argued that the location-context task can be solved using familiarity only, which would not be episodic-like (Easton & Eacott, 2010). Rodents spending more time with a novel object compared to an old object might simply do so because the new configuration appears less familiar. However, there is some evidence that mice use recollection-like processes to discriminate the temporal order in which objects have been presented (see Zlomuzica, Dere & Dere, 2013; Davis et al., 2013). Remembering the temporal order of events is seen as feature of episodic memory by some researchers and in Zlomuzica et al.'s study (2013), H1R-KO and control mice were examined in a temporal object memory task. Performance of control animals implied that they used a recollection-like discrimination strategy in seeking out the familiar objects of the temporal order sequence, because memory traces are strengthened when an event is reinforced (based on the dual-process theory put forward by Yonelinas (2002)). Using the broader definition of episodic-like memory (see Chapter 1) animals can be assessed on tasks for objects in specific

places and within different contexts. In particular scene memory in monkeys has been considered to be similar to episodic-like memory (Gaffan, 1994). In Gaffan's task animals learn about the location of specific objects within unique backgrounds. This task relies on the fornix (Gaffan, 1994), which is important for episodic memory. However, scene learning requires learning and pre-training, which is not episodic-like. Eacott and Norman's (2004) object-location-context task on the other hand relies upon rats' innate preference for novel configurations (see Chapter 1 for details) and has proven to be a useful task to assess episodic memory in rodents. It might be argued that object-location-context tasks can be solved on the basis of familiarity alone. However, we know that in humans, episodic memory relies on the recollection of past events (Tulving, 1983) and therefore the same could apply to animals. Literature would suggest that contextual retrieval is part of episodic memory in humans (see Perrson, Ainge, & O'Connor, 2016), but the association of only objects in contexts does not mirror episodic memory in animals (Norman & Eacott, 2005). An OC association alone does not trigger as much recollection as an OLC combination in humans (see Ameen-Ali et al., 2017). Object-context and object-location context can be distinguished in animals, as the OC task is not seen as episodic, whilst the object-location-context task is episodic in nature and therefore is more likely to use recollection-like processes (Eacott & Gaffan, 2005). Eacott and Gaffan (2005) have argued that OC does not have to be solved using an episodic mechanism, whilst OLC does. However, there is no reason to believe that the OC task could not be solved using recollection, as it has been shown to be the case in humans (see Perrson et al., 2016).

Novelty preference paradigms require the presentation of objects to animals in order to measure the discrimination and the presence of objects during the test trial might be in itself a source of familiarity like processes. Eacott, Easton and Zinkivskay (2005) demonstrated recollection processes in an episodic task using a modification of the 'what-

where-which' task. In an E-shaped maze (Figure 1.6 in Chapter 1), rats were exposed to two different objects in specific locations and a particular context. They were then presented with copies of the previously seen objects in swapped locations and a different context. Rats were also held in a holding cage with a copy of one of the object, which gave them time to habituate to it. After a delay animals were returned to the maze for the test phase and they were exposed to one of the previously seen contexts and copies of the two objects presented in the same location as before. When the objects were in sight, rats preferentially explored the object that was not presented to them in the holding area. When the objects were out of sight from the start arm, the rats turned towards the non-habituated object in the test phase. When the objects were visible animals could have shown a preference for the non-habituated object based on familiarity only. However, when the objects were not visible, the rat had to recollect their prior experience of the object's location, which means that their decision to turn to the non-habituated object must be based on recollection.

The aim of the current study was to measure memory performance on the original episodic-like (what-where-which or object-location-context) memory task without the presence of some objects at test. A slightly modified version of this task allows me to assess the memory for location of objects in unstable conditions within given contexts across multiple events. Based on previous research and our own observations in the lab the current study was a follow-up of study 2 (Chapter 3). It was found that in the unstable object-location-context task, animals tended to revisit the location where an object used to be in a specific context at sample phase. In the usual novelty preference paradigm objects are present at every sample and test phase to measure the discrimination ratio. However, the pure presence of an object at test may trigger familiarity-like processes and not recollection. Based on the finding in Chapter 3, it should be possible to test an animal's memory for the history

of object-location-context configurations. This can be achieved by using the unstable OLC task, because during the test trial object C is no longer present. For example, if object C was in the top right corner in context X in the first sample phase and in the bottom right corner in the second sample phase in context Y, rats would explore the top right location at test in context X (Figure 5.1, Material & Method in Chapter 5). This suggests that animals can discriminate between many different objects in different contexts. Using recollection, they remember where an object was placed previously by exploring that location. It could be argued that this behaviour is driven by the odour of the open field. To avoid any odour traces, the floors of the contexts were separated from the walls, which means that they could be rotated. If the animal were following the odour of the object then when the floor is rotated the animal will not preferentially explore the previous location of the object at test but instead would explore the region of the odour trace, which has now been rotated to a new position. However, if the animal were recollecting where the object was in the sample phase, then we would expect it to explore the location of C even when the floor is rotated. Overall, this study sought to determine whether it is possible to assess an object-location-context task at the same time as a location-context task and to show that animals use recollection like processes in this preference paradigm.

5.2. Materials and Method

5.2.1. Subjects

Eight naïve Lister hooded rats supplied by Harlan were used in this experiment. They were housed in groups of four in rooms maintained on a 12hr light/dark cycle. Testing was carried out during the light phase and water was available throughout the day. Animals were food restricted to 85% of their free-feeding body weight of age matched controls. Animals started testing when they were 9 weeks old.

5.2.2. Apparatus and objects

The animals were tested in the same square-shaped open field as for previous studies (2-4). For details refer to study 2 in Chapter 3. Inserting four different contexts could change the features of the apparatus (see Figure 3.1). Following careful counterbalancing of the testing schedule, objects were picked based on previously explained criteria (see Figure 2.1 and Chapter 3.2.2). The camera placed above the maze allowed offline scoring of the animals' exploratory behaviour for analysis.

5.2.3. Habituation and pre-training

As previously, animals were handled daily for three days prior to habituation. Pre-training involved four phases and habituation was carried out in the same way as in study 2 (Chapter 3). Behavioural testing took place in a separate room under dim white light and white noise in the background to cover environmental noise.

5.2.4. Test protocol

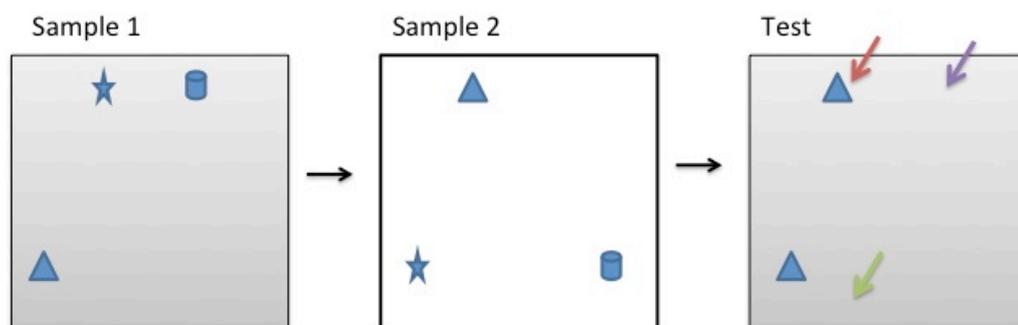
Animals were tested on one task of episodic memory (unstable object-location-context). A testing session consisted of 12 trials (lasting two hours), and animals repeated the same task three times over three weeks. Normally, rats were tested between 8am and 12pm and between 1pm and 5pm. It was ensured that each rat was tested at the same time of day in each week. For example, rat 1 was tested 8-10am on Monday in week 1, followed by another testing session on the same day and at the same time in week 2 and then again in week 3 (ensuring that there were always seven days in between testing sessions for individual animals). Animals were divided in to two groups: rotation and no rotation. In the first week, all animals were exposed to the unstable OLC task without rotating floors. In week 2 half of the animals encountered the 'rotation' followed by the 'no rotation' in week 3. The other half encountered the 'no rotation' in week 2, followed by 'rotation' in week 3.

At the start of each session, the animal was placed in the holding area. The door would then open to allow the animal to move to the testing area. In both exposure phases and the test phase animals were given 2 min of exploration. Between phases rats were in the holding area while the arena was changed. The rotation of the floors took place between the second sample phase and test. The floors were separate from the walls, which allowed rotations at 90 or 180 degrees in either direction (Figure 5.1). As before, objects were baited with food pellets to encourage exploration and movement between the compartments. Exploration was taken when the animal was at a distance of 1cm of the object and actively exploring it. The duration of exploration was measured off-line by holding down a key pad on the computer. The testing contexts, the novel object and placement of the novel object were counterbalanced.

5.2.5. Unstable object-location-context (unstable OLC)

This task was carried out as previously described in study 2 (Chapter 3). Given the nature of this task it is also possible to test context-location memory (unstable LC). To see if the exploration of object C was driven by the residual odour of the object, or memory of the previous location of C in a specific context, the floor inserts were rotated. The rotation was counterbalanced within and across animals – rotations were at 90 or 180 degrees in either direction.

Figure 5.1. Demonstration of the unstable object-location-context task.

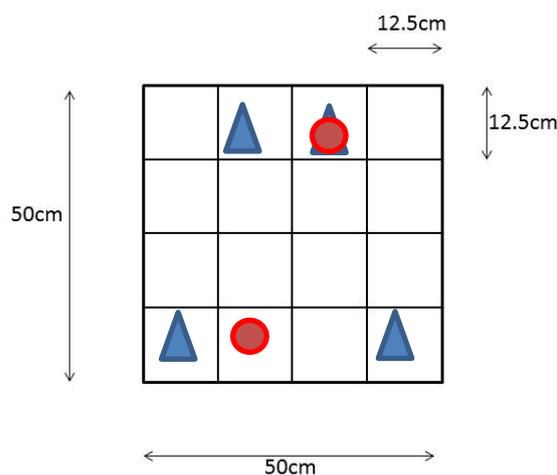


Rats encounter three objects in sample 1 in three different locations. In sample 2 the same three objects change locations. At test one object is removed and one pair of objects is shown. The red arrow shows the novel object-location-context configuration. The green arrow shows where the animal would be exploring if it followed the odour trace of the object from sample 1 (floor was rotated 180 degrees). The purple arrow shows the location where the object used to be in that context.

Red = uOLC, Purple = uLC, Green = odour of object C when rotated 180degree

The average and cumulative D2 scores were calculated (Chapter 3.3) D2 scores for the unstable OLC task were calculated by dividing the D1 score by the total exploration. D2 scores for the unstable OLC task were calculated by dividing the D1 score by the total exploration time. For example, in Figure 5.1, this would be the discrimination ratio between the triangles in the test phase The D2 scores for the unstable LC task were calculated in the same way; however, at test phase unoccupied locations were scored. The D2 for the uLC was made up of the location of the object based on the sample phase and the location of the odour. For example, in Figure 5.1, the uLC D2 score would be made up of scoring the exploration of the area in the test phase where the green arrow (odour of the cylinder based on its location in sample 1 after rotation) and purple arrow (physical location where the cylinder was in sample 1) is. For calculation purposes in this example, the green arrow represents the ‘novel exploration’, and the purple arrow the ‘familiar exploration’. Hence, in both cases it was measured how long animals were exploring empty spaces, but only one of them was purely based on memory (defined as uLC). Floors were divided up into different quadrants and the time spent in those was measured (Figure 5.2). Sniffing in this area was counted as exploration and as a demonstration that the animals remembered this specific location.

Figure 5.2. Dimensions of the apparatus when it was divided into quadrants.



The floor of the apparatus was divided up into quadrants, which measured 12.5cm x 12.5cm. Times spent in these quadrants were scored off-line.

Based on **Figure 5.1** -Triangles (and circles) represent the locations of objects and the locations which were scored. Circles represent the odour of where an object could have been after rotating and where it was originally.

5.3. Results

One animal was not included in the data analysis, because it failed to shuttle reliably towards the end of a session. Therefore, the analysis is based on the remaining seven animals.

The following questions were of interest in this study and will be analysed in the results: do rats remember an object's location without its physical presence? Can we use the unstable OLC task to assess the unstable LC task? Overall, by scoring all possible exploration times, rats spent more time with the actual objects, but the discrimination ratios were reliable enough to produce a comprehensive data set. On average, rats spent 20 seconds exploring the novel and familiar objects in the unstable OLC, and 2 seconds exploring empty spaces in the unstable LC.

5.3.1. Unstable OLC

5.3.1.1. Average D2 scores & cumulative D2 scores

Seven rats received a total of 36 trials of the unstable object-location-context task over three weeks, with 12 trials run within a single session each week. To determine if performance of the animals was above chance, one sample t-tests were carried out (one-tailed) and average and cumulative D2s were compared against zero.

When the three weeks of unstable OLC were combined the results were highly significant, demonstrating reliable object-location-context discrimination as measured by the D2 ratio $t(20) = 5.505$, $p < 0.001$. The cumulative D2 was 0.21. The combined average D2 was also significant $t(20) = 6.008$, $p < 0.001$ with the same D2 of 0.21. However, a week by week analysis showed clear differences in performance (see Figure 5.3). A one-way ANOVA by week revealed a significant difference between the D2s in week 2 and 3 of testing (Bonferroni, $p = 0.027$).

In week 1 average D2 scores were significantly above chance, showing a clear preference for the novel object-location-context configuration. Cumulative D2 scores showed

a similar trend. Over the 12 trials animals showed a clear preference for the novel configuration. The average ($t(6) = 4.930, p = 0.002$) and cumulative ($t(6) = 5.482, p = 0.001$) D2 scores were similar (0.219 and 0.221, respectively).

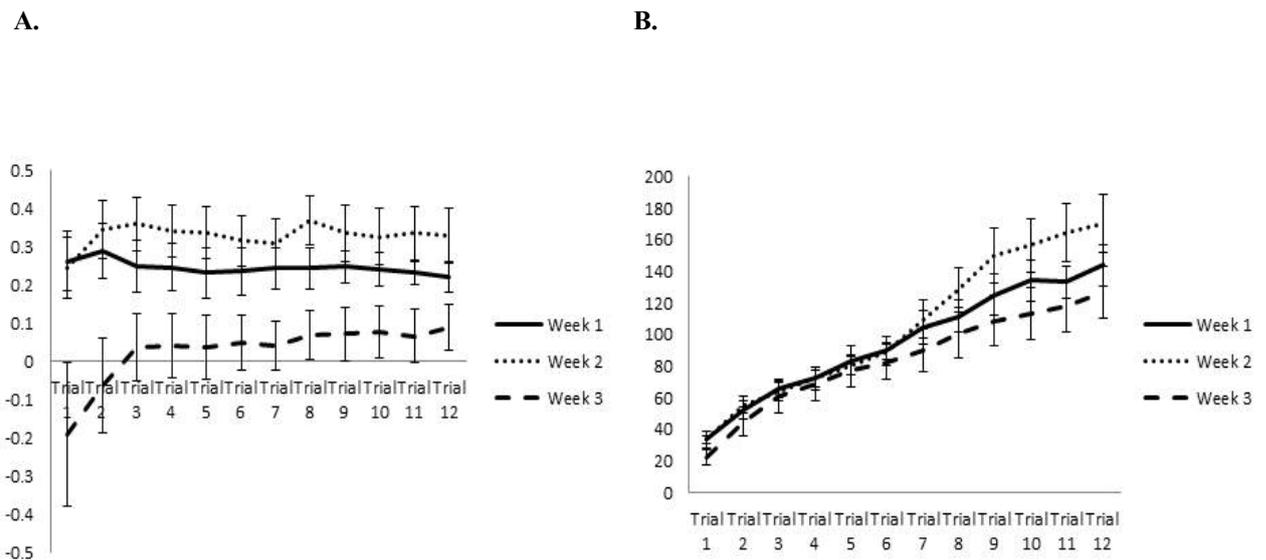
In the second week, another 12 trials of unstable OLC followed and the results are comparable to the first week's performance. The average D2 was 0.317 and animals significantly discriminated between objects $t(6) = 4.986, p = 0.001$. The cumulative D2 was 0.329 and animals demonstrated clear memory for the novel object configuration $t(6) = 4.618, p = 0.002$.

However, in the third week of testing animals D2s dropped drastically for the average (0.086) as well as the cumulative score (0.089). The average D2 was significant $t(6) = 2.279, p = 0.031$, but the cumulative score did not approach significance $t(6) = 1.515, p = 0.091$.

5.3.1.2. Cumulative exploration times

Exploration of the rats was compared to determine if performance could have been affected by running the same task multiple times. Involvement in the same task, could have affected the exploration during the last week of testing. The results showed no significant difference between the exploration rates over three weeks $F(2,35) = 1.395, p = 0.262$. Therefore, while the rate of exploration did decrease over the three sessions and any decrease in performance can affect memory, this is unlikely to explain the poor performance in Week 3 (Figure 5.3).

Figure 5.3. Cumulative D2 ratios and exploration rats over three weeks of testing in unstable OLC.



A. Shows the difference in cumulative D2 scores over 12 trials over the three different weeks. However, in the third week animals' performance started very low and remained close to chance. **B.** Shows the cumulative exploration (in sec) over 12 trials over three weeks, which remained constant.

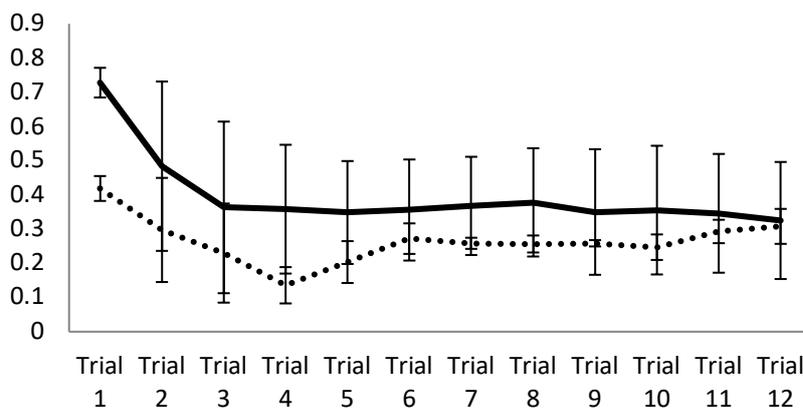
5.3.2. Unstable LC

5.3.2.1. Average D2 scores & cumulative D2 scores

The main interest of this study was to see if rats can recall an object's previous location without its actual presence at test. The unstable OLC task was used to answer this question, because it allows us to investigate the effect of the disappearance of an object (Figure 5.1). Overall seven rats received 36 trials of the unstable OLC task over three weeks and within this task was an unstable LC task. In the first week of unstable LC no rotations of the floor took place and as observed in a previous experiment rats were able to determine the prior location of an object $t(6) = 3.343$, $p = 0.01$ (one-tailed) (Figure 5.4). However, this recollection of location could be caused by the odour traces of objects. To investigate this, animals were split in to two groups – four rats encountered the 'rotation' condition in week 2, followed by 'no rotation' in week 3; three rats encountered the 'no rotation' condition in week 2, followed by 'rotation' in week 3.

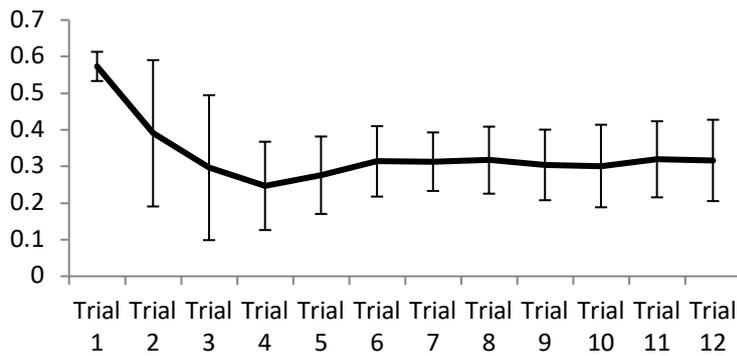
To determine if performance of animals was above chance in the location-context task both weeks were combined and one sample t-tests were carried out (one-tailed) (Figure 5.5). The average D2 score was significantly above chance $t(6) = 3.980, p = 0.003; D2 = 0.325$. Furthermore, the cumulative D2 was calculated for comparison: $t(6) = 3.397, p = 0.008; D2 = 0.318$. To investigate this significance further a paired t-test was used to see if there was a difference between the rotation and no rotation condition. When both weeks were collapsed (as it does not make a difference to the research question if rats performed better in the second or third week) it was shown that there was no significant difference between the rotation ($D2 = 0.34$) and no rotation ($D2 = 0.53$) condition $t(6) = -1.579, p = 0.166$. This demonstrates that rats are doing both conditions by memory and not only by odour cues.

Figure 5.4. Cumulative D2 in the unstable LC during rotation.



This graph shows the discrimination ratio of all rats in the unstable LC task (cumulative D2). The shape of the curve is different to the unstable OLC task. In the LC task animals demonstrated a very good memory for the history of an object's presence in the first trial, but it then decreased until it remained at a constant discrimination ratio level. Black line=Rats 3, 4, 7, 8. Dotted line=1, 2, 6

Figure 5.5. Cumulative D2 in the unstable LC during rotation when both weeks of testing were combined.

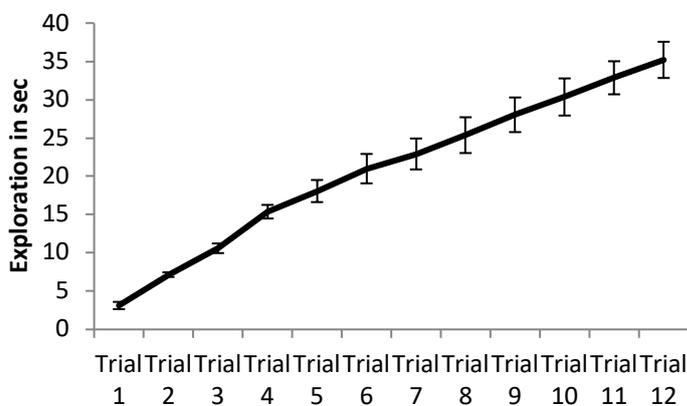


Rats demonstrated a very good memory for the non-presence of an object in the first trial, but it then decreased until it remained at a constant level.

5.3.2.2. Cumulative exploration times

Animals remain interested in the task, as the exploration times continue to increase (week 2 and 3). While it was not predicted that the animals would spend large amounts of time exploring a position in which there was no object present, memory for the previous occasion could be demonstrated by a relatively small increase in time spent exploring this position over unoccupied positions (Figure 5.6).

Figure 5.6. Cumulative Exploration in unstable LC



Exploration rates (sec) increased throughout the session, suggesting that animals remained interested in the environmental configuration. Error bars represent SEM.

5.3.3. Effect of rotation on OLC and LC

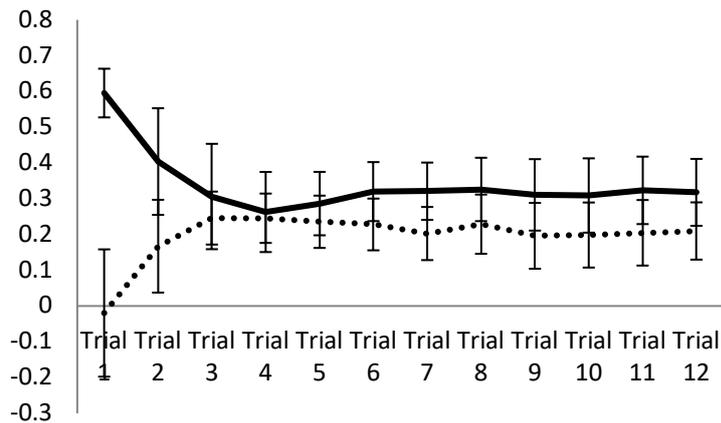
In order to investigate the effects of rotating the floor of the maze on performance in the unstable OLC and LC tasks multiple statistical analyses were run (Figure 5.9). Given previous results in this chapter it could have been possible that rotation affected one task more than the other.

To determine if animals' performance was affected by the rotation of the floor, separate t-tests (one tailed) were run using the average D2s. Animals could be above chance without rotation and at chance with rotation, but rats significantly explored the novel object-context-place configuration in both conditions in both tasks: OLC-rotation $t(6) = 2.388$, $p = 0.03$; OLC-no rotation $t(6) = 3.526$, $p = 0.01$; LC-rotation $t(6) = 3.980$, $p = 0.004$; LC-no rotation $t(6) = 8.881$, $p < 0.001$.

A repeated measures ANOVA (rotation x task) showed that there was no significant effect of task $F(1,6) = 0.986$, $p = 0.359$, but there was a significant effect of rotation $F(1,6) = 28.182$, $p = 0.002$. No interaction was found $F(1,66) = 0.258$, $p = 0.630$. A paired t-test showed that there was no significant difference between OLC ($D2 = 0.184$) and LC ($D2 = 0.325$) when the floors were rotated ($t(6) = 2.185$, $p = 0.072$), but a significant difference between OLC ($D2 = 0.219$) and LC ($D2 = 0.418$) tasks when the floors were not rotated ($t(6) = 2.975$, $p = 0.025$). This suggests that rotation of the floor affected the OLC task more than the LC task.

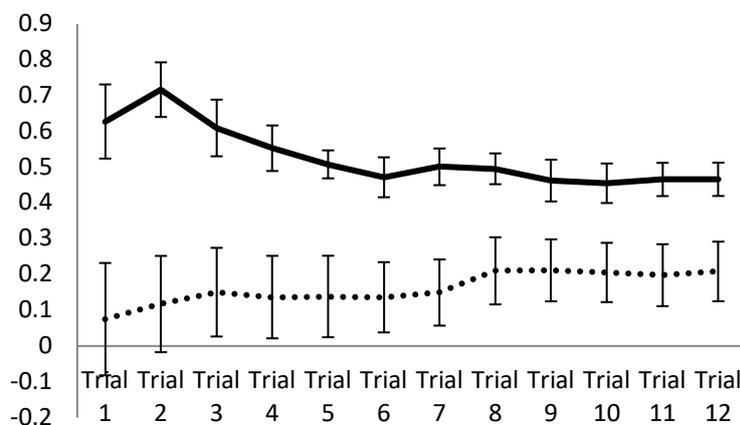
Investigating this effect further, in the figure 5.7 and 5.8 it can be seen that the group of animals that had no rotating floors showed that performance started low in the unstable OLC task compared to the unstable LC, but animals' performance drastically increased on the second trial. This is the opposite of what happened when the maze was rotated. Another steep increase was seen on trial 8. Overall the performances of animals that do not rotate show a good performance throughout both tasks.

Figure 5.7. Cumulative D2 during rotation in LC and OLC.



This graph shows the cumulative D2 score of the unstable LC task and the unstable OLC task over 12 trials for both groups. The unstable LC started with a very high discrimination ratio which then decreased but remained very good and stable throughout the task. The unstable OLC started off at a lower discrimination ratio than the LC task, but after three trials the performance remained constant. Black line=LC; Dotted line=OLC

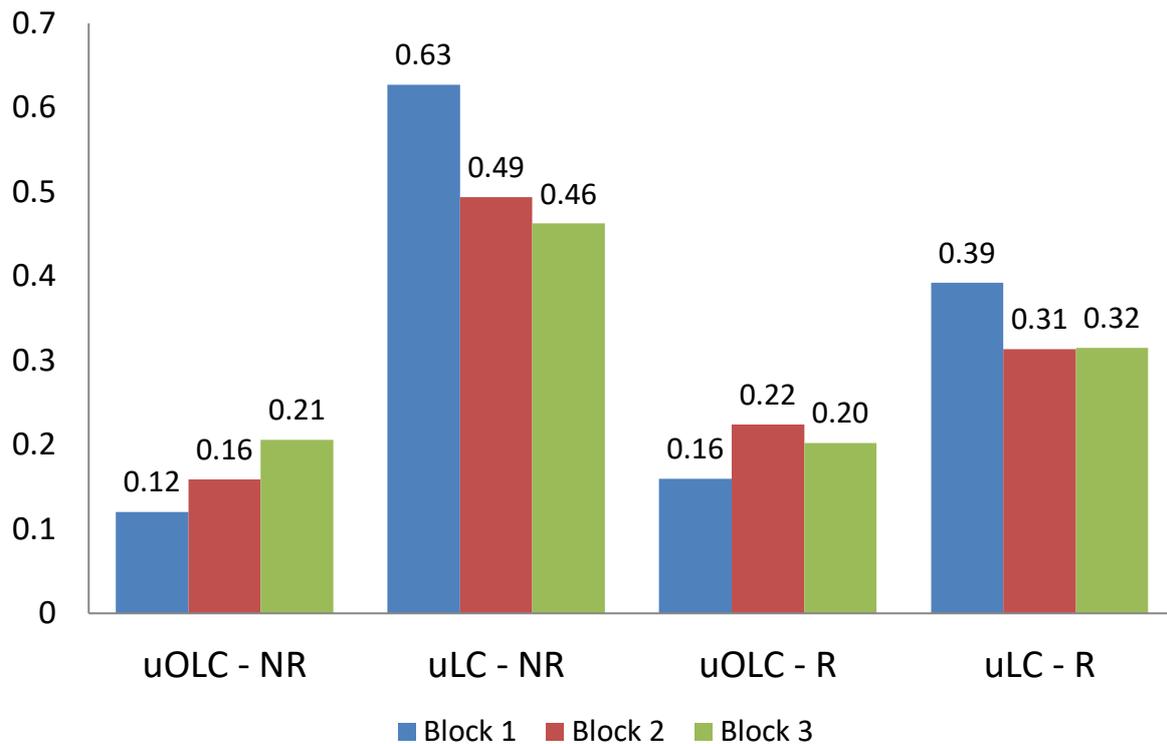
Figure 5.8. Cumulative D2 during no rotation in LC and OLC.



This graph shows the cumulative D2 score of the unstable LC task and the unstable OLC task over 12 trials for both groups when the floor was not rotated. The results were similar to the Rotation condition. The unstable LC task started with a very high D2, whereas the unstable OLC started off with a lower D2. Black line=LC; Dotted line=OLC

When the data were analysed further it was noticed that there could be a correlation between the performance (as measured by the D2 ratio) in the uOLC and uLC tasks. Indeed, there was a relatively strong positive correlation between the unstable OLC average D2 and the unstable LC average D2 in the first week (0.3, $p = 0.009$). There was also a strong positive correlation between the two scores in week 2 (0.5, $p = 0.001$). No correlation was found in the third week (0.1, $p = 0.643$) and in week 3 animals did not perform above chance on the uOLC task, suggesting that one task influenced the performance of the other.

Figure 5.9. Summary of average D2s divided up in blocks in the rotation and no rotation condition.



NR = no rotation; R = rotation; uOLC = unstable OLC; uLC = unstable LC

This graph provides a summary of the average D2 scores across all tasks (divided up in three blocks of four trials) and conditions for comparison.

5.4. Discussion

The standard object-location-context task also tests memory for the history of configurations, however, the primary aim of this experiment was to determine whether rats could show memory for the non-present objects in this task. In the unstable OLC task animals encounter locations within an environment where an object used to be present. I aimed to measure performance while the objects to be remembered were absent during the test trial. It was found that it is possible to assess an object-location-context task at the same time as a location-context task and this also confirms that animals use recollection-like processes in these preference paradigms. By investigating the effects of rotating the floors of the apparatus on the object-location-context and the location-context task it was demonstrated that performance in recognition and episodic memory tasks are based on memory and not merely

on detectable odour traces. To eliminate any confounding variables in this thesis, as rats have the remarkable ability to trace themselves by following odour traces and this ability can also affect memory retrieval of objects, it was essential to assess the recollection-like processes in rodents' memory tasks.

In two out of three weeks, the animals demonstrated reliable memory for the novel OLC configuration. However, when animals encountered the same task for a third time their performance drastically decreased. Nevertheless, overall the animals demonstrated reliable OLC discrimination, with a cumulative D2 of 0.21. Exploration times were investigated, and as expected exploration decreased over three weeks, but this change in exploration was not significant and is unlikely to explain the poor performance in week 3. Animals encountered two different conditions: rotation and no rotation. It was found that animals did the unstable LC task, which is a task that can be tested within the OLC task, very well. Their discrimination ratio remained stable over 12 trials. In comparison in the OLC task animals showed poorer performance in the first few trials, but after three trials the performance picks up and remained constant. This suggests that there might be something about constantly rotating the maze, which interferes with the animals' memory in the beginning (or even later when the animals encounter the task in week 3). However, because of this extraordinary performance (cumulative D2 of 0.3) in such a complex task it is very unlikely that the performance was driven by the odour of the objects or the rats' odour traces. Thus, animals must be using recollection-like processes. When the floors were not rotated in week 2 uOLC performance started low, but animals' performance increased on the second trial, which is the opposite of what happened when the maze was rotated. However, I had relatively few animals, and when looking at individual trials this leads to very little power, suggesting this

might be an effect by chance. Overall the performances of animals that do not rotate show good a performance throughout the task.

In week 3 (as in week 2) the unstable LC task started off with better D2 scores than the unstable OLC. This clearly shows that something is happening when the apparatus was rotated. The performance in the OLC task improved but remained close to chance. The discrimination ratio in the unstable LC was very high on the first trial, but then dropped off, before it returned to a good level. When the uOLC performance was divided into three blocks of four it was shown that the first showed the lowest D2 (-0.08), performance then slightly improved. Interestingly, the unstable LC performance remained intact and stable. In the no rotation condition the unstable OLC performance of animals in week 3 performances started off very low, was then at chance for a few trials until it increased slightly in the second half. Something similar can be seen in animals that had the no rotation condition in Week 2. However, it is questionable why animals cannot do the same episodic memory task three times (at least 5-7 days were in between testing sessions). This could be related to interference or an increased memory load, which would suggest that animals remember this task for a very long time. Another reason for their poor discrimination could be the unexpected outcome at test phase when object C disappears. Disappearance kept them interested in the LC task in week 3, but not OLC. It is remarkable to see that an animal can demonstrate memory for the history of events, without the cues actually being present at the point of decision-making. This is an intriguing result as it could be argued that rodents tend to spatially change their response when exploring different environments. If a spatial location has previously been explored the animal could be less likely to explore the same location again and alternate its response by exploring another spatial location. This kind of behaviour is important in the wild, because if resources have been previously found there and have not been depleted it might seem very efficient to return (Gaffan & Eacott, 1986). Using different

kind of maze tasks in which animals are required to alternate their response patterns has shown that this behaviour is evidence of spatial working memory.

The aim of this study was to investigate the effect of odour cues on performance in the OLC and LC task by rotating the floor of the apparatus. Rodents have the outstanding ability to use their olfactory system to solve spatial tasks (Maaswinkel & Whishaw, 1999; Means et al., 1992; Wallace et al., 2002). Rodents preferentially use visual information for tasks such as place learning over olfactory trails (Schenk, 1997). Based on behavioural observations it has become quite clear that rats track olfactory cues while navigating an environment; they are able to self-track is as remarkable as it is problematic for behavioural tasks (Wallace et al., 2002). Odour cues can be used to locate food locations or can be used as stimulus. This is especially relevant for paradigms, which require the rat to alternate between different arms of a radial maze (Ainge et al., 2007; Lavene & Schenk, 1998; Wallace et al., 2002). The ability to detect if the turn has been made based on odour is an important cue. Olfactory traces have been considered contextual cues in spontaneous alternation tasks, which help the rat to locate itself (Lavenex & Schenk, 1998). Even when odour cues are not relevant they may affect retrieval of memorised information, and help the animal to identify objects and locations (Lavenex & Schenk, 1995). One possibility to eliminate non-controlled odour cues is by rotation of the floor of the maze (see Lavenex & Schenk, 1995). Lavenex and Schenk (1995) assessed the influence of olfactory cues on place learning in rats and compared the use of odour and visual cues. They compared both types of cues on the discrimination of food sources and the goal was either in a stable or unstable location. The results obtained indicate that rats, which were trained in darkness and irrelevant olfactory traces were less efficient at finding the correct food location compared to animals trained in white light. Rats were unable to identify the goal's original position. When olfactory cues

remained stable, rats in darkness and white light performed equally well at finding the correct food location, showing that rats can rely on long-lasting traces for orientation, but only when the maze is not rotated and in white light.

If rats use odour traces to identify specific contexts then rotating the floor of the maze can have an impact on their performance in episodic memory tasks. As such, Still and Macmillan (1975) have shown that when maze odours changed between trials in a spontaneous alternation task performance declined, indicating that rats used odour as a contextual cue. In the unstable OLC task, an effect of rotation of the floor was found, and animals showed poor performance in the last week of testing. Indicating that odour traces on the floor may affect retrieval of past experiences. Nevertheless, it is surprising that rotating the floors only affects the OLC task, where objects are present and not the LC task, which is based on memory for locations without objects.

By eliminating the possibility that the effect that I have seen in previous LC trials was due to odour trials I have established that recollection-like mechanisms are used in the episodic memory tasks. Based on the results of this study it has become clear that performance in the LC task must be driven by recollection of an absent object and its location. Using the E-maze Eacott et al. (2005) have shown that rats demonstrate reliable object recognition memory using their ability to recollect past experiences. However, a demonstration of recollection processes in the what-where-which task as developed by Eacott and Norman (2004) was still open. Recognition memory reflects two distinct memory processes: recollection and familiarity (Yonelinas, 2002). For example, you might recognize a person as familiar but you are unable to recollect who the person is or where you have met them before. Recollection on the other hand brings back details of the experience. SOR tasks require the presentation of different objects in order to measure the discrimination ratio. However, the pure presentation of objects might trigger familiarity like processes, which would not support the hypothesis

that the (unstable) OLC task is episodic in nature. Recollection is well suited to support learning of novel associations, and familiarity is only expected to support novel learning under limited conditions (Yonelinas 1997, 1999; Yonelinas, Kroll, Dobbins, & Soltani, 1999). In the LC task animals significantly explored the previous location of an object more than its odour location. It appears that memory performance at this stage for location-context associations was strong and represents the strength of the animals' object preference. Over multiple trials animals maintained this strong preference. By selecting the 'where-which' location rats demonstrated recollection of an item and its location in a particular context. In contrast to other tasks this paradigm can only be solved using recollection of past events, as the object is not present at test. One control condition for this experiment could have been to use another group of animals, which would have been placed into the open field, but with a different context. Animals should randomly explore the open field. This would substantiate the notion that animals use recollection like processes in the unstable OLC task.

The results of this study have wider implications. Recognition tasks are particularly suited to study 'place cells' and 'trace cells' in the hippocampal formation. As such, Tsao, Moser & Moser (2013) were interested in whether memory of specific experiences is detectable in the activity in the entorhinal cortex. The lateral entorhinal cortex (LEC) is the main interface between the hippocampus and the neocortex. Previous research has shown that LEC neurons provide information about the content of the spatial-contextual environment (for example: Deshmukh & Knierim, 2001; Tsao et al., 2013; Wilson, Langston, Schlesiger, Wagner, Watanabe, & Ainge, 2013; Young, Otto, Fox, & Eichenbaum, 1997). It is unclear whether these neurons provide information about the history of events. To address this, Tsao et al. (2013) recorded from the lateral entorhinal cortex neurons in an open field where they presented rats with objects on several trials. It was found that some neurons only fired at objects (object cell) and others fired at places where an object had been located on a previous

trial (trace cell), demonstrating the recollection of past experiences in that environment. Interestingly, these cells did not respond to the presence of the objects, meaning that object cells and trace cells are different. Trace cells differ from mismatch cells, because such cells fire when an animal detects a new object or finds that a familiar object has been removed. Mismatch cells only fire for a few seconds, whereas trace cells fire for a longer period of time. Trace cells also differ from place cells, because place cells remain stable and remap when an object is moved in an environment; trace cells follow the object. Lateral entorhinal cortex neurons provide information about the presence of discrete objects at specific spatial locations, and information about the history of locations of objects. Trace activity may be linked to history-dependent firing in the hippocampus; such as the continuous firing in place cells after spatial cues are absent (Tsao et al., 2013). Properties of the trace cells in LEC are very similar to cells in the anterior cingulate cortex (ACC). Some cells in the ACC respond to where an object was located in a spatial environment on previous trials. Weible et al. (2009) recorded ACC neuronal activity while rats were doing tests of novel object and novel location recognition tasks in an open field. During novel location test, some neurons followed the familiar object to the new location and others fired where the object had been. One of the neurons fired not only where the objects are, but also where the object had previously been. Another neuron never fired where an object actually is but fires instead to where the object had previously been. ACC neurons do not exhibit spatial correlates such as found in other parts of the brain (place cells in the hippocampus).

In conclusion, I am able to use the object-location-context task to assess rats' memory for a previous event, even when the objects being recalled are not physically present. From this point of view, it is like the E-maze study by Eacott et al. (2005) where objects were present but out of view at the decision point, but uses a spontaneous task which is more reliable. The E-maze task was not as reliable in terms of robust and strong results, whereas

the continuous trial apparatus is. These results can now be used to further investigate the involvement of acetylcholine in learning and spatial memory. Eliminating the possibility that memory in episodic tasks is based on odour traces is essential, as rats frequently use olfactory traces to solve spatial tasks which could influence the outcome of the experiment by interfering with the memory retrieval of the novel environmental configuration.

Chapter 6

Study 5: The Effects of Multiple Contexts in an Episodic Memory Task

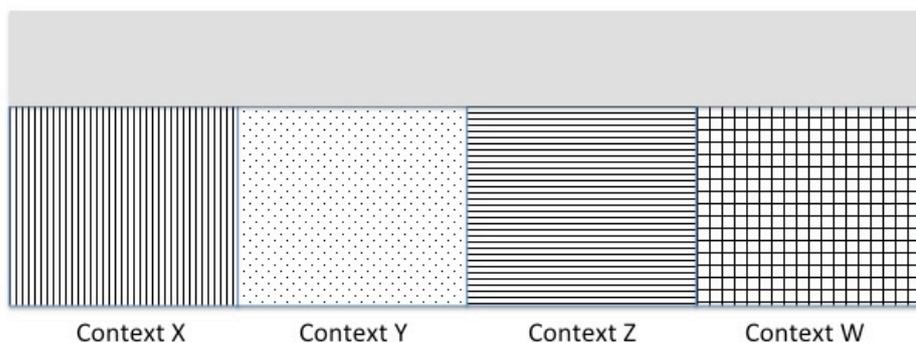
6.1. Introduction

After the successful demonstration of episodic memory in animals on the object-location-context task in a continuous trials apparatus, it was of interest whether multiple sample and test phases could be conducted without compromising performance and if rats maintain preference for novel OLC configurations over multiple sample and test phases.

Performance on multiple sample phases, which are followed by two test phases, has implication for understanding episodic memory in animals as well as humans. The ability to use information flexibly is often referred to by researchers in conjunctions with episodic memory in humans (Tulving 1985; Clayton, Dickinson, & Bussey, 2003). We can ask humans multiple questions about a single event and it is therefore useful to show the same capacity in rodents when investigating neural mechanisms. From a behavioural perspective, it is interesting to see if rodents can distinguish between very similar events and the continual trials methodology used in previous experiments has already shown that multiple trial can successfully be run in one day (Chapter 3). This increases efficiency and speeds up the data collection process and has a significant impact on the 3Rs (refinement, reduction, replacement). It is now of importance if this protocol can be made even more efficient by having multiple test phases associated with each sample phase. This would not only increase data collected for each animal, but also create a situation similar to some human tests where multiple questions are asked about a single event. Additionally, this procedure would also create a new methodology for different sort of experiments that involve pharmacological manipulations. Psychopharmacological researchers often conduct only one trial per day over many weeks, which is very time consuming. Based on this, Vandrey (2014, unpublished MSc

project) further explored the idea of multiple trials by producing a multiple continual trial protocol. Vandrey (2014) used an apparatus, which was designed in our lab by Robertson et al. (2015). This maze is a multi-compartment environment based on Spiers et al.'s (2013) work on place cell activity. The apparatus is made of a corridor, which connects four chambers and each chamber is configured as a different context (Figure 6.1). Robertson et al. (2015) have found that rats can demonstrate reliable OLC memory in this apparatus using multiple trials. As a result of this finding interesting questions have emerged and Vandrey's (2014) project aimed to investigate if animals can answer multiple questions about a single experience by changing the structure of the protocol. The two sample phases were preceded by four (instead of two) possible tests.

Figure 6.1. Diagram of Robertson et al.'s (2015) continuous apparatus.



Context X, Y, Z and W represent the four stationary chambers. The grey area is the corridor, in which animals are held in between phases. Each chamber is separated from the corridor by a door, which can be opened and close by the experimenter (see also Spiers et al., 2013).

Keeping the problem of interference in mind, it is essential that I can present multiple pieces of information within a single test session, whilst the animal is still capable of distinguishing between similar events. This is necessary for drug interventions between encoding and retrieval. If successful, this would indicate a robust memory, which would not be interfered by the similarity of preceding events. However, if unsuccessful then there are two types of interference, which can cause non-positive D2 scores: proactive and

retrospective. Proactive interference means that information from an earlier event affects and disrupts memory for new experiences (Underwood, 1957). This type of interference is a major source of confusion and cause errors in processing information. Continuous trials that were used in Chapters 3-5 are likely to be vulnerable to build up of proactive interference due to presentation of multiple stimuli in a very short period of time. It may be possible that forgetting over time or suppression may be needed in order to overcome interference, because similarity among target items produces competition and performance can decline quickly if all trials use similar material (Craig et al., 2013).

Retroactive interference means that new experiences have disruptive effects on memory for an earlier event (Dewar et al., 2007). In the current experiment, it is possible that presenting similar phases before the test phase might interrupt the animal's performance. It has been shown that a novel object-context association can be impaired if subjects explore a second and different object-context association within a short timeframe, as retrospective interference is exerted (Martinez et al., 2014). In other words, when similar experiences are being processed in an overlapping time window, competition between targets occurs.

When human subjects are presented with items in a particular context, three kinds of information are to be encoded: object information, context information and object-context association (Murane & Phelps, 1995) When asked to carry out an old/new recognition task, a feeling of familiarity emerges when a previous context is presented again with a new or old item. This feeling increases confidence in the memory response. A large body of research has looked at contextual reinstatement in infants and most findings support the notion that the context within an association can influence memory retrieval at a later stage. As such, Rovee-Collier et al. (1985) have investigated reinstatement in a series of experiments in infants. They are suggesting that contexts function as retrieval cues and the contexts has to be

connected to the stimulus in order to recognize its significance. Contextual cues are important for preverbal infants and for young infants, contextual cues serve as a framework for organising memories (Rovee-Collier et al., 1985). Infants use reinstatement mechanism to recall early memories over a long period of time (Campbell & Jaynes, 1966). As long as they continually encounter reminders of the nature of the event, they can maintain the memories for a significant period. This does not only apply to human infants, but also to young rats. Campbell and Campbell (1962) found that young rats showed poorer retention than older rats after progressively longer delays. This difference in memory performance was independent of rats' ability to learn. In another study Campbell and Jaynes trained two groups of rat pups in a fear-conditioning task. Both groups received inescapable shocks in the black compartment and were then placed in the white compartment. This was repeated and rats received a total of 30 shocks. An untrained group of young rats was treated in the same way, but did not receive any shocks. This procedure was repeated periodically and the results showed that at test the trained group (which received the shocks) exhibited fear responses in the black box trying to spend more time in the white compartment. In contrast the group, which received no shocks, spent an equal amount of time in the black and white box showing no fear or stress. This led them to conclude that 'any learnt response, whether acquired in infancy or adulthood, conscious or unconscious, instrumental or autonomic, joyful or traumatic, can be maintained at a high level by an occasional reinstatement' (Campbell & Jaynes, 1966, p.480).

In the current study four sample phases and two test phases (Test 1 and Test 2) were used. Good discrimination on Test 2 (T2) is a reliable indicator for stable episodic-like memory. However, too many test phases may increase retrospective interference, because the animal may recall information from Test 1 (T1). If events are too similar it increases interference and events become less distinguishable. The aim of this study was to investigate

the extension of the sample phase of a single trial by presenting these sample phases within different contexts. A multiple sample phase protocol was used to determine if it could elicit episodic-like strategies in rats or if it is susceptible to interference. The protocol included four sample phases followed by two test phases (Figure 6.2). Experiment 1 looked at the use of merely two contexts, experiment 2 used four contexts. Preferential exploration of novel object-location-context configuration should be robust over multiple trials if memory for sample and test phases is resistant to build up of interference. However, some build-up of interference might be expected if the OLC memory is sensitive to highly similar events as it is the case in humans (see Dewar et al., 2007). On the other side, multiple contexts can help the animals to distinguish similar events and when a rat is placed in the same context at test as it was in the sample phase, it could boost its memory by reinstating previous experiences.

6.2. Method and Materials: Experiment 1 & 2

6.2.1. Subjects

Twelve naïve Lister hooded rats supplied by Harlan were used and were housed in groups of four in diurnal light conditions. Testing was carried out during the light phase and animals had ad libitum access to water throughout the study. Animals were food restricted to 85% of their free-feeding body weight of age matched controls. Animals participated only in one test session per day and each group was tested for four days.

6.2.2. Apparatus and objects

The animals were tested in the same square shaped open field as for previous experiments. For details refer to study 2 (Chapter 3.2.2). Identical duplicates were used during testing so that each animal never saw the same object within an experiment. Two

objects were used per day, but objects were never reused in any of the tasks. The order of contexts was counterbalanced across animals.

6.2.3. Habituation and pre-training

Animals were handled daily for three days prior to habituation by the experimenter. Pre-training involved four phases. Phase 1 involved placing the animals in pairs or threes into the apparatus for 15min in each of the two contexts. In phase 2 animals were placed singly into the apparatus and were given 10 minutes of exploration in each context. In phase 3 the shuttle training began and animals were trained to move between the testing area and the holding area. This phase consisted of two sessions (one for each context) and involved placing pellets (20mg, Putrified Diet) on the floor and using the doors to control the animal's movement. In the last phase, an object was introduced and baited with a pellet. The object was placed in the centre of the open field in each context and animals were given 10 minutes to explore.

6.2.4. Data analysis

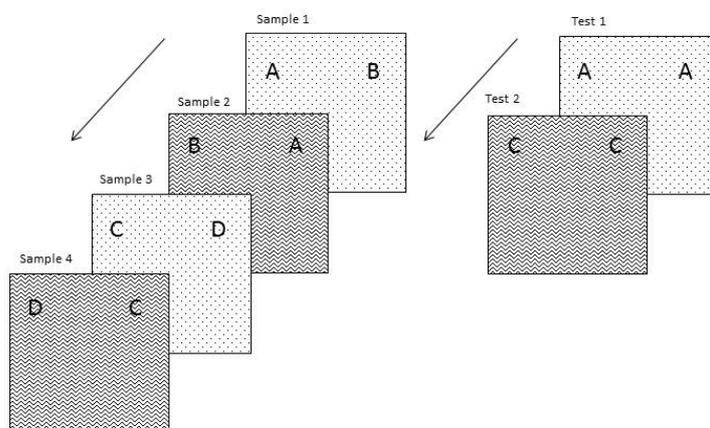
As in the previous experiments D2 scores were calculated. D2 is the discrimination ratio and calculated by dividing the D1 score by the total exploration time. In experiment 1, there were four days of testing and two contexts were used over four sample phases. The effect of using merely two contexts in the open field was analysed by looking at the average discrimination ratio and average exploration times over four trials. If animals cannot seek out the novel environmental configuration in either test 1 or test 2 then that would suggest that there is some degree of interference. Given that there were four sample phases in this study, it could have had an effect on the animal's memory, meaning that if the test phase was based on sample 1 and not sample 4 it could have been worse, than when the test was based on sample 4 than on sample 1. Hence the effect of order of sample phases was investigated and whether

animals show better performance in the OLC task after four days due to increased experience and experiencing two test phases. In experiment 2, the analysis was similar, but four contexts were used to investigate the effects of interference on the rat's memory.

6.2.5. Test protocol

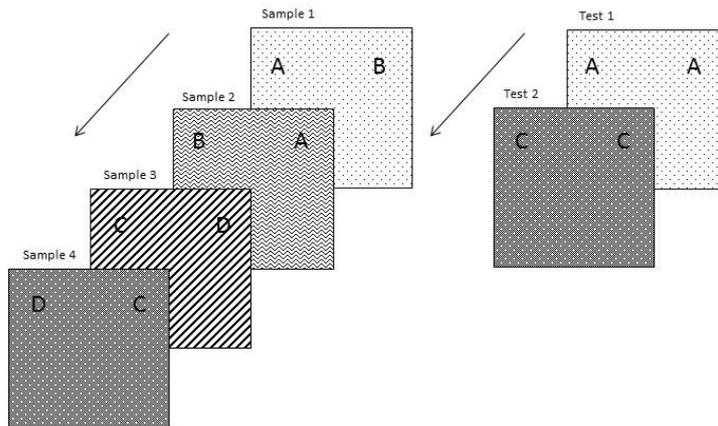
Testing began when animals were approximately 10 weeks old and lasted for four days for each context group. On each testing day (which was carried out in the morning) all animals underwent a single trial that was comprised of four sample phases and two test phases (T1 and T2). The procedure remained the same as previously explained. In experiment 1 two contexts were used, which meant that each context was seen twice in the sample phase (Figure 6.2). Within each trial the animal saw both contexts and both objects at test. T2 was based on T1, meaning that the distinction between memory for sample phases and behaviour driven by exploration can be made. In experiment 2 of this chapter four contexts were used, but the procedure remained the same (Figure 6.3).

Figure 6.2. Representation of a single trial using two contexts.



This task included four sample phases (left) while using two contexts and two test phases (right). In sample 1 objects A and B were in context X followed by sample 2 in which they swapped locations in context Y. Sample 3 contained object C and D in context Z, followed by sample 4 in context W and swapped object locations. In this example, Test 1 has the novel object (A) is on the right in context X. Test 2 has the novel object C in context Z on the right.

Figure 6.3. Representation of one trial using multiple contexts.



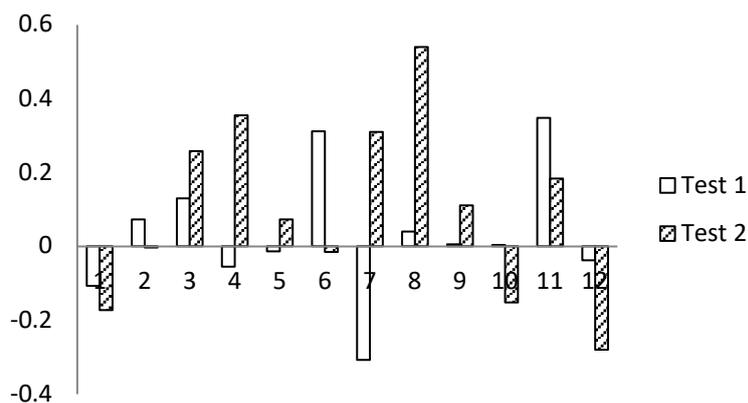
The task is the same as in Figure 6.2, but it included four sample phases (left) using four contexts and two test phases (right).

6.3. Results: Experiment 1 (2 contexts)

6.3.1. Discrimination measure

To determine if animals performed above chance a one sampled t-test (one-tailed) was carried out. Rats did not significantly spend more time exploring the novel object-context-place configuration in T1 $t(11) = 0.649, p = 0.265$, or in T2 $t(11) = 1.452, p 0.087$. Rats remained at chance in T1 with an average D2 of 0.03. They showed a trend to explore the novel OLC configuration in T2 with a D2 average of 0.1, see Figure 6.4.

Figure 6.4. Individual rats' performance on OLC using two contexts.

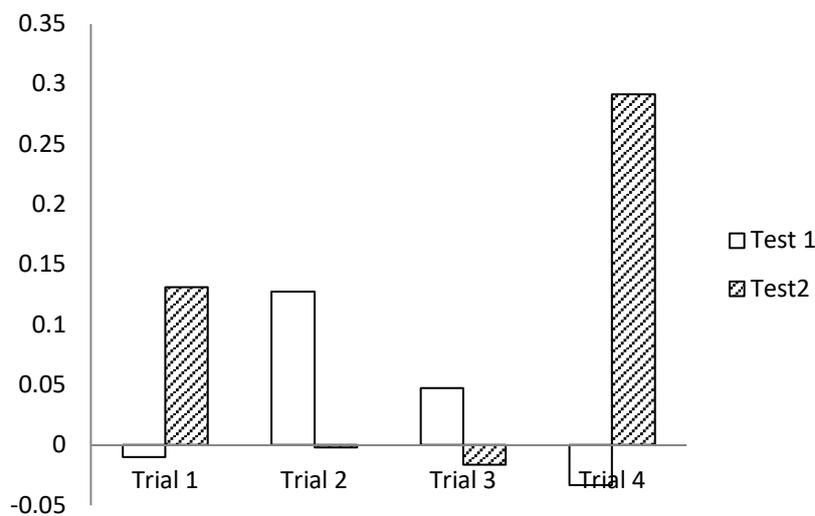


Rats showed a trend to explore the novel OLC configuration in T2, but this was not significantly above chance.

6.3.2. Performance change with experience

To determine the performance of the animals over four trials a repeated measures ANOVA (trial x test phase) was conducted. Performance did not change with more experience for T1 $F(3,33) = 0.286$, $p = 0.836$ or T2 $F(3,33) = 1.831$, $p = 0.161$. The graph shows that performance on the last trial improves, however this change was not significant, see Figure 6.5.

Figure 6.5. Change in performance on the OLC task using two contexts over four trials.

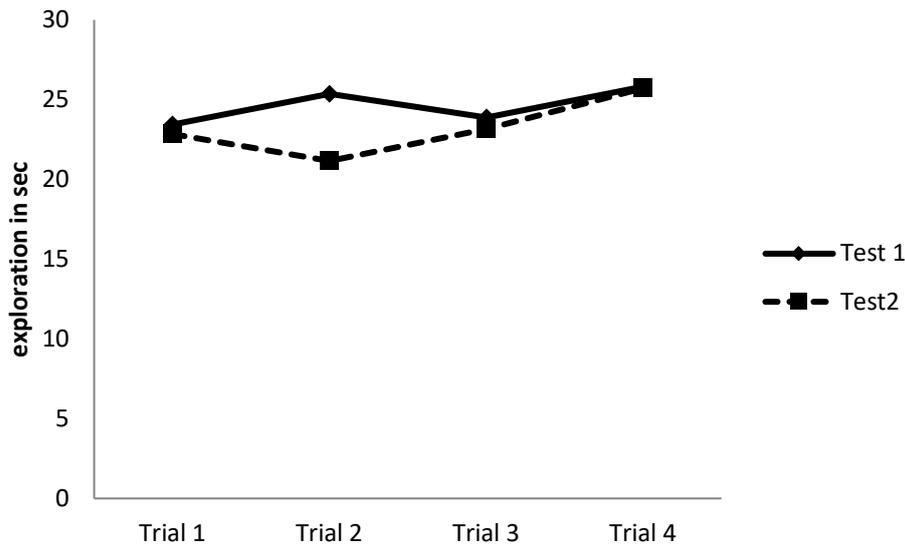


Performance did not change over four trials, but the discrimination ratio is higher on the last trial in Test 2.

6.3.3. Exploration change

Exploration times were calculated for each test phase over four trials to rule out the possibility that performance remained at chance because of low exploration times. A repeated measures ANOVA (exploration time x performance at tests) was carried out and has shown that exploration remained stable throughout the task over multiple days for T1 $F(3,33) = 0.131$, $p = 0.941$. The average exploration times varied between 23 and 25 seconds. There was also no change in exploration times in T2 $F(3,33) = 0.367$, $p = 0.777$, where the exploration times varied between 21 and 25 seconds, see Figure 6.6.

Figure 6.6. Change in exploration over four trials on the OLC task using two contexts.



Exploration (in sec) remained stable throughout the task over multiple days, as the change was not significantly above chance.

6.3.4. Sample phase tested and effect of order

This analysis looks at the performance of the test phases based on the context used in the sample phases. I.e. did animals perform better in the test phase if it was based on sample 4 than sample 1? Each sample phase was tested an equal number of times across animals, but not every animal experienced the sample phases equally. This was due to counterbalancing constraints. To determine whether performance changed depending on which sample phase (1-4) was tested, D2 scores for each phase were compared across days using a two-way repeated ANOVA (sample phase x test x performance). An effect of sample phase order was found $F(3,96) = 3.614$, $p = 0.016$. Performance based on sample 3 (average D2 = 0.27) was significantly better than for example performance based on sample 4 (average D2 = -0.08). Bonferroni: $p = 0.023$). There was no effect of test order $F(1,96) = p = 0.225$. There was no interaction between performance at test and the sample phase tested $F(3,96) = 1.912$, $p = 0.134$.

6.4. Discussion: Experiment 1

The aim of this study was to determine if rats could answer multiple questions about a single event. The OLC task was modified in a way, which allowed me to present animals with multiple sample phases and test phases. Multiple samples are a means of extending and improving existing models of multiple trials protocols. In this experiment, I used four sample phases and two test phases to investigate animals' episodic memory. In the first part of the experiment only two contexts were used (see Figure 6.2), which meant that contexts were repeated and animals saw each context three times in one trial. It was found that when the protocol only included the use of two distinct contexts animals did not preferentially explore the novel OLC configuration. There was a trend towards preferential exploration, but this was not significant and the set-up of the task did not prove useful in improving existing testing protocols of object-location-context tasks. Rats did not significantly spend more time exploring the novel object-context-place configuration in T1 or in T2. Animals remained at chance in T1 with an average D2 of 0.03. They showed a trend to explore the novel OLC configuration in T2 with a D2 average of 0.1. As previously stated, the accumulation of information may be the cause of the animals' inability to distinguish the test phases from each other. A significant effect of sample phase order was found, suggesting that animals have a better memory of the second phase of the task (Figure 6.5). Only the last sample phases were remembered as animals performed significantly better on the third sample. This pattern suggests that the introduction of additional sample phases introduced interference and the memory workload was too high.

Furthermore, the use of merely two distinct contexts may have increased the build-up of interference. By the time the animals reached the test phases, the environment (contexts and objects) was highly familiar, which interferes with previous experiences. Exploration of the preceding phases led to interference and a mismatch of the novel and familiar objects (Dewar et al, 2007).

Overall, the results suggest that it may not be possible to run multiple samples and tests in one animal in this way, because of similarity-based interference. However, given that only two contexts were used and previous experiments have shown that animals successfully show episodic memory in continuous trials it was worth investigating whether the use of four contexts might help their performance. It is possible that there were not enough cues for animals to separate the events. Therefore, the next experiment will look at multiple sample phases using four contexts.

6.5. Method: Experiment 2

6.5.1. Subjects

The same twelve naïve Lister hooded rats supplied by Harlan were used in experiment 3. Testing was carried out during the light phase and animals had ad libitum access to water throughout the study. Animals were food deprived to 85% of their free-feeding body weight of age matched controls.

6.5.2. Testing protocol

Testing began when animals were approximately 13 weeks and lasted for four days. On each testing day an animal underwent a single trial that was compromised of four sample phases and two test phases (T1 and T2). The procedure remained the same as previously explained (experiment 1 of this chapter). In experiment 2 four contexts were used, which meant that each context was seen only once in the sample phase (Figure 6.3). Within each trial the animal saw two of the contexts at test. As previously, T2 was based on T1.

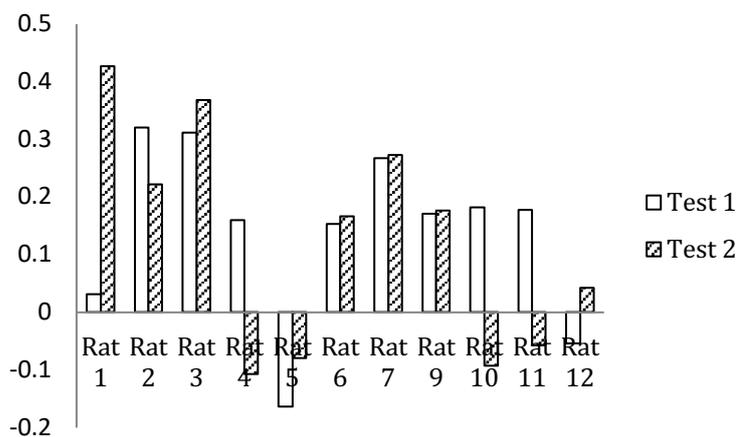
6.7. Results: Experiment 2 (4 contexts)

One animal was excluded because it failed to shuttle. Therefore, the following analyses are based on eleven animals.

6.7.1. Discrimination measure

To determine if animals performed above chance a one sampled t-test (one-tailed) was conducted to compare the average D2s for each test. Rats spent significantly more time exploring the novel OLC configuration in T1 (Average D2 = 0.14; $t(10) = 3.140$, $p = 0.006$) and in T2 (Average D2 = 0.12; $t(10) = 2.102$, $p = 0.031$). There was also no difference between T1 and T2 $t(10) = 0.341$, $p = 0.740$ (two tailed), see Figure 7.7

Figure 6.7. Individual rat performance (D2s) on the OLC task using four contexts

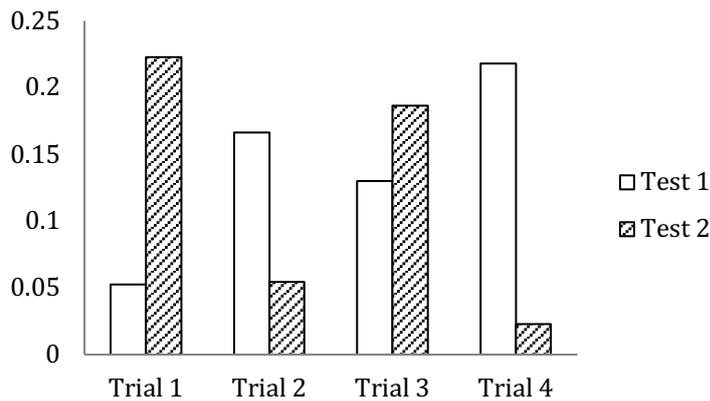


Individual rats spent more time exploring the novel object-location-context configuration in Test 1 and Test 2, however the performance in test phases were not different from each other.

6.7.2. Performance over four trials - performance change with experience?

To determine the performance of the animals over four trials a repeated measure ANOVA was conducted (trial x test phase). D2 scores for each test phase were compared across four days. Performance did not significantly differ with experience for T1 $F(3,30) = 0.429$, $p = 0.734$ or for T2 $F(3,30) = 1.232$, $p = 0.315$, see Figure 6.8.

Figure 6.8. Performance change over four trials using four contexts.

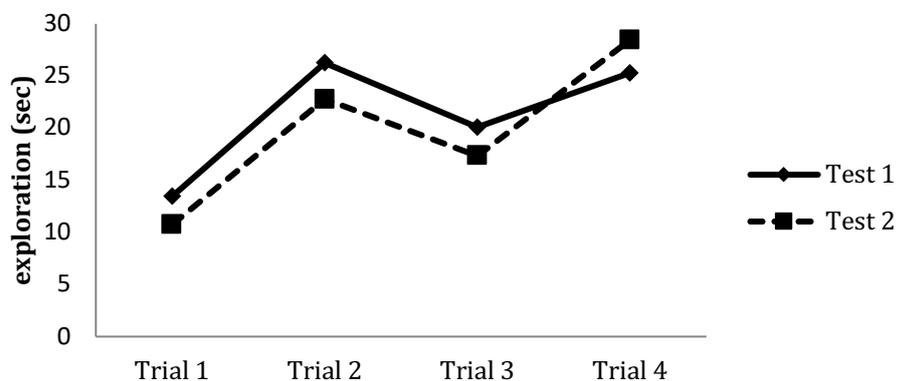


Discrimination ratios did not vary across the four trials neither in Test 1 nor in Test 2.

6.7.3. Exploration change

Exploration times were calculated for each test phase over four trials to investigate if animals remained interested in the task. A repeated measures ANOVA (exploration x test phase) showed that exploration in T1 $F(2.019, 20.185) = 1.810, p = 0.189$ (Greenhouse-Geisser corrected) remained stable. In T2 a significant change in exploration time was found $F(3,30) = 3.724, p = 0.022$. Animals explored significantly more on the last day (mean = 28.4 sec) than on the first day (mean = 10.75 sec), see Figure 6.9.

Figure 6.9. Exploration change over four trials using four contexts.



In Test 1 exploration times remained stable across trials, but in Test 2 exploration was significantly higher in trial 4 in Test 2.

6.7.4. Sample phase tested

In episodic memory both sample phases are needed to test an object-location-context configuration. Therefore, sample 1 and 2, 3 and 4 were combined. Due to counterbalancing

restrictions, there were an uneven number of tests based on sample 1/2 and sample 3/4. There was no effect of test order $F(1,88) = 0.422, p = 0.518$. There was a marginally significant effect of sample phase order $F(1,88) = 3.951, p = 0.05$. No interaction between test and sample phase was found $F(1,88) = 0.025, p = 0.874$.

6.8. Discussion: Experiment 2

Investigate performance on multiple test phases as in this study has implications for our understanding of memory processes that involve many similar components. If rats have the ability to use earlier acquired information in a flexible manner then this would resemble human episodic memory. Humans are able to consciously recall many details about past experiences. Therefore, the protocol for this experiment aimed to resolve the issue of interference by using four distinct contexts.

It was found that rats spent significantly more time exploring the novel OLC configuration in T1 (mean $D2 = 0.14$) and well as in T2 (mean $D2 = 0.12$). This finding supports the hypotheses that the OLC task can be used to demonstrate a flexible memory in rats and further validates the episodic memory task. Similar to the 2-context condition, there was a marginally significant effect of sample phase order. However, this time animals performed better in sample 1&2 than in sample 3&4. By using distinct cues we were able to reduce the amount of retrospective interference. One limitation of the counterbalancing is that the novel configuration is always located in the opposite location from the previous trial. This makes it impossible to determine if the preference for novelty is due to spatial alternation or recollection for the sample phase.

6.9. General discussion

Many studies have investigated the rats' innate preference for novelty to test memory (Albasser et al., 2010; Dere, et al., 2005; Dere et al., 2006; Eacott & Norman, 2004; Ennaceur and Delacour, 1988; Easton et al., 2011) The object-location-context task was developed by Eacott & Norman (2004) to demonstrate episodic-like memory in rodents can last for up to an hour (see also Eacott et al., 2005). Context may not only have an effect on memory retrieval but also on event separation. The experiments in this chapter aimed to explore multiple sample phases as a potential of extending and improving existing continuous trials protocols. The original object-location-context task was modified to include four sample phases and two test phases. Animals preferentially explored the novel OLC configuration when four distinct contexts were used in the sample phases, but not when only two distinct contexts were used. Therefore, rodents have the ability to remember multiple events when presented in clearly distinctive environments.

When contexts were repeated (experiment 1), animals were unable to demonstrate episodic memory. The animals showed a trend towards preferential exploration of the novel configuration, but this was not significant. The repeated use of the same context may have hindered retrieval by increasing interference from previous events. Animals were influenced by their own exploration in the preceding phases, which led to a mismatch of the novel and familiar objects. This finding compares to previous research, which found similarity based retroactive interference in humans (Dewar et al., 2007). Therefore, experiment 2 used four contexts and animals' performance was maintained throughout the task and they performed above chance. Somewhat surprisingly, there were no differences between the successful performance on the four contexts OLC task and the two contexts OLC task when compared using post hoc tests.

Humans recall several details about a single event and thereby demonstrate flexibility of their conscious recollection (Clayton et al., 2003). The present findings suggest that rats have the same ability to some extent. Interference decreases performance in memory tasks in humans and rodents. Proactive as well as retroactive interference can influence memory for past experiences. In the present experiments, retroactive interference is most likely to affect D2 ratios. RI is affected by the similarity of the objects to be remembered (Dewar et al., 2007). The sample phases are very similar and may not provide enough contextual cues for the animals. The large number of similar events may have caused retroactive interference, and this notion was supported by the significant effect of sample phase order. If contextual interference causes the poor performance in experiment 1, then it would be of interest to investigate this further.

Context is an integral part of learning and memory and it is well known that the hippocampus is critical for encoding contextual information. Memories for experiences that occur in a particular context become associated with hippocampal representation. Returning to that specific context will cause the hippocampus to recognize the relevant memories from that context. As a result, these memories are being recalled and interference from similar events are reduced. Learning new information is associated with learning about the contextual and environmental information and the context later acts as a retrieval cue (Smith, 1988). Items learnt in one context are better recalled when the testing takes place in the same context (Godden & Baddely, 1975; Smith, 1988). Much of the reinstatement research has been carried out with young infants, as it is of importance how early memories influence children later on in life. Campbell & Jaynes (1966) have argued that infants' early memories can be maintained over a significant period of development by periodic reminders (which could be compared to exposing rats to multiple contexts at test). An association is learnt which can influence the retrieval of that association later. Even in very young infants the setting of an

event impacts their retrieval of the event. For example, Hartshorn (2002) has demonstrated that infants' memory can be maintained by repeated exposure, demonstrating that a reinstatement of a previous event helps the subject to identify that the original experience has not changed.

A great number of experiments have also shown beneficial effects of contextual cues in adults (e.g.: Godden & Baddeley, 1975; Smith, 1979). However, if memory tests rely on recognition processes a more complex picture emerges (Hockley, 2008; Smith & Vela, 2001). Computational models suggest that such reinstatement (also referred to as "pattern completion") is mediated by the hippocampus (see studies by Staresina et al., 2012; Manning et al., 2011). When an individual remembers a past experience they not only recall features of the event itself, but also contextual features that provide information about 'neighbouring events' (Manning et al., 2011). It follows that when at test, the context which is re-presented with the same item it was paired at study, will trigger a process of association (Hanczakowski et al., 2014). However, Hockley (2008) has argued that successful context-dependent discrimination requires specific instructions in humans, meaning that the amount of attention devoted to encoding the item-context association is essential. If this is done successfully then context reinstatement assists later retrieval (Godden & Baddeley, 1975).

Patients with hippocampal lesions have problems recognising changes in context. For example, when control participants were trained in one context, but tested in another they gave fewer correct responses than patients with hippocampal damage (Freeman et al. 1997; Honey & Good, 1993). If context contributes to memory in the same way as it does in humans is open to interpretation. However, it has been established that context contributes to episodic memory (for discussion see Smith & Bulkin, 2014) and conditioning. As explained previously, fear-conditioning research has shown that learnt behaviours are linked to the learning context. Similar to the human study in patients with hippocampal damage who did

not respond to context changes, control rats quickly learn to avoid an environment in which they have received shocks, but animals with hippocampal lesions are not affected by this and appear to be insensitive to changes in context (David, Walker, Miles, & Grillon, 2009).

Overall, these experiments have shown the limits of the episodic-like memory task in rats. Findings suggest that regular reminders of a previous event can help memories to be maintained and distinct contexts enhance recollection. However, if we consider the interference theory proposed by Mueller & Pilzecker (1900) it is possible that memories, which are experienced close in time compete for representational space, meaning they would interfere with each other (Lechner et al., 1999). With great similarity between interpolated and original events the degree of disruption increases (Lechner et al., 1999; Mueller & Pilzecker, 1900). Newly formed memories persist in a fragile state and need time to consolidate, meaning that new information could be interrupted by old information if there is not enough time to consolidate (Mueller & Pilzecker, 1900).

This chapter provided a framework for future work to develop designs, which use contextual reinstatement by exposing rats to multiple tests to increase the amount of data points in episodic memory tasks. Animals preferentially explored the novel environmental configuration when four distinct contexts were used. Therefore, rodents have the ability to remember and separate multiple events when presented in clearly distinctive environments.

Chapter 7

Study 6: Event Separation and Interference in the Location-Context Task

7.1. Introduction

This chapter was originally designed to be the link between the rodent and human experiments in this thesis; however I was unable to finish the experiment due to a severe allergy to rodents. Therefore, the results are merely a trend, because there are many limitations (such as the low number of animals and lack of statistical power) due to unforeseen circumstances.

In the previous chapter, it was found that rats could not perform well when they were only presented with two contexts in multiple exposure phases, but could do so with four contexts, suggesting that contextual cues help to delineate episodic memories. Study 6 used this finding to further investigate the ways rodents separate events, or trials, from each other. Remembering a sequence of overlapping events is critical to behaviour, whether that is in humans or rodents. Context can include elements like spatial, tactile, olfactory cues, which are necessary for the hippocampus in storing experiences of events. The hippocampus has an important role in representing temporal and spatial sequences. Animal studies offer the ability to assess neuronal activity as rodents move through an environment and lesion work shows that the hippocampus is crucial for sequence memory (see DuBrow & Davachi, 2013 for review). For example, electrophysiological studies looking at the role of place cells and trace cells allow researchers to assess the necessity of certain brain regions for a behavioural episodic memory task. Elucidating the unique contributions of the medial temporal lobe to encoding, retrieving and segmenting episodic memory is beyond the scope of this chapter and the allergies will not allow any further animal testing. However, the rest of this thesis will

investigate various theories of memory segmentation by providing various contextual cues in behavioural tasks.

The event-segmentation theory suggests that continuous actions, such as moving from one room (or compartment in rats) to another, are separated into different events. Episodes in memory become organised through an event-segmentation mechanism while the current experience (moving within a room for example) is on-going (APS, 2011). Segmentation consists of a continuous evaluation of future events and if these events are separated well it enhances the processing and understanding of an occasion (see Kurby & Zacks, 2008). Studies have shown that humans make sense of the world by separating events into a modest number of meaningful units (APS, 2011). As previously explained in Chapter 1, events can be separated by time, location or context the question is now how do we know when the human (or the rat in this experiment) perceives that a new event or a new trial has begun. Updating event boundaries within an event model requires one identify a pre-boundary and post-boundary which will lead to a discontinuity in representation (see studies by Radvansky and colleagues.)

Episodic memory requires us to bind sequential experiences, because this structures the perception of our daily life. The ability to integrate and identify events that occur across different points in time, location or context is important for the retrieval of experiences (DeVito & Eichenbaum, 2011). In a study by DeVito & Eichenbaum (2011) mice showed strong preferential exploration of odours that they had experienced earlier within the same sequence of events but did not prefer an odour that they experienced hours earlier the same day in a different sequence. This finding suggests that across repeated sequence presentations, animals link the five different odours within each sequence. The hippocampus and the medial prefrontal cortex have been claimed to contribute to remembering the order of experiences, but the same areas are not necessary for object recognition (Devito &

Eichenbaum, 2011; Langston & Wood, 2010). Furthermore, the prefrontal cortex is involved in predicting future and sequential events (Eichenbaum, 2017).

Sequence coding by the hippocampus may be especially important when the sequences have overlapping elements through which memory of earlier elements must be remembered to complete each distinct sequence (Levy, 1996). Levy's task involved two series of events that overlap in the middle items and a critical feature of this task was the free choice; In that test animals were required to remember their choices from the first two pairings of the current sequence during the ambiguous components of the trial and then use the earlier information to guide the correct odour selection.

By developing a series of tests in which rats were required to recall up to 30 different odours, Panoz-Brown et al. (2016) showed that rats remember items in contexts using only episodic memory. Rats were exposed to a sequence of odours and taught to learn new odours. Their task resembled the object-location-context task. For example, rats were put into an arena A with an odour (such as chocolate), followed by another arena B with two odours (chocolate and strawberry). Finally, they were then placed in arena A again with flavour chocolate and strawberry. The novel configuration in this example would then be the blueberry flavour. This result clearly shows that flavours/odours can be used to define an event and that rats have the ability to remember multiple episodic memories.

Based on the finding that animals use earlier information to guide behaviour, it is hypothesised that animals perform well on pseudo trials (trials within trials, see Method for details) in the location-context task (LC), as these trials are part of a sequence. The experimenter defines the sequence; nevertheless, animals might be able to distinguish between trials that happen to be there through careful counterbalancing.

In the continuous trials apparatus rats move between two compartments and perform the same task multiple times. In the location-context task the object changes on every exposure. This is because the object's identity is not relevant in this task. Because of this continuous change of objects, trials are not as clear-cut, as in the object-location-context task. In the LC task sample phases and test phases are not separated in the same way and there is no distinction between phases, which means they are indistinguishable from an animal's perspective. Within a testing session sample phases can be test phases and vice versa and rats may perform above chance on these 'within trials' or pseudo trials. For example, if each trial consists of two sample phases and a test phase and the counterbalancing is done carefully it is possible to have more than the original test phases. For example, in trial 1 sample 2 could also be sample 1, test would then be sample 2 and therefore trial 2 sample 1 would be the test phase (see Figure 7.3 for details). Previous exposures of experimenter-defined configurations may be influencing the rats' behaviour across trials. Therefore, it was of interest how animals separate events (in this case trials) from each other and to what extent behaviour is influenced by interference from previous events/trials.

In this study, I was interested whether rats can separate multiple trials by flavour. Some of the trials were combined using flavour and others were separated by flavour. One testing session consisted of 12 trials, but there are also test phases within the trials (Figure 7.1 and Figure 7.2). The counterbalancing was done in a way that ensured that there were six additional test phases within a testing session (pseudo trials).

Figure 7.1. Example of four trials in the flavour-segmented condition (different).

| | | | |
|----------------|---|---|--|
| Trial 1 | Sample 1 (banana) (e) | Sample 2 (banana) (e) OR Sample 1 (p) | Test (banana) (e) OR Sample 2 (p) |
| Trial 2 | Sample 1 (berry) (e) OR Test (p) | Sample 2 (berry) (e) | Test (berry) (e) |
| Trial 3 | Sample 1 (bacon) (e) | Sample 2 (bacon) (e) | Test (bacon) (e) OR Sample 1 (p) |
| Trial 4 | Sample 1 (pina colada) (e) OR Sample 2 (p) | Sample 2 (pina colada) (e) OR Test (p) | Test (pina colada) (e) |

Each trial was assigned its own flavour. The order of the encountered flavour was counterbalanced across and within animals. In this condition, it was predicted that the experimenter-defined trials would be better than the pseudo trials (here shown as the ‘OR’ option).

For example, rats would be able to determine the novel LC configuration in the traditional test phase on Trial 1. However, rats would not be able to determine the novel LC configuration in the additional test phase in Trial 2. The ‘e’ represents the experimenter-defined trials, and ‘p’ the pseudo trials.

Figure 7.2. Example of four trials in the non-flavour-segmented condition (combined).

| | | | |
|----------------|---|--|--|
| Trial 1 | Sample 1 (banana) (e) | Sample 2 (banana) (e) OR Sample 1 (p) | Test (banana) (e) OR Sample 2 (p) |
| Trial 2 | Sample 1 (banana) (e) OR Test (p) | Sample 2 (banana) (e) | Test (banana) (e) |
| Trial 3 | Sample 1 (berry) (e) | Sample 3 (berry) (e) | Test (berry) (e) OR Sample 1 (p) |
| Trial 4 | Sample 1 (berry) (e) OR Sample 2 (p) | Sample 4 (berry) (e) OR Test (p) | Test (berry) (e) |

It was predicted that performance would be better in the non-flavoured segmented condition than in flavour-segmented condition. Pseudo trials are represented as the ‘OR’ option. In the flavour-segmented condition, the experimenter defined trials, the original test phases, would be better than pseudo trials. However, for the non-segmented condition there should be no difference between performances.

In this example animals should be able to recognize the novel LC configuration in the test phase in trial 1, but also the test in trial 2.

The ‘e’ represents the experimenter-defined trials, and ‘p’ the pseudo trials.

It was hypothesised that performance in pseudo trials will be significantly better in the combined (non-flavoured segmented) condition than in the different condition (flavoured segmented). Furthermore, performance in the traditional experimenter-defined trials will be better in the flavour-segmented condition than in non-flavoured segmented condition.

In the flavoured-segmented condition, experimenter-defined trials will be better than pseudo trials but for the non-segmented condition (combined condition) there should be no difference between performances across these trial types.

7.2. Method and Materials

7.2.1. Subjects

Five naïve Lister hooded rats supplied by Envigo were used in this experiment. They were housed in groups maintained on a 12hr light/dark cycle. Testing was carried out during the light phase and water was available ad libitum throughout the study. During habituation animals were food deprived to 90% of their free-feeding body weight. Animals started testing when they were 8 weeks old. A within-subjects design was used, which meant that each rat encountered both conditions in a counterbalanced manner (flavoured-segmented vs non-flavoured segmented).

7.2.2. Apparatus and objects

The animals were tested in the continuous trials apparatus, which was used in Chapters 3-5. For details on the apparatus, objects, and counterbalancing refer to section 3.2.2.

7.2.3. Habituation and pre-training

Each animal was handled daily for three days prior to habituation. Rats were habituated to moving between rooms and cage covers were used to minimise stress, the testing room, the open field, the objects, the flavoured pellets and contexts. Behavioural testing took place in a separate room under dim white light and white noise in the background to cover environmental noise. Pre-training involved four phases aimed to habituate the animals to the environment, which lasted 8 days. Phase 1 involved placing the animals in threes into the

apparatus for 30 minutes in each context with the flavoured pellets (Bioserv) scattered on the floor. This allowed them to explore the open field freely and be introduced to the different flavours. In phase 2 animals were placed singly into the apparatus and were given 15 minutes of exploration in each context with the six different flavoured pellets. For phase 3 the goal was to train the animals to shuttle between the two areas of the apparatus: the testing area and the holding area. This phase consisted of four sessions (one for each context) and involved placing pellets (20 mg, Purified Diet; BioServ – non-flavoured) on the floor and using the doors to control the animal's movement. In phase 4 an object was introduced and baited with pellets. The object was placed in the middle of the open field in each context and animals were given 10 minutes to explore.

7.2.4. Test protocol

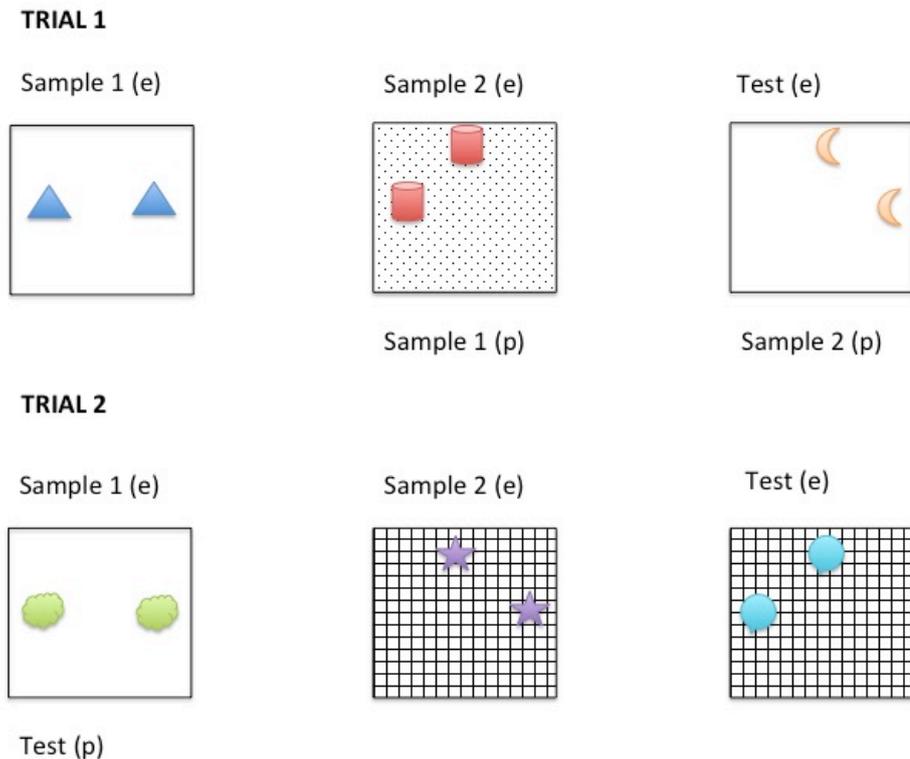
Animals were given two test sessions for the object-location (OL) task. A testing session consisted of 12 trials and lasted two hours. As in previous experiments rats were tested between 8am and 12pm and between 1pm and 5pm. It was ensured that each rat was tested at the same time of day for each task, but due to issues with the allergy this was not always possible. At the start of each session, the animal was placed in the holding area. The door would then open to allow the animal to move to the testing area. In both exposure phases and the test phase animals were given 2 min of exploration. Between phases rats were in the holding area while the arena was changed. Objects on each trial were baited with a food pellet to encourage exploration. In this experiment, different flavoured pellets were used to differentiate trials (bacon, marshmallow, banana, berry, chocolate and Pina colada), but were not used as rewards. The usage of pellets is explained in Figures 7.1 and 7.2. Exploration was taken when the animal was at a distance of 1cm of the object and actively exploring it (i.e. sniffing at or touching it). As previously explained, actions such as sitting on the objects or

using the items as support during rearing were not considered exploratory behaviour. The duration of exploration was measured off-line by holding down a keypad on the computer. The testing contexts, the novel object and placement of the novel object were counterbalanced. The criterion for ending a session was if the animal failed to shuttle between the two areas after three minutes. The data of that animal would not be included in the analysis.

7.2.5. Location-context (LC)

In the location-context (LC) task, rats receive two exposure phases in which they see two identical copies of an object in different places and in different contexts. As this task is independent of the object's identity and reflects the novelty of place-context configurations, distinct objects are used in each phase. Because the object changes every time the animal enters the maze, it is possible to have trials within trials. Figure 7.3 shows two typical trials, which consist of two sample phases and a test phase. However, in trial 1, sample 2 can also be seen as sample 1 of a "pseudo trial", a non-experimenter defined trial. Equally, the test phase of the experimenter-defined trial can also be seen as pseudo trial sample 2 and therefore the experimenter defined trial 2 sample 1 would be the test phase of the pseudo trial.

Figure 7.3. Schematic representation of the location-context task.



The sample and test phases were either segmented by flavour or not. Normally, each trial consists of two sample phases and a test phase. However, by counterbalancing the trials in the location context-task it is possible to have more than one test phase (pseudo phases). The experimenter-defined trials are emphasised with ‘e’. Other trials are pseudo trials, which are emphasised with ‘p’. Using the example above, in trial 1 sample 2 (e) can also be sample 1 (p), test (e) can be sample 2 (p) and therefore trial 2 sample 1 (e) would be the test phase (p).

7.2.6. Data Analysis

As in the previous studies D2s were calculated (Chapter 3.3, see also Albasser et al., 2010). One-sample t-tests were used to determine if the performance of the animals was above chance.

7.3. Results

7.3.1. Performance in standard and pseudo trials

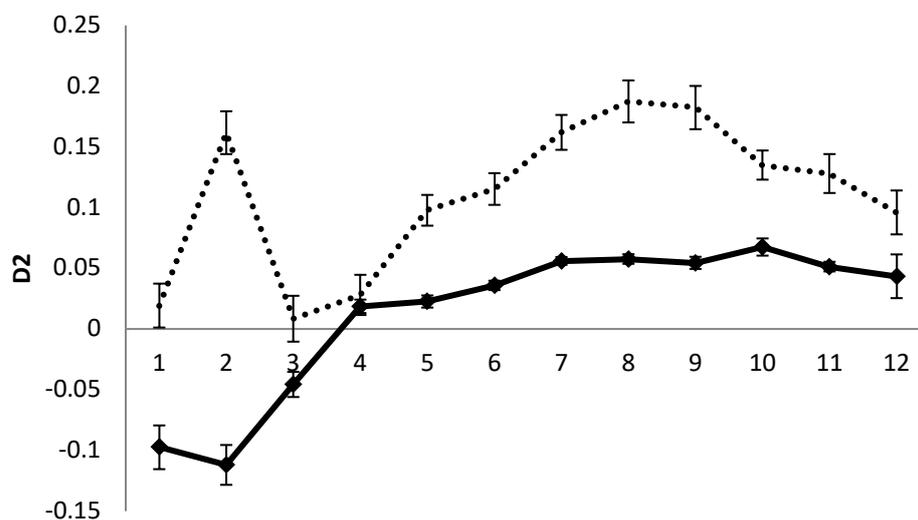
The average and cumulative D2 were analysed using one-tailed t-tests to investigate the performance in flavoured and non-flavoured segmented trials.

Animals were tested over two weeks – two animals were tested in the non-flavour segmented condition in week 1 and the other three animals were tested in the flavoured condition in week. 1. In week 2 the order was reversed. There was no overall difference between week 1 and week 2 $t(4) = 1.792$, $p = 0.148$ and therefore the data was collapsed over the two testing weeks.

Next, it was of interest whether animals can seek out the novel location-context configuration in experimenter defined trials (Figure 7.3). One tailed t-tests showed that performance was not significantly different above chance when every trial had a different flavour (i.e. trials were flavour segmented) $t(4) = 1.479$, $p = 0.11$ (one-tailed), but significantly different from chance in the non-flavoured segmented trials $t(4) = 3.038$, $p = 0.02$ (one-tailed). In other words, animals were able to seek out the novel location-context configuration when the trials were not flavour-segmented (i.e. multiple trials had the same flavours).

Using a paired t-test no significant difference between condition non-flavoured ($D2 = 0.13$) and condition flavoured ($D = 0.09$) was found $t(5) = -0.885$, $p = 0.426$. Therefore, performance in the experimenter defined trials was not better in the flavour-segmented than in the non-flavour segmented condition (Figure 7.4).

Figure 7.4. Cumulative D2 when the two weeks of testing were combined over 12 trials.



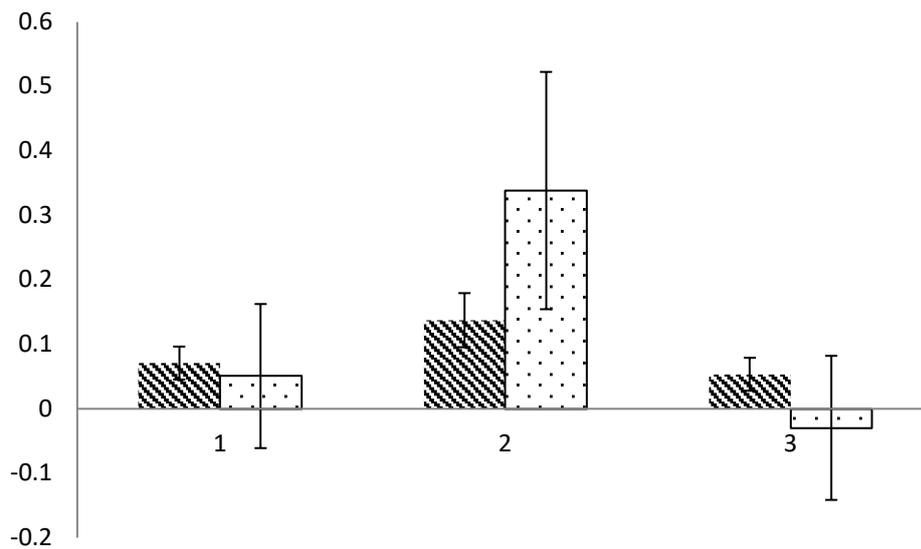
A significant difference between the flavour segmentation conditions was found. Animals performed significantly better in the non-flavoured condition than in the flavoured. Black – flavour segmented condition. Dotted – non-flavoured segmented condition. Error bars represent the SEM.

A one tailed t-test using the average D2 ratios was carried out to analyse the performance of rats in pseudo trials showed that neither the flavour segmented condition $t(4) = -0.803$, $p = 0.23$; $D2 = -0.04$, nor in the non-flavoured segmented condition $t(4) = -1.030$, $p = 0.18$; $D = -0.11$ was significantly above chance and there was no significant difference between them $t(4) = 0.640$, $p = 0.557$. Therefore, performance in pseudo trials was not significantly better in non-flavoured than in the flavoured condition.

7.3.2. Interference and effect of block

In order to see if performance changed over a testing session, especially in the combined flavour condition, the D2 scores for each rat were separated into three blocks of four trials. Regardless of condition we would expect block 1 to have the lowest interference and block 3 the highest interference. For each animal, an average D2 score was calculated within that block. Using a repeated-measures ANOVA (condition x block) it was found that there was no main effect of condition $F(1,4) = 0.794$, $p = 0.423$, no main effect of block $F(2,8) = 4.199$, $p = 0.06$, and no interaction between condition and block $F(2,8) = 1.844$, $p = 0.220$. The effect of block was near significance and therefore Figure 7.5 shows the D2 ratios in condition flavour and non-flavour segmentation. In both conditions block 2 had the highest D2 ratio, whereas block 3 had the lowest discrimination ratio, indicating some interference.

Figure 7.5. Effect of block in condition flavour and non-flavour.



D2s were divided up in three blocks of four trials. In both conditions block 2 had the highest discrimination ratio, whereas block 3 had the lowest discrimination ratio. The striped bars represent condition flavour segmented and the dotted bars represent condition non-flavour segmented. Error bars represent SEM.

7.4. Discussion

This chapter provides a link between the previous animal studies and the upcoming human study of this thesis. However, due to an allergy to rats the study had to be stopped before it could be finished, resulting in loss of power. Therefore, this experiment should be seen as a theoretical link between chapters and what could have been achieved if it had not been for the allergies.

In this study, I was interested whether we can separate multiple trials and events by flavour. While running multiple trials has lots of advantages, there is a risk of increased interference and investigating how trials might be segmented into events is highly relevant to how humans separate their episodic memories. Some of the trials in this study were combined using flavour and others were separated by flavour. One testing session consisted of 12 trials, but there were also test phases within the trials (pseudo trials). It was hypothesised that performance in pseudo trials would be better in the non-flavoured (combined) condition than

in the flavoured (different) condition; and that performance in the experimenter defined trials would be better in the flavour-segmented condition than in the non-flavoured segmented condition. However, due to the low number of animals (five overall) there was not enough statistical power to draw firm conclusions and no basic effects were found. It is surprising that rats performed the experimenter-defined trials above chance and the point was if there were no flavour cues to segmentation, experimenter-defined and pseudo trials are the same.

The location-context (LC) task consists of two sample phases and a test phase. Every phase contains a new pair of objects, which makes the trials less distinguishable and ideal for investigating event separation in rodents. Because of this continuous change of objects, trials are not as clear-cut, as in the object-location-context (OLC) task. In the LC task sample phases and test phases are not separated in the same way and sample phases can be test phases and vice versa (called pseudo trials in this study). Using different flavours, such as chocolate, banana or bacon pellets, to segment trials from each other, it is possible that interference was introduced and made context configurations less distinguishable. In previous tasks (Chapters 3-6) all sample and test phases had the same neutral flavoured pellet to encourage exploration and shuttling between the compartments. This experiment used six different flavours of pellets to separate or combine trials depending on testing schedule.

Unlike in previous experiments, animals did not perform above chance on the location-context task in the experimenter-defined trials. Interestingly, when conditions were separated by flavour-segmented vs non-flavour condition it was found that animals performed above chance when the trials were combined by flavour. However, given the low numbers this difference may be driven by distinct animal differences. Nevertheless, if flavour is used to segment events or trials in this case, then different flavours might prevent animals segmenting the sequence into pseudo trials and will only segment into real trials, which were

defined by the experimenter. Yet, the results clearly show that there was no evidence that in either condition are pseudo trials being performed above chance. The interference theory proposed by Mueller & Pilzecker (1900) explained that it is possible that memories, which are experienced close in time compete for representational space, meaning they would interfere with each other (see also Lechner, 1999). With great similarity between interpolated and original events the degree of disruption increases (Lechner, 1999; Mueller & Pilzecker, 1900). Newly formed memories persist in a fragile state and need time to consolidate, meaning that new information could be interrupted by old information if there is not enough time to consolidate (Mueller & Pilzecker, 1900). Running multiple trials over a short period of time increases the amount of data that can be collected from one animal, however in this case it could have interfered with the overall aim of the experiment.

When events are bound by flavour I am cueing animals how events should be segmented. Thus, pseudo trials become less obvious and performance in pseudo trials could be at chance. When the same flavour is used for every trial the rat could have been unable to differentiate the experimenter-defined trials from pseudo trials and they could have interfered with each other. However, as the pseudo trials were not above chance, this is unlikely. It is surprising that the combined (non-flavoured condition) worked in previous studies but not in this one, as the methods remained the same. Given the low number of animals a direct comparison with previous data is not possible.

A block analysis was run to see if there was a difference in performance over the testing session, because of the use of flavour to segment trials. Three blocks, made up of four trials, were compared to each other in both conditions. In block 1 we would only expect low interference, as it is the beginning of a testing session, whereas in block 3 we would expect high interference, with block 2 being intermediate. The block analysis showed that there was

no effect of condition (flavour or non-flavour segmentation) on the performance in the experimenter-defined LC task. However, it is worth mentioning that the data suggest that there was some evidence of interference towards the end of a testing session in the non-flavoured segmented condition.

If the experiment did not have come to an end early due to allergies, the number of animals would have been higher. Eight to ten rats have previously been shown to provide reliable results and statistical power (Ameen-Ali et al., 2012 for example). If animals did not have to be excluded from the study, because the animal numbers had to be reduced due to the severity of the allergies, I would have an equal number of rats in each condition, which would have provided a more reliable way of testing the effects of flavour segmentation on location-context memory. If this experiment were to run again other ways of segmenting trials, such as auditory cues, could be useful for comparison, as auditory cues have been shown to support spatial navigation and contextual conditioning (Rudy, 1993). Theoretically, any type of information that accompanies encoding and retrieval and may help memory performance and segmentation of events. There is growing knowledge about the involvement of neural mechanisms in encoding and retrieval of memories. However, the interaction between encoding, consolidation and retrieval has not been investigated in great detail. Additionally, the design of this experiment provides an excellent opportunity to monitor the activity of different brain regions (such as sub-regions of the hippocampus, or the prefrontal cortex) while a rat is carrying out the location-context task. Immediate early gene c-fos would be a powerful tool to investigate neuronal activity of neurons within defined areas of the brain during experimenter-defined and pseudo trials.

Different flavours of pellets were used to segment trials, which aimed to resemble the idea of a doorway acting as a boundary (see Radvansky & Copland, 2006). Moving into a

new environment, whether that is defined by flavours or by doorways, interferes with the brain's working memory and an overload of information increases the chances of interference.

Based on the experiments with rodents the next study will investigate the effect of event separation in episodic memory in humans. A series of virtual environment and real-life experiments were run by Radvansky and colleagues (Radvansky & Copeand, 2006; Radvansky et al., 2011) where they demonstrated the influence of changing events and its boundaries on memory. In their very first experiment Radvansky and Copeland (2006) asked participants to move through a multi-room virtual environment. The environment consisted of different rooms, which had one or two tables in them. The participant was asked to walk towards the table and pick up and set down objects. The purpose of the experiment was to determine if there was a difference in performance whether participants walked through a doorway on their way to the table or not. The results showed that participants' memory for objects was worse when a shift in location occurred than when there was no shift. They called this the 'location updating effect'. The doorway acts as a boundary, which helps us to separate events from each other. By segmenting a series of events, we reduce interference from having to distinguish between too many memories. However, Radvansky and colleagues do not look at the episodic nature of memories in a virtual environment. Hence, the next chapter will investigate the role of recollection and familiarity in location updating effects and episodic memory.

Chapter 8

Study 7: Location Updating Effects in Episodic Memory

8.1. Introduction

Animal models are crucial in memory research and the work in previous chapters successfully demonstrated different ways of testing rodents' behaviour in variations of the OLC task. These experiments were well controlled and aimed at reducing animal numbers used in memory research. Due to ethical concerns associated with human experimentation, animal models of memory have often been used. However, improving the translation between human and rodent models provides researchers with an opportunity to further reduce the number of animals used. Episodic memory is the memory for events in a person's life and previous research in this thesis has shown that using a content-based description of episodic memory allows us to assess episodic-like memory in animals, too. Specifically, in Chapter 3 it was demonstrated that rats are capable of showing episodic memory in a multiple trial apparatus. In this maze animals are required to move between two compartments. Animals are shown different objects (what) in different locations (where) in different contexts (which). Many recognition memory tasks have successfully been carried out in rodents, however episodic memory tasks in animals still remain controversial. The final experimental chapter is an attempt to translate an episodic memory task in animals to a human equivalent, as in the human tasks the exact episodic nature has been under explored.

The role and associated effects on behaviour of spatial boundaries in environments has been explored in animals to a great extent and some studies have aimed to develop similar behavioural tasks in humans. For example, Holland and Smulders (2011) designed a what-where-when task for humans, which is based on the idea of food hoarding in birds. Participants were asked to hide two items on two separate occasions. They were then tested for their memory of what was hidden where and when over two testing sessions. There were

two conditions, active and passive. The active condition was based on the animal's version of the what-where-when task and participants were instructed to memorise information. In the passive condition participants were not asked to memorise and were not aware of the fact that their memory would be tested at a later point. Both groups used mental time travel to solve the episodic memory task, and locations were better remembered from the first testing session than the second session. However, the subjective experience of participants was not investigated, which can make a crucial difference to the interpretation of their results. Furthermore, Holland & Smulders task was not a test of recognition per se and is not comparable to previous studies using remember/know judgements. In humans memory tasks are often carried out very differently compared to animal studies as participants are able to express themselves verbally. As previously explained in Chapter 1, in humans, one way of assessing recognition memory is by asking participants to either make a 'remember' or 'know' judgement about a previous experience or occurrence. Yonelinas (2002) has argued that recognition reflects two distinct processes, recollection and familiarity, and Aggleton and Brown (1999) suggested that these two processes depend on different regions in the MTL. The hippocampus is claimed to support recollection, and regions within the parahippocampal gyrus support familiarity. However, other researchers argue that recognition is a single process, where 'knowing' simply reflects a weaker memory trace and 'remembering' is associated with a strong memory trace (see Squire, Stark & Clark, 2004). To explore the two different arguments, earlier experiments tested recognition memory tasks in non-human animals by reproducing the MTL damage that is found in human patients with amnesia by using selective lesions (Haist et al., 1992; Parkin & Leng, 1993; Squire & Knowlton, 1995). Reproducing this kind of impairment in animals helps us to understand the underlying neuroanatomical mechanisms of memory. However, developing tasks that are comparable between species is challenging and we must be sure to test the same kind of memory that is

lost across different species due to amnesia, illness or injury. Human patient studies have proven to be useful in determining the brain structures underlying recognition, but most research focuses on animal models. Given the procedural differences across different experiments, it was necessary to develop consistent procedures.

As such, (Easton, Webster, & Eacott, 2012) assessed humans' experience of episodic-like recognition memory based on memory tasks that have been used in animals. The basic paradigm for recollection in memory involves asking people to make a judgement about the nature of their memories. Based on Rajaram (1993) description 'remember' refers to those items of which we have a conscious recollection and therefore reflects episodic memory. Remember is the ability consciously recollect and become aware of aspects of contextual details of an encoded event (Yonelinas, 2001). For example, one might recall what was experienced at the time the object was presented during the experiment. Remembering requires a deeper level of processing and should bring back to mind a particular piece of information about the image from the time of study. "Know" refers to items that are familiar, but one cannot recollect the actual occurrence and is therefore not episodic in nature. Knowing is a feeling of unconscious familiarity and responses should be made when one recognises that the tested object was in the study but cannot recollect any details about its actual occurrence (Yonelinas, 2001). When one is certain of recognising the object but the object does not bring to mind any specific details then a 'know' response should be given. Using this approach, participants can be asked about their subjective experience in a more objective manner. By adapting the animals versions of the what-where-which and what-where-when tasks Easton et al. (2012) examined the experience of human participants based on animal studies by Eacott and Norman (2004); (Kart-Teke, Dere, Brandão, & Huston, 2007). Participants sequentially viewed PowerPoint slides on screen, which consisted of a number of symbols shown in different locations on different backgrounds. The same symbols

were used on the next screen, but the location and backgrounds on which they were presented changed. Subjects were tested on their memory for symbols (what), location (where), the contextual background (which) and also for their memory of the first or second screen (when). Additionally, participants were asked to make a remember/know judgement and rate their confidence in their response. It was found that tasks relying on contextual information to discriminate events could only be performed using recollection, meaning that participants had to use episodic strategies to solve the task. Tasks using temporal information could be performed using recollection and familiarity, which suggests that temporal tasks are prone to non-episodic strategies. Hence, Study 7 will not use temporal cues as a measure, but extent on the spatial and contextual aspects involved in episodic memory. That is based on the neural responses during episodic-like tasks where neurons in the medial temporal lobe are of relevance, as these fire when a rat is close to spatial boundaries and place cells cluster around doorways in a multi-compartment apparatus (Spiers, Hayman, Jovalekic, Marozzi, & Jeffery, 2015). Spiers et al. (2015) recorded place cells in rats as they were exploring a four-compartment environment. There was a clustering of place cell activity around the doorways of the compartments, implying that doorways play a role in isolating subcomponents of representations. In addition to place cell firing across and near different compartments, grid cells have shown to repeat their firing patterns when a spatial shift was encountered, such as an entry to corridors (see Derdikman, et al. 2009; Derdikman & Moser, 2010). Doorways may segment neural representations of space by separating various episodes in an environment. One hypothesis is that in order to discriminate between similar fragments of an environment, like offices along a corridor in the Psychology Department, is to use spatial locations. Our brains are said to store memories in episodes, which is why they are called episodic memories and walking through doors triggers memory a segmentation of episodes.

Many (but certainly not all) memories are episodic in nature, suggesting that when we go from one room to another our brain signals that we are entering a new space.

Previous research has suggested different theories about this segmentation and encoding of episodic events. When participants are asked to watch a short video clip they are able to segment the video into events and participants largely agree on where the boundaries are and this happens naturally (Newtson, 1973; Zacks et al., 2001). Typically participants perceive an event boundary when they are unable to predict what is going to happen (Zacks, Kurby, Eisenberg, & Haroutunian, 2011). Segmentation of short-term memory events is a useful method to navigate a complex environment, such as the Psychology Department in Durham. Another line of research investigated situation models which were tested in studies about reading of narratives. These suggest that readers automatically create representations similar to those that would be created in their real life and help the reader to make sense of the text (Zwaan, 1999). Within the situation model, the event index model claims that situations are centered around daily events (Zwaan, Langston, & Graesser, 1995) and the events are segmented by space and time. Given that episodic memory is mainly spatiotemporal in nature (Tulving, 1983), this study investigates the role of spatial boundaries. Episodic memory requires the demarcation of events and this evidence is both neural and behavioural. This has led to tasks where memory is explicitly affected by walking across boundaries.

Radvansky and Copeland (2006) demonstrated a detailed assessment of the influence of changing events on memory, which formed the basis of the current study. In Radvansky and Copeland (2006) experiment participants moved through a multi-room virtual environment. The environment consisted of different rooms and had one or two tables in them. The participant was asked to walk towards the table and pick up and set down objects. They then walked to the next table, which was either in the same room (no shift) or in another

room (shift). At any given point participants needed to remember only two objects: the one they were currently carrying and the one they had just put down. The purpose of the experiment was to determine if there was a difference in performance in the shift condition compared with no shift. The results showed that participants' memory for objects was worse when a shift in location occurred than when there was no shift. People also responded slower to probes in the shift than in the no shift condition, indicating that this compromised memory. Radvansky and Copeland called this the 'location updating effect' and suggest that spatial shifts require people to rethink their understanding of the situation and the need to create a new situation model.

Based on these findings it is suggested that mental updating of a dynamic event disrupts memory. While some studies have examined the subjective experience to demarcate recollection from familiarity, and others have examined the nature of spatial boundaries in episodic memory, the two approaches have been combined. The experimental task used in this experiment is loosely based on the work of Radvansky and Copeland (2006), where people moved through rooms in a virtual environment, but we will also assess episodic memory. As it is impractical to have people move through a large space, and because real rooms are not very flexible and cannot easily be controlled, I had people move through virtual reality. Virtual reality is a useful tool for studying navigation and spatial memory. The technology allows researchers to define the cues that carry information, whereas this is not possible in the real world experiment (Minderer & Harvey, 2016). Virtual reality can fill the gap between natural behaviour and conventional approaches (Radvansky & Copeland, 2006). Recent work has shown that performance in virtual spaces is comparable to that for real spaces (Sun, Chan, & Campos, 2004; Waller, Loomis, & Haun, 2004). Using human participants, I tested their memory of objects and their experience associated with it. The aim of this study is to investigate the ability of people to retrieve information about objects as

they move through a virtual environment. Based on a series of studies by Radvansky and colleagues it is believed that information about an object is less available when people move from one location to another, which is referred to as the location updating effect or doorway effect (Radvansky & Copeland, 2006).

The present study will further investigate the role of recollection and familiarity in location updating effects and episodic memory. It was of interest if a shift in space impacts episodic memory. In the experimental task, participants moved from room to room and were asked to pick up objects on the way. Once an object was picked up it was carried to the next room and then put down on a table. A new object was to be picked up and carried to the next table (either within a room or the next room) etc. At various points participants were probed for their memory. During the probes, an example of an object appeared on the screen and participants were required to report if this was an object they were currently carrying/putting down, if they remembered/knew/guessed and rate their confidence in the response given. Probes either happened half way through a room (no shift) or after a doorway (shift). The expectation was that episodic memory would be worse after a shift, meaning that participants would give fewer 'remember' than 'know' responses. Given that moving through actual space is rather difficult, because it requires many different rooms, the experiment was carried out on a laptop using a virtual environment. The most crucial part of this experiment is the ability to assess the participants' strategies of solving this task, as most experiments in the past have not looked at the episodic nature of memories and the subjective experience of participants.

8.2. Method and Materials

8.2.1. Participants

Fifty participants from the University of Durham were recruited through the online Psychology subject pool and given partial course credit for their participation. Four participants stopped early after reporting motion sickness and one participant was excluded for not following the instructions. This leaves a total of 45 participants. The experiment was approved by the Durham University Psychology Ethics Committee. All participants gave informed consent and were offered either participant pool credit or the chance of winning £50.

8.2.2. Materials and apparatus

The virtual environment was constructed, compiled and displayed using Mazesuite software (Ayaz, Allen, Platek, & Onaral, 2008; www.mazesuite.com), which was run on a standard Dell laptop, running Microsoft Windows 7. Using the 0–255 RGB scale employed by Mazesuite, the coloured walls used in the experiment were defined as RGB 204, 178, 127. The experiment was carried out on a laptop in a quiet room in the Psychology Department. Participants were asked to navigate through a computer-generated virtual environment from a first-person perspective using the cursor keys to generate movement. The virtual space was a 55-room environment that had rooms of two possible sizes to examine the location updating effect – large rooms were twice as long as small rooms. The small room took 2 seconds to walk on the short side and 3.2 seconds on the long side. In the large room (which was twice as long as the short) took 6.5 seconds to walk and the short side took 2 seconds (which was the same time as the small room on the short side). It was assumed that people walk at 2m/s and the height of the walls was 2.5m. These dimensions were based on Radvansky et al. (2010). The difference in room size allowed us to control for the time and distance travelled. The small rooms contained one rectangular table and the large room contained two

rectangular tables. They were arranged so that the distance between the last object and the door was the same in both the long and the small room. In addition, the distance between the last object of table 1 in the long room and the first object of the table 2 was the same. The distance between picking up the object and the probe was identical for shift and no shift. Each table was placed along a wall (see Figure 8.1). At one end of the table the object was to be put down and at the other half of the table the object was to be picked up.

The objects were made by combining different colours and shapes from a pre-determined pool. The shapes were a cube, wedge, pole, disc, cross and cone. All objects were made in Blender software and imported into MazeSuite. Following the RGB scale used by Blender, the colours were defined as follows: red (5 0 0), orange (5. 5 0), yellow (8 ,8 ,0), green (0 ,8 ,0), blue (0 ,5 ,10), purple (.4 0 8), white (8, 8, 8), brown (.6 .4 .0), grey (.7 .7 .7) and black (0 0 0). Not all colour and shape combinations were used as probes.

8.2.3. Procedure

Before the experiment began participants were given an information sheet with the following information:

What is the purpose of the study?

We are interested in the recollection of events and the aim of this study is to investigate the ability of people to retrieve information about objects as they move through a virtual environment.

Do I have to take part?

Your participation is voluntary and you do not have to agree to take part. You can withdraw, without giving a reason, at any time if you decide you would no longer like to take part. You can also withdraw your data by contacting either me or my supervisor.

What will I do if I take part?

You will be asked to navigate through a computer-generated virtual environment, which will take about 30min. The virtual environment will be presented on a computer screen and consists of different rooms, which vary in size. You move around using the arrow keys and you will see different objects on tables in the rooms. Depending on the size of the room there will be either one or two tables. Your task is to walk towards the table and pick up the object. Then you take the object to the next table, where you will put the object down and pick up a new one. At different points you will be asked questions about events you have encountered. For example, you will be asked what object you are currently carrying and you will be asked to make a remember/know judgment. Instructions on how to discriminate between remembering and knowing will be given to you before the experiment starts and examples will be provided. As a reimbursement for taking part in this study you will be given participant pool credit or have the chance of winning £50.

Will my data be kept confidential?

All information obtained during the study will be kept confidential and if the data is published it will not be identifiable as yours. You will be allocated an anonymous number for data collection, which will not be connected to your name or identity.

After reading through the information sheet, participants were asked to sign a consent form and then asked to read the information on how to distinguish between remember/know/guess judgements. Training was provided on responding to this aspect of subjectively experienced events, which consisted of a written explanation and examples. Written instructions for the remember/know judgement were the following (taken from Rajaram, 1993):

Remember: If your recognition of the object is accompanied by a conscious recollection of its prior occurrence, then press 'Remember'. Remember is the ability to become consciously aware again of some aspect or aspects of what happened or what was experienced at the time the object was presented. In other words the 'remembered' object should bring back to mind a particular association, image, or something more personal from the time of the study, or something about its appearance or position.

Know: Know responses should be made when you recognize that the object was in the study but you cannot consciously recollect anything about its actual occurrence or what happened or what was experienced at the time of occurrence. In other words, press 'Know' when you are certain of recognizing the object but the object fails to evoke any specific conscious recollection.

This was followed up by an example from the experimenter to make sure the participant had understood the difference. The following real life example was used: A 'remember' response would be given if you walked down a street and saw a person you know very well and you recall details of this person (i.e. their name, how you met them, where you saw them last etc.). A 'know' response would be given in this scenario if you had a feeling of familiarity when you see them but could not recall any details about them (i.e. do not recall how you met them, do not know their name etc.).

Participants were made aware of the fact that the experiment would be in two halves of 24 trials, due to limitations in the maximum programme size. They were instructed to tell the experimenter when they had finished the first half so that the experimenter could open the second file which contained the other half of the experiment. The break in between the first and second half was minimal, approximately 20 seconds. Following this, participants were seated in front of the laptop screen and provided with the chance to move around the

environment for one trial using the four keyboard cursor keys. Pressing the ‘up’ and ‘down’ cursor keys allowed the participant to move forwards and backwards. Pressing ‘right’ and ‘left’ cursor keys permitted the participants to rotate within the room.

The experiment started immediately after the first test trial, which was embedded within the experiment and participants were told about this subtle transition. In the virtual environment, the participants were asked to pick up an object from the table, move to the next table by either walking across a large room or passing through a doorway to the next room and then drop off the object on the table in front of them (Figure 8.1). Picking up and putting down the object was achieved simply by walking towards the correct end of the table. Once the new object was picked up it disappeared so that participants were not constantly reminded of what they were carrying. It was of no relevance whether the participant put the current object down first and then picked up the new object, or vice versa. Once the participant reached the correct table the object was put down but no two objects could be held at the same time. In order to reach the next room, the person had to turn away from the table, which ensured that participants would not go through the already-encountered door. The 55-room environment contained 48 probe trials, which means that participants were not probed at every location. Half of the probes followed a shift across a boundary, while the remainder involved no shift. On each probe trial, participants were required to pick up or put down an object and answer the probe questions using the cursor keys as described above. There was no time limit on moving from room to room or answering the questions, but participants were instructed to complete the task as quickly and accurately as possible.

Probe trials were used to assess the location updating effects on episodic memory and consisted of the following:

1. **Picture of an object in the middle of the screen** (*Is this an object you are carrying or recently have put down?*)

Immediately upon either travelling halfway across a large room (shift condition) or entering a new room (no shift condition), participants were presented, in the centre of the screen, with the choice of one of the recently handled objects and a negative probe/foil made from a combination of another shape and colour that had not been experienced recently. Negative probes (which required a 'No' response) were generated by recombining the object and colour name for the two positive objects. For example, if the carried object was a red cone, and the set-down object a blue cube, the probe might be a blue cone. Participants were instructed to use the 'up' and 'down' cursor to respond 'yes' if the probe was either the object that was currently being carried or the one that had just been set down (positive probe). They were to respond 'no' to all others (foil/negative probe). Half of the probes occurred after a spatial shift and half did not. There were 24 positive probes and 24 negative probes. The order of objects and questions were not counterbalanced across participants due to restrictions of the program. However, the appearance of shift and no-shift probes was counterbalanced.

2. **Recollection judgement** (Do you remember / know / guess this?)

Participants were then asked to make a 'remember', 'know', or 'guess' judgement by using the 'up' and 'down' keys. As previously explained the instructions were based on Rajaram (1993) and have been used successfully by Easton et al. (2012).

3. **Confidence rating** (*How confident are you in your response?*)

Following this, participants also rated their confidence in their own responses (ranging from very confident, confident, unconfident to very unconfident), by using the 'up' and 'down' keyboard cursor keys. Participants were encouraged to use the whole range of ratings.

The experimental procedure lasted between 20 and 25 minutes. After the final trial a message appeared on the screen, which indicated to the participant that all trials had been completed. They were given a debrief sheet and given the opportunity to ask any questions they had.

Figure 8.1. Screenshot of the virtual environment with the tables and objects that were to be picked up and dropped off.



8.3. Results

Due to limitations in the maximum programme size, the procedure was divided into two halves. There was a short break between the first 24 trials and the last 24. It was therefore of interest whether this made a difference to participants' performance. The proportion of the errors made in the two halves, which was the dependent variable, were calculated and a paired t-test was used to compare performance. There was no difference between the first and the second half of the experiment, either in the shift or in the no shift conditions ($t(44) = 0.441, p < 1$; $t(44) = -0.680, p < 1$ respectively). Hence the data were combined and analysed together.

The response time and the proportion of errors are summarised in Table 8.1. Overall, participants were probed for their memory either halfway across a room (no shift error rate = 0.09) or after leaving a room (shift error rate = 0.14). The proportion of errors was significantly higher in the shift than in the no-shift condition $t(44) = 3.131, p = 0.003$. Thus,

there was a location-updating effect, with participants performing worse after a spatial shift than without one. Participants responded faster when there was no spatial boundary (RT = 3439 milliseconds) than when there was a boundary (RT = 3753 milliseconds) and this difference was significant $t(44) = 3.476$, $p = 0.001$. When response times were separated in to incorrect and correct responses, it was found that the difference came in the correct responses $t(44) = 2.477$, $p = 0.017$. The incorrect responses in shift and no shift, showed no difference in reaction time $t(44) = 1.463$, $p = 0.151$.

Table 8.1. The response time and the proportion of errors in the shift and no-shift condition.

| A. | | |
|-------------------|----------------------|------------------------|
| Probe type | Error | Response Time |
| <i>Shift</i> | 0.1389 (SEM = 0.016) | 3753.09 (SEM = 135.54) |
| <i>No Shift</i> | 0.0972 (SEM = 0.017) | 3439.74 (SEM = 119.68) |

| B. | | |
|-------------------|-------------------------|---------------------------|
| Probe type | Correct Response | Incorrect Response |
| <i>Shift</i> | 3680.74 (SEM = 135.33) | 4822.12 (SEM = 1012.37) |
| <i>No Shift</i> | 3450.37 (SEM = 119.77) | 3229.35 (SD = 331.37) |

A. Mean response time (in milliseconds) and errors (in proportion) with standard deviation for the shift and no-shift condition. **B** Additionally, mean response times for correct and incorrect responses were analysed separately in the shift and no-shift condition.

Probe trials were either positive (in which a recently handled object was presented, and a correct response was ‘yes’) or negative (in which a foil object was presented, in which case the correct response was ‘no’). Incorrect responses were analysed separately for their responses and their recollection/confidence judgement. To investigate whether participants performed better in positive or negative probes, a repeated measures ANOVA was conducted using condition (shift or no-shift) and probe type (positive or negative) as factors. Participants made fewer correct responses after a spatial shift (see Table 8.1), but there was no effect of probe type $F(1,44) = 0.860$, $p = 0.359$), suggesting that it made no significant difference to

the proportions of errors whether participants incorrectly respond ‘yes’ or ‘no’ to the probe. No interaction between condition and probe type $F(1,44) = 0.827, p = 0.368$ was found.

Table 8.2 breaks down the number of correct and incorrect responses according to recollection judgements. Each participant underwent 48 trials and each response was reported as one of three types of subjective experience (remember, know, guess). Overall, remember made up 78%, know 17%, and guess 1% of the total responses.

Table 8.2. Breakdown of the mean number of correct and incorrect responses, in which participants said they remembered, knew or guessed the object shown on the laptop screen.

| | Correct | | Incorrect | |
|-----------------|--------------|-----------------|--------------|-----------------|
| | <i>Shift</i> | <i>No Shift</i> | <i>Shift</i> | <i>No Shift</i> |
| Remember | 16.16 | 18.07 | 1.67 | 1.44 |
| Know | 3.71 | 2.91 | 1.07 | 0.47 |
| Guess | 0.62 | 0.49 | 0.56 | 0.36 |

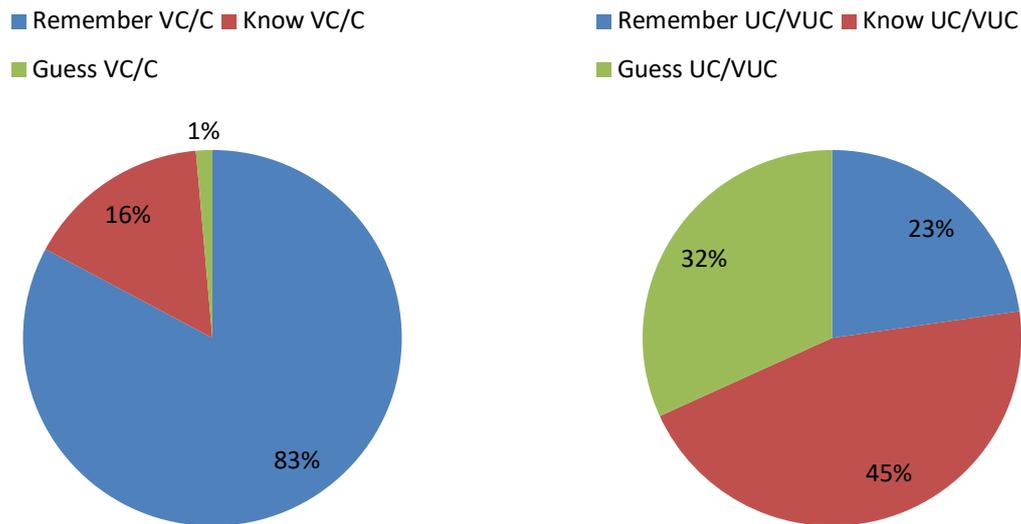
This is a breakdown of the number of correct and incorrect responses according to recollection judgements. Each response was reported as one of three types of subjective experience (remember, know, guess).

Breaking down the correct and incorrect responses (see Table 8.2), and using condition (shift or no-shift) and recollection judgement (remember, know, guess) as factors a repeated measures ANOVA showed that there was a significant effect of shift $F(1,44) = 10.46, p = 0.002$, a significant effect of recollection judgement $F(2,88) = 295.02, p < 0.001$, and a significant interaction between the main effects, $F(2,88) = 10.21, p < 0.001$. A pairwise comparison showed a clear difference between remember and know ($p < 0.001$) and between remember and guess ($p < 0.001$). There was a higher proportion of remember responses in the no-shift condition than in the shift. There were also a higher proportion of know responses in the shift than in the no-shift condition.

Given the low number of number of guesses, it was decided to analyse only the correct remember and know response. A repeated measures ANOVA (condition x recollection judgement (without guesses) showed that there was a significant effect of recollection judgement $F(1,44) = 196.883$, $p < 0.001$ and a significant interaction between recollection judgement and shift condition $F(1,44) = 10.851$, $p = 0.02$. The data suggest that more remember responses were given in the shift condition (16.16, $SD = 4.76$) than know responses (3.71, $SD = 2.91$). In the no shift participants were more likely to remember (18.07, $SD = 4.41$) and gave more remember responses than know responses (2.91, $SD = 3.08$) when correct answers were given compared to the shift condition.

Participants were also asked to rate their confidence in their judgement of whether the objects shown had been encountered previously on a scale ranging from very unconfident to very confident. The confidence ratings of the answers given were considered to investigate the possibility that know responses were less accurate simply because they represented low-confidence answers (see also Easton et al. 2012). Subjects could be more confident in their own response when they indicated they remembered an object rather than when they merely knew about the object. The proportions of the confidence levels and all recollection judgements are summarised in pie charts in Figure 8.2 which shows the percentage of very confident and confident responses when a correct remember, know or guess judgement was made. It also shows the percentage of very unconfident and unconfident incorrect remember, know and guess responses. Only those responses rated as very confident and confident were further analysed.

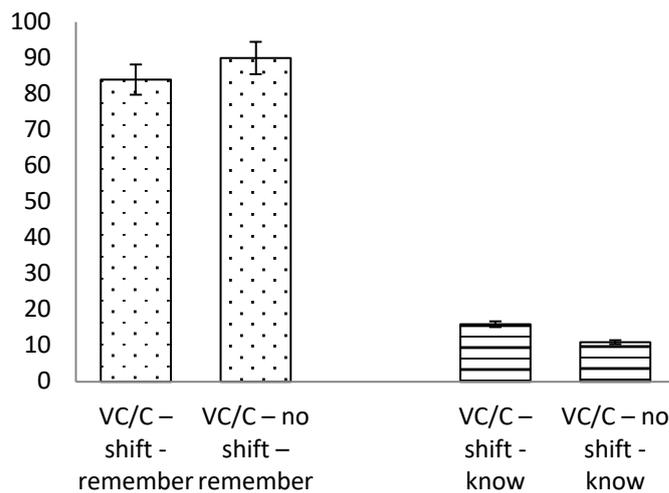
Figure 8.2. Confidence levels and recollection judgements (in percentage).



Percentage of very confident of correct (left) and incorrect (right) responses. Proportion of very unconfident and unconfident of correct and incorrect responses.

The confidence levels of participants were looked at more closely, because it may have been that the ‘know’ responses merely reflect a lack of confidence and by comparing only ‘confident remember’ and ‘confident know’ responses I control for this problem. An effect of shift was found $F(1,44) = 4.596, p = 0.038$, an effect of recollection $F(1,44) = 227.342, p < 0.001$ and an interaction $F(1,44) = 9.574, p = 0.003$ (Figure 8.3). A paired t-test showed that there was a difference between know and shift/no shift condition ($t(44) = 2.063, p = 0.045$). Furthermore, there was a significant difference between remember and shift/no shift ($t(44) = -3.207, p = 0.002$). This means that participants gave more correct answers in no-shift when they remembered and less so in the shift condition. However, they show the opposite pattern when they responded ‘know’. More correct know answers were given in the shift condition than in the no shift condition.

Figure 8.3. Correct responses (in percentage) in the two conditions and associated high confidence levels



Percentage of correct responses in shift and no-shift depending on their recollection judgement. VC = very confident; C = confident.

Overall, this experiment demonstrated location-updating effects in a virtual environment, which replicated Radvansky et al's (2006; 2010) finding of a doorway effect. People made errors following a spatial shift than when they merely moved across a room, supporting the idea that people need to update their event model following a change in location. Furthermore, it was shown that episodic memory relies on remember responses.

8.4. Discussion

This experiment assessed the role of location updating effects in episodic memory. Participants showed poorer memory for items after a spatial shift than after no spatial boundary, suggesting the need to update an event model affects one's ability to remember. This aspect of the study replicated the findings of Radvansky and colleagues (for example Radvansky & Copeland, 2006). The novel aspect of the current study was that this effect was only apparent for events that participants reported as 'remembered'. As such, Radvansky's claim that the location updating effect is a reflection of episodic memory processes is

supported by the results of this study. Furthermore, moving from room to room affected people's recollection of an event, but their confidence played no major role in performance.

Moving from one location to another serves as an event boundary, which changes the availability of information. The results of this study have shown that participants made more errors in the shift condition than in the no shift condition. The shift in location had an impact on episodic memory and the data are in line with other findings in which remembering information was affected by the process of updating an event model (see Radvansky & Copeland, 2010; Swallow et al., 2009). Participants also responded faster when there was no spatial shift compared to a spatial shift. This is in line with studies looking at text comprehension which included spatial shifts and no shifts in reading passages. People read more slowly when they encounter a spatial shift in a text (Zwaan, Magliano, & Graesser, 1995) and they organise the events within the text by spatial information (Zwaan, Langston, & Graesser, 1995). Furthermore, a higher proportion of *remember* responses were given in the no-shift than in the shift condition, but a higher proportion of *know* responses were seen in the shift than in the no-shift condition. Additionally, when *remember* responses were given, more correct answers were recorded when there was no spatial shift and fewer correct answers after a spatial shift. However, when *know* responses were given, more correct answers were recorded in the spatial shift condition and fewer correct responses after no spatial shift.

The current experiment also looked at *remember/know/guess* judgements and the associated confidence ratings, as it is essential to investigate the possibility of the remember judgement just reflecting a higher confidence level. Subjects could be more confident in their own response when they indicated they remembered an object rather than when they merely knew about the object. Episodic memory relies on recollection of an event and participants

should have indicated that they *remember* when the memory is truly episodic. *Know* responses on the other hand indicate the process of familiarity without a recollection aspect. *Knowing* could simply reflect lower confidence ratings and would therefore be more likely to be incorrect and *remember* responses are typically higher in confidence (Wixted and Squire, 2011). Considering this as a confound variable in this experiment, I only investigated the high confidence responses and compared them with the overall responses (including all confidence ratings) (see also Easton et al., 2012). When only high confidence answers were examined the results remained unchanged in the shift and no-shift condition. Therefore, confidence in itself cannot explain the difference between the spatial and no spatial shift.

In view of the wider implications of this study, the theory of event segmentation explains the location updating effect. Event segmentation occurs when an event boundary is encountered and a new event model is required. The event model for the previous event then declines in availability until it transfers to a background level (Radvansky et al. 2011). In this case it is assumed that only one event model can be active at a time. Therefore, the event model that is currently active is promoted and retrievable, whereas the previous one is not. Information that is being actively processed in the current event is more available (Glenberg et al, 1987). Considering the availability of information, another explanation of diminished episodic memory after a spatial shift could be the overloading and interfering memories from previous events. When participants carry one object from one room to another (spatial shift condition) then the object is associated with two locations (the picked up location and the one where it is being carried to), introducing interference and competitive retrieval at the next probe. Thus, when a probe appears after a shift, two different kinds of information will compete and make retrieval slower (which is what was found in the current study) (Bower & Rinck, 2001; Radvansky, 1999). According to the event horizon model information may be

less available after a shift because people segment actions into events based on event boundaries. As part of recognition we are trying to select a single memory trace, but two events (moving from room to room) interfere with one another, which results in increased error rates and slower response times. Attention may move from one event to the next and the shift influences the available knowledge about the objects (Rinck & Bower, 1995). When there is no spatial shift then there is only one event involved, but when there is a spatial shift there are two events – that is, the current and the previous location. Overall, doorways act as a spatial boundary, which initiates the updating of people’s event models. This updating reduced the information, which was available about objects and shows that the structure of our environment affects our experiences.

An alternative explanation of the location updating effects involves the involvement of a much simpler memory process. The current task can also be seen as a short-term memory task, which is believed to last only for a few seconds. Using doorways as a transition from one context to another, the task could be solved by rehearsing the name and colour of the object as one moves through the virtual environment, which could improve performance in the shift condition if no distractor task is used. However, the current task only involves a small memory load, but yet there was a very significant difference in error rates between the shift and no shift condition. Therefore, the structure of the environment does have a major impact on episodic memory.

The event-indexing model suggests that situations are based on how an event is perceived and humans separate events according to space and time (Radvansky et al., 2011). Human episodic memory is spatiotemporal in nature (see O’Keefe and Nadel, 1978) and short-term memory is interrupted by the presence of a spatial boundary. If participants see two objects that are separated by a boundary (spatial or temporal) it will be the association between the object and the boundary which will determine how the event is segmented. Generally

speaking it is more difficult to remember two sequential and similar events that are separated by a boundary, than to remember two separate events that happened within the same boundary (Zacks et al., 2001). It has been shown that temporal order of objects is affected by event boundaries (DuBrow & Davachi, 2013). It is possible that the last object seen in the previous room will no longer be available in working memory when the next object is encountered in the other room.

I was interested in how people monitor space and boundaries and also how people change their perception of an event, as these changes can affect the availability of information. The analysis has shown that participants more often responded correctly with a *remember* judgement in the no-shift condition, which is what was predicted. In contrast, participants showed the opposite trends when they responded with *know*. An increased number of *know* answers were given when a spatial boundary was encountered, compared with an increased number of *remember* responses when no spatial boundary existed. This study shows that episodic memory relies on *remember* responses rather than *know*. It is possible that performance of participants is better for *know* when they go through a door (spatial shift), because they lose some of their episodic experience of the event. By shifting from one location to another the nature of the experience has changed and participants effectively transfer from a conscious recollection process to a process of familiarity. However, this interpretation is somewhat speculative because there was no explicit prediction about how the spatial shift would affect performance and *know* responses. It was simply predicted that there would be a location updating effect for *remember* responses, but no effect for *know*. Further exploration of this effect is necessary to determine its nature.

Due to unexpected participants' behaviour, changes to the experimental set-up are necessary. It was observed that some participants did not proceed through the rooms in the right order

and were excluded from the study. Therefore, it is vital to make sure that the program used to design the virtual environment leaves the experimenter with enough flexibility to ensure that for example the door to the next room should not be open until the participant has put down and picked up the new object and to have an invisible barrier that would prevent participants from returning to a previous room. Another change includes the presence of objects on tables. When a participant entered a room objects that were to be picked up could be seen immediately, which could have affected memory. By seeing objects in advance it could have increased interference and memory load. If the participant focuses too much on the object ahead they might forget about the object they are carrying.

The colour or pattern of walls could also be changed to add in contextual differences (see Horner et al., 2016). This might enhance recollection in this study, as it could help the participants to distinguish different rooms and its associated objects and therefore decrease interference. Furthermore, contextual reinstatement could be tested by having the participant return to an earlier room to test environmental context-dependent memory. Chapter 6 of this thesis investigated contextual reinstatement in rats. The object-location-context (OLC) task was set up in a way which allowed us to test multiple test phases. Although this study did not show reliable context reinstatement as it was hypothesised, it was found that when a context was repeated (Chapter 6, Experiment 2), animals were unable to seek out the novel object-location-context configuration (i.e., they showed no episodic memory). The use of the same context may have hindered retrieval by increasing interference from previous events. Therefore, a follow-up experiment (Chapter 6, Experiment 2) used multiple contexts and animals' performance was maintained throughout the task. Context is an important part of learning and memory and it is well known that the hippocampus is critical for encoding contextual information. Returning to a previously encountered context will cause the hippocampus to facilitate pattern completion and to enable associative retrieval of the

relevant information. Learning new information is associated with learning about the contextual information in an environment (virtual or real life) and the context acts as a retrieval cue (Godden & Baddely, 1975; Smith, 1988). Therefore, it would be worth investigating how different contexts would affect the location updating effects in this episodic memory task for humans in a virtual environment.

Another way to overcome interference is through pattern separation, which was also investigated in Chapter 4. The hippocampus will be expected to play a key role when pattern separation is required in episodic memory, especially when interference must be overcome. Doorways could interrupt pattern separation and future studies using fMRI could examine how participants overcome this kind of interference using behavioural pattern separation.

In conclusion, this chapter investigated the role of location updating effects in episodic memory. People showed poorer memory for items after a spatial shift, suggesting that updating an event model affects one's ability to remember. In an earlier study by Radvansky and colleagues it was shown that memory declines when people move from one location to another and this effect was replicated. Furthermore, moving from one location to another affected people's recollection of an event, but not their confidence in their response

Chapter 9

Discussion

9.1. Aims & findings

The overall aim of this thesis was to explore episodic memory, interference caused by similar events and its demands on hippocampal function by using different methodological and practical approaches in rats and humans.

The first objective of this thesis was to develop different tasks of episodic memory to investigate its demands on the hippocampus without causing interference and to address methodological issues in previous studies. Different tasks of episodic memory, such as stable object-location-context, unstable object-location-context, stable location-context and unstable location-context, were tested in rodents to replicate previous findings by Easton et al. (2011) in Chapter 2. The long-term aim of this study was to test hypotheses on place cell remapping and to speed up testing procedure to make the tasks more suitable for testing neural mechanism. These tasks were to provide us with an opportunity to test animals with the same lesions as in Easton et al.'s (2011) study to see if the previously found difference in performance was due to the task or to the stability of locations. However, no reliable object discrimination was found in any of the tasks when they were run over two testing sessions within the same day. This was a surprising result given that other studies have reported data in which SOR tasks and episodic memory tasks were run in a single day. It was argued that the repeated use of similar tasks alternating within a day caused interference. Hence, future studies should consider the effects of proactive and retroactive interference carefully, as these can heavily influence rodents' memory for novel environmental configurations. The method of running two different tasks within a day was re-evaluated based on the non-significant

findings and therefore Chapter 3 focused on the use of multiple trials in a newly developed continuous trial apparatus for rodents.

Multiple trials have been successfully carried out before, thereby reducing the number of animals used, but reliable measures of discrimination were only found in simpler versions of recognition tasks (Albasser et al., 2010; Ameen-Ali et al., 2012). Albasser, et al. (2010) developed a new object recognition test, using a 'bow-tie maze' in rats. They combined features of the spontaneous recognition task with the delayed nonmatching-to-sample task. The bow-tie maze was successful in detecting novel object discrimination in rodents, however the design of it would not allow to investigate spatial components of memory. Therefore, Ameen-Ali et al. (2012) developed a continuous apparatus that allows for multiple trials per testing session, which relied on measures of preferential exploration of novel objects and in novel contexts. Whereas this approach was successful in testing typical spontaneous recognition tasks it failed to provide reliable results in an episodic-like memory task in rodents.

Study 2 (Chapter 3) addressed an alternative approach asking whether episodic memory tasks can be tested over multiple exposures to similar events (i.e. trials). In order to understand the neural processes involved in memory it is essential to develop tasks that can be run closer together in time. Furthermore, this reduced the number of animals used in an experiment and made significant contribution to the 3Rs. All episodic and spatial tasks were performed above chance, adding to the work of Ameen-Ali et al. (2012). Most importantly, no interference was found, despite running many similar trials after another.

The use of multiple trials yielded to an interesting study, which is summarised in Chapter 5. Interestingly, when the unstable OLC task (see Chapter 5) was investigated more closely it was found that animals showed a tendency to re-visit the location in the arena which was previously occupied by an object. This observation led to Study 4 (in Chapter 5), which

investigated the effects of odour on a model of episodic memory. are investigated by using floor rotation in the unstable object-location-context task. Determining whether animals use odour to solve episodic and spatial memory tasks is essential, as lesion studies need to be carried out with reliable tasks that can be run closely in time.

If we want to understand episodic memory fully, we cannot only consider data on a behavioural level. The behaviour must be linked to neuronal mechanisms underlying it. Therefore, to study the role of particular neurotransmitters (such as acetylcholine), we must develop reliable memory tasks that test episodic memory and understand the underlying brain mechanisms and structures which support that kind of memory. By using four different contexts it was possible to investigate episodic memory in rats, which can now be used to enhance our understanding of neuronal activity in hippocampal sub-regions (CA1 and CA3). Overall, the first aim was achieved by providing a reliable methodology to test episodic memory in rats. Using this approach, we are able to investigate neural processes in the hippocampus and its surrounding areas in a continuous trials apparatus.

Using the approach which was developed in Chapters 2 and 3, the second aim (Chapter 4) of this thesis was to investigate the effects of interference between trials on rats with cholinergic lesions to the medial septum. Based on a study by Easton, Fitchett, Eacott, & Baxter (2011) it was hypothesised that MS/vDB lesions could impair episodic memory in rats, because of the built up of interference caused by multiple trials. This would be in contrast to the one-trial/day version, where rats with cholinergic lesions were unimpaired in an OLC task, but impaired in the LC task. Rats with selective lesions of basal forebrain neurons in the medial septum and vertical limb of the diagonal band, which caused a cholinergic depletion of the hippocampus, were tested. Furthermore, this study aimed to build on the results of study 2 (Chapter 3) to determine how effectively episodic memory can be tested in a multiple trial

apparatus in intact as well as lesion animals. It was found that earlier findings by Easton et al. (2011) can be replicated in this new apparatus, leading to a significant reduction in animals used in lesion experiments. Studies in animals have widely supported the role of acetylcholine in episodic memories and indicate a role for ACh within the hippocampal system and its surrounding structures (e.g. Baxter, 2001; Drachman, 1977, Easton et al., 2011; Leutgeb et al., 2004). The localisation of the cholinergic function has been investigated by using a various testing methods, such as local infusions of scopolamine, application of cholinergic agonists or selective lesions (Hasselmo, 1999; 2005; 2006). Lesions of cholinergic neurons can be induced by injections of the toxin Saporin. Selective cholinergic lesions of the medial septum (as in Chapter 4) do not cause damage to some versions of episodic memory tasks, as it was shown in Study 3. It has been suggested that the neurotransmitter GABA can be used to substitute ACh in spatial memory tasks (Pang & Nocera, 1999), but lesions to the cholinergic and GABAergic system causes major impairments (Pang et al., 2001). Hence, the cholinergic neurons may be involved in some memory processes, but memories can be retrieved and formed without cholinergic projections (Parent and Baxter, 2004). The model presented in Chapter 4 aimed to provide a framework for understanding the role of ACh's input from the medial septum in a model of episodic memory using multiple testing sessions. Crucially, there was no evidence of increased interference caused by the overlap of similar events in the continuous trials apparatus, making it ideal for further lesion studies. When testing associative memory interference during encoding is an important issue, especially in region CA3, but also in other regions of the hippocampus). Therefore, we need to overcome the problem of interfering memories during encoding, which requires a clear separation of events. If encoding and retrieval are not separated fully, recollection of memories can fail.

Given that ACh is involved in responding to novel information and in encouraging learning of new (over old) configurations (see Easton et al., 2012 for review), it would be beneficial to investigate place fields in the subfields of the hippocampus (such as CA1, CA3 and DG). These areas are particularly responsive to changes in the environment (Lever et al., 2010; O'Keefe & Nadel, 1978) and cholinergic lesions of the hippocampus lead to different firing patterns in CA1 and CA3 (Ikonen et al., 2002). In Chapter 4 the discrepancy in performance could not have been due to the tasks per se, but due to the use of different of locations. In the location-context task objects change locations between exposures and test, meaning there is no stability of object location, whereas in the OLC task locations of objects remain stable. Rats could have been unimpaired in the task in which locations remained constant, because place cell maps did not have to be remapped (Easton et al., 2011). However, when locations continued to change between events, remapping became essential and rats with cholinergic lesions performed at chance (Easton et al., 2011).

Future research involving the OLC and LC task could involve the investigation of direct and indirect pathways between the prefrontal cortex and the hippocampus (see Eichenbaum, 2017 for review). These areas of the brain have been claimed to play a complementary role in episodic memory in rodents and humans, as these interactions are involved in remembering and learning events. Given the uncertainty around acetylcholine's involvement in memory, observations into neurobiological pathways between the hippocampus and the PFC could further guide the examination of the role of this neurotransmitter and its connections to different components of the brain system. The aim of demonstrating the reliability of the dissociation within the hippocampus based on cholinergic function within the hippocampus, and the verification the new apparatus as assessing episodic-like memory in the same manner as earlier studies, without causing proactive interference, was achieved and can now be used

to investigate cognitive deficits in patients with hippocampal brain damage or mental illnesses.

The final aim of this thesis was to provide a link between human and rodent research, to address how animals and humans separate events and how they use contextual information for segmentation to avoid interference in episodic memory.

Study 4 measured memory performance in an object-location-context task without the presence of some objects at test. This study is a follow-up to study 2 (Chapter 3), where it was found that animals showed memory for objects, which were not physically present in the maze by showing an interest (i.e. by increased exploration) in that area. An alternative version of the OLC task allowed me to assess the memory for location of objects in unstable conditions across multiple trials. Animals were tested over three weeks and it was found that the rats' episodic memory was not based on odour in this task, but on recollection. The presence of an object at test could merely introduce a familiarity decision, which would not be truly episodic. Investigating the effects of odour cues on performance in the OLC and LC task by rotating the floor of the apparatus was crucial, as rats have the ability to use their olfactory system to solve spatial tasks (Maaswinkel & Whishaw, 1999; Means et al., 1992; Wallace et al., 2002). The task in Study 4 (Chapter 5) was similar to the E-maze by Eacott et al. (2005) where objects were present but out of sight at the point of decision-making in order to investigate recollection and familiarity based mechanisms in rodents. However, the E-maze was not a very reliable apparatus in terms of providing robust data. Considering the wider implications of Study 4 and its use for future studies, it is noteworthy that this kind of recognition task is suited to study place and trace cells in the hippocampal formation. Especially the lateral entorhinal cortex (LEC) has been shown to provide information about the spatial environment (Deshmukh & Knierim, 2001; Kuruvilla & Ainge, 2017; Tsao et al.,

2013; Young, Otto, Fox, & Eichenbaum, 1997). Neurons in the LEC area could be used to investigate recollection of past experiences in rodents. Object cells are known to fire at objects and trace cells fire where an object had been on a certain trial (Tsao et al., 2013). Object and trace cells must serve different purposes, because only an object cell responds to the object itself. Unlike place cells trace cells also follow an object when it has moved location. This suggests that lateral entorhinal cortex neurons provide information about the presence of specific objects at specific spatial locations, and contexts.

Using contextual cues to seek out novel environmental configurations was investigated in Study 5 (Chapter 6). Contextual information which can take any form and does not have to be tactile in nature (see Study 6) is an essential part of learning and memory.

The hippocampus is critical for encoding information about contexts and one of its role is to prevent pro- and retrospective interference by using contextual information to separate events. In study 5 it was of interest whether multiple sample and test phases can be conducted without disrupting memory or rats. A slightly different protocol for the object-context-location task was developed in order to see if preference for novel configurations could be maintained. It was of interest if rats could maintain preference for novel OLC configurations over multiple sample and test phases. Interestingly, it was found that rats are able to demonstrate episodic memory when the environment contains multiple contexts (two vs four contexts were tested). This finding suggests, that memories for an event that occurs in a specific context, will cause the hippocampal context code to be re-expressed when the relevant context is revisited (Smith & Bulkin, 2014). Context acts as a retrieval cue and minimised interference from previous events. In human and animal research the definition of context is very broad. We need to be clear about the nature of the context and how we use it in experiments. Research question, procedural demands and layout of tasks will determine what nature of context to use.

As such, Study 6 tested rats tested on the location-context task in which contexts were not only defined in terms of its physical properties, but also in terms of flavours. The aim of this experiment was to investigate how animals separate the trials (i.e. events) in the LC task, as this task requires object change on every exposure. Some of the events were experimenter defined, but others were pseudo trials (i.e. trials within trials). Events are therefore less distinguishable than in the OLC task that only changes objects after each trial. Six different flavours were used to encourage animals to segment trials; however this did not lead to results above chance. This chapter was supposed to be the link between the rodent and human experiments in this thesis. However, due to developing severe allergies to rats the study could not be run as intended and ended early. The effects of flavour to separate events (trials) could not be demonstrated, but this study still provides a good link to the next chapter, which looks at location updating effects in episodic memory in humans. Despite not fully achieving the aims of this study, it provided a good starting point for future work on using variants of contexts for event segmentation in rodents.

Moving away from animal research, the aim of the last study was to investigate the ability of people to retrieve information about objects as they move through a virtual environment and link it to previous findings of animal studies. Improving the links between human and rodent models provide researchers with an opportunity to further reduce the number of animals used, but human memory tasks are often carried out very differently as participants are able to express themselves verbally. In humans the basic paradigm for recollection in memory involves asking people to make a judgement about the nature of their memories. Using a virtual reality task Radvansky and Copeland (2006) demonstrated a detailed assessment of the influence of changing events on memory. Leading on from research in Radvansky's lab, I tested participants memory of objects and their experience associated with it in a virtual environment. In previous studies the episodic nature of memories was not considered. As in

animal studies, Radvansky's virtual reality task using doorways could be solved in several ways using familiarity or recollection-like processes. Hence, Study 7 builds on their work by including a recollection and confidence judgment when a shift in location was encountered. It was found that doorways impact memory negatively, meaning that participants' memory for objects were worse after a shift in spatial location than after no spatial shift. Hence, there is the need to update an event model which affects one's ability to remember. This aspect of the study replicated the findings of Radvansky and colleagues (for example Radvansky & Copeland, 2006). Furthermore, the novel aspect of Study 7 was that this 'doorway effect' was only apparent for events that participants reported as 'remembered', which suggests that the location updating effect is a reflection of episodic memory processes.

Despite the importance of context for episodic memory (as shown in Chapters 2-7), very few experiments have investigated all three aspects of object-location-context at the same time. Various studies, including animals, neuroimaging and patient studies, have shown that the medial temporal lobe plays an essential role in encoding and retrieving memories (Kirwan & Stark, 2007). Additionally, some frontal lobe regions may play a role in episodic memory retrieval, but this was beyond the scope of this thesis, hence I suggested to look at the involvement of PFC in Chapter 4 as a follow up study (for reviews see: Eichenbaum, 2017; Preston & Eichenbaum, 2014). Most theories of episodic memory either focus on the role of the MTL (Squire & Zolan-Morgan, 1994) or on the role of the PFC (Eichenbaum, 2017; Fletcher et al., 1998), without explaining the interaction between these two brain structures. Another way to investigate different brain regions involved in episodic memory and interference is functional magnetic resonance imaging. Using functional magnetic resonance imaging (fMRI) and a virtual reality environment Burgess, Maguire, Spiers and O'Keefe (2001) looked at a computer-generated environment in which participants were presented with characters and objects in different locations. During scanning participants

were tested on their memory for the three components. A network of areas was identified to be involved including prefrontal areas and hippocampal formation. This is consistent with the idea that those areas are required to overcome interference caused by similar events. During a fMRI scan it would be possible to run the task used in Study 7 (Chapter 8) to investigate the activation of for example, the parahippocampal gyrus during retrieval of spatial location information, or the prefrontal cortex during the retrieval of contextual and spatial information. Further evidence is needed to explain the neurobiological mechanisms underlying interference and how it is solved. Other animal studies have shown that the hippocampus (sub regions CA1 and CA3) is strongly involved in pattern separation (and completion), however there is little evidence in humans (Buckner et al., 2001; Kirwan & Stark, 2007; Lee Hunsaker, Kesner, 2005; Leutgeb et al., 2004; Zeineh et al., 2000). High resolution fMRI could provide useful insights, but the overlapping nature of episodic memories are challenging and studies have to be designed carefully.

9.2. Conclusion

Exploring the broader issue of interference, event separation and its demands on hippocampal function, research has shown that contextual inference plays a critical role in episodic memory. Throughout this thesis it was demonstrated that context plays a profound role in memory and it can cue memories associated with it in rats and humans. Studies such as by Godden and Baddeley (1975) have supported the notion of contextual cues in memory. When participants were asked to learn item in context X then they were better recalled when they were tested in the same context X and not in context Y (see also Smith, 1988). Several theories have been proposed to explain this phenomenon. It is now well known that the hippocampus is heavily involved in contextual processing. More specifically, animal studies

investigating brain lesions have shown that the hippocampus is greatly implicated in spatial mapping and its associated environmental context (for example: Nadel & MacDonald, 1980).

In this thesis, I focused on the nature of contextual representation and its function to prevent interference (for more research see also Eichenbaum et al., 2012; Rudy, 2009). Firing patterns in the hippocampus, such as seen in place cells, time cells, trace cells and border cells, are highly sensitive to context. Clearly, neurons not only change their activity patterns in response to spatial switches, but also to changes in task demands, time, and olfactory cues (Eichenbaum, Otto, & Cohen, 1994; Wood et al., 2000). As it has been explained previously in Chapter 1, context can take many different forms and there is an obvious need to define the concept of context, but neuronal response patterns are normally unique to a specific context. Hippocampal context representations must have an adaptive value for rats and humans, but it still remains to be established what exactly that is. Based on the findings in studies in Chapters 2-8, I have concluded that context is critical in separating and recognising similar events. The primary function of contextual representations may be that encode new exposures to contexts and recognise familiar contexts which we have already been exposed to. Hence, context plays a critical role in episodic memories, which are all unique but sometimes very similar. Episodic memory is part of our daily lives and very prone to interference, as it functions at a very high mental level and requires a lot of capacity. Using contextual retrieval cues can assist priming of relevant memories when needed and reduce interference from other memories. Pattern separation and completion are two hippocampal mechanisms for preventing interference and as it has been shown in Chapter 4, animals with lesions to the cholinergic system were unable to form memories associated with contexts and places. Due to rat allergies, it was not possible to further test the involvement of hippocampal neurons in Chapters 5 to 7, but it is to be expected that they will be able to differentiate contexts and episodic memories for individual trials in the presented experiments.

Overall, this thesis focused on three separate aims, which were drawn together in the end by explaining the effects of interference on episodic memory in different behavioural tasks in rats and humans. Issues with previously used spontaneous recognition tasks were evaluated and new models of episodic memory were developed. This groundwork was necessary so that these tasks can now be utilised in the continuous trials apparatus. The apparatus made a significant contribution to memory research, as it is possible to run multiple trials within one animal without causing interference from previous experiences. Being able to run multiple trials with variations of the object-location-context task will be advantageous in lesion studies, which further could investigate effects of acetylcholine in the hippocampal formation and the prefrontal cortex. Furthermore, the apparatus has shown to be valuable in evaluating the effects of contextual information as a measure to prohibit interference in episodic memory. The behavioural tasks have told us a lot about their demands on hippocampal function in rodents, but it is also essential to link animal findings to humans to enhance our understanding of neurological diseases. Consequently, the successful work on location updating effects in episodic memory in a virtual environment provides an excellent starting point for a new direction of research, including functional brain imaging and patient studies, based on this thesis.

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