Associative Processes in Recognition Memory

Emma Whitt, BSc.

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

December 2011

Abstract

Recognition memory, or the discrimination between novelty and familiarity, is well predicted by an associative model of memory (Wagner's SOP). In this thesis I examined predictions from this model concerning priming of stimuli, and stimulus spacing, in rats' object recognition. Priming of an object resulted in a bias in behaviour towards the non-primed object. This may be due to associative processes, as described by the SOP model. Spacing stimuli in a sample stage of an object recognition task resulted in longer-lasting or better discrimination in a test of familiar versus novel object, as predicted by the model. Incorporating a short or long delay between sample and test led to better discrimination after a short delay, though differences in stimulus spacing conditions at each delay were not significant. I also examined recognition using stimulus generalisation. Generalisation of a conditioned response occurred between stimuli that shared elements of familiarity. Although not significant, familiarity generalisation may have been less apparent in animals with lesions to perirhinal cortex, providing some support for the suggestion that perirhinal cortex has a role in novelty/familiarity discrimination. The main conclusion was that recognition memory, as measured by the object recognition and generalisation tasks, might involve associative processes.

Experiment 10 of Chapter 4 was published in the following article:

Robinson, J., Whitt, E. J., Horsley, R. R., & Jones, P. M. (2010). Familiarity-based stimulus generalization of conditioned suppression in rats is dependent on the perirhinal cortex. *Behavioral Neuroscience, 124*, 587-599.

Experiments 3 and 4 of Chapter 2 are in press:

Whitt, E., Haselgrove, M., & Robinson, J. (2011). *Indirect Object Recognition: Evidence for Associative Processes in Recognition Memory.* Manuscript accepted and in press.

Acknowledgements

My gratitude goes to my two funding bodies: the School of Psychology, The University of Nottingham and the Biotechnology and Biological Sciences Research Council (BBSRC, Doctoral Training Award in Integrative Physiology).

I thank my supervisors Jasper Robinson and Mark Haselgrove for guiding my through the last three years and introducing me to the world of associative learning. Much gratitude goes to Peter Jones for conducting the surgical procedures described in this thesis, for his instruction and assistance in histological procedures, and help throughout my PhD. Thanks also to Rachel Horsley for assistance in histology, Adam Lester-George for conducting Experiment 6, and Alex Walker for assistance running Experiment 7. I thank my examiners Charlotte Bonardi and David Sanderson for their helpful discussion, comments, and suggestions.

Thank you to the Behavioural Neuroscience group for helpful lab-talks and discussions. Thanks also to everyone who worked in office B31 for day-to-day support. Thank you to Dave Keatley for his support, motivation, and understanding during the past three years.

Contents

Preface

My thesis concerned the examination of recognition memory and investigated associative processes in memory. An associative model of memory (e.g. Wagner, 1976, 1981) predicts the effects reported in experiments that use object recognition to test memory (e.g. Ennaceur & Delacour, 1988). Object recognition involves discrimination between novel and familiar items; the associative model predicts this discrimination. I have tested predictions from the model concerning priming of stimuli (Chapter 2) and stimulus spacing (Chapter 3), and generally found support for the idea that recognition memory (as that shown in object tasks) involves associative processes. I have also examined generalisation on the basis of novelty and familiarity. Contemporary models of recognition memory (e.g., Cowell, Bussey, & Saksida, 2010a, 2010b), while successful in explaining some recognition effects, fail with regards to findings presented in this thesis, perhaps suggesting the need for alteration or extension.

Glossary

US: Unconditioned stimulus

UR: Unconditioned response

CS: Conditioned stimulus

CR: Conditioned response

OR: Object recognition

SOP: Standard Operating Procedures

RH: Representational-Hierarchical

MTL: Medial temporal lobe

T: Tone

C: Clicker

PeRh: Perirhinal lesion

Sham: Sham (control) surgery

S: Scopolamine

MS: Methylscopolamine

ITI: Inter-trial interval

ANOVA: Analysis of variance

SME: Simple main effects

SEM: Standard error of the mean

Chapter 1. General Introduction

1.1 Recognition memory

Recognition is the ability to remember and distinguish past items and events. According to Mandler (1980), recognition memory concerns the identification of a stimulus or event (its familiarity) and judgement of its prior occurrence. These combined processes result in successful memory: the first retrieves the familiarity value of the event; the second is a search and retrieval process that determines whether the target item was originally presented. Familiarity may occur without the retrieval process, a stimulus would be familiar but there would be no knowledge of the context it was encountered in. Both these processes occur simultaneously and result in discrimination between a stimulus that has been previously presented (familiar) and one that has not (novel). It is this discrimination between familiar and novel stimuli that I have focussed on in this thesis.

1.1.1 Testing recognition memory.

Object-based tasks.

In order to test recognition memory, tasks that involve discrimination between novel and familiar stimuli are used. The delayed match-, or non-match-, to-sample task (e.g., Buckley, Gaffan, & Murray, 1997; Gaffan & Murray, 1992; Meunier, Bachevalier, Mishkin, & Murray, 1993; Mumby & Pinel, 1994; Otto & Eichenbaum, 1992; Zola-Morgan, Squire, Amaral, & Suzuki, 1989) is an object-based task in which an animal is first presented with two sample objects. After a delay, the animal is presented with the sample object and a novel object and is required to either identify the familiar object, or the novel object, depending on

whether a match-to-sample or non-match-to-sample rule is used. The objects stand on food wells, so if the correct object is chosen, the animal earns a reward. The animal must remember the familiar object in order to solve the task. This task has been mainly used with primates and sometimes with rats. The main limitation of the task is that learning the matching rule may be difficult, and so require several pre-training sessions in order for the subject to learn the rule. This also means that variability between subjects' performance increases (Clark & Martin, 2005).

Another object-based task is object recognition; this is used primarily with rats and mice. An object recognition task comprises two stages: a sample stage and a test stage. In the sample stage, the rat is exposed to two identical objects for a duration of time. A delay follows, normally out of the testing apparatus. In the test stage, rats are then exposed to a copy of the familiar sample object and a novel object. Rats typically explore the novel object more than the familiar (e.g., Ennaceur & Delacour, 1998; Dix & Aggleton, 1999; Norman & Eacott, 2005). This higher exploration of the novel object indicates that the animal has recognised the familiar object. There are several advantages of using object recognition: it requires no training, because rats have an inclination to explore novel objects, which means there are no rules the animal has to learn. The task does not use rewards; this means that food restriction is not required during the experiment. However, there are some problems using this task that relate to comparison and replication. Many laboratories that run object recognition experiments currently use different apparatus, such as open field arenas, or Y-mazes, and all varying in size. The objects

themselves are described as ‘junk objects’ and are everyday objects that seem to have no natural affordances to the animals. Affordances refer to qualities of the objects that may encourage rats’ natural behaviours such as climbing or chewing. A rat may be more attracted to an object that can be climbed on compared to one that cannot. Pairings of objects should avoid these inadvertent preferences (Ennaceur, 2010). The variety of apparatus used makes it difficult to replicate experiments that have been conducted in other laboratories. However, this variety of arenas and objects could also be a positive aspect, if the same results are being reported across all of these laboratories, it may mean that overall findings are reliable.

The object recognition task has been used with stimuli other than objects. For example, Forwood, Bartko, Sakisida, and Bussey (2007) used two-dimensional stimuli, these included photographs, shapes, and patterns. Using this type of stimulus meant that rats would not be able to use tactile cues to help discrimination, and so being more akin to experiments using human participants. Rats were able to discriminate between novel and familiar stimuli with these three types of two-dimensional stimuli. This suggests that the novelty/familiarity discrimination procedure can be performed with any type of stimuli, whether a two-dimensional picture, or a three-dimensional object.

Familiarity generalisation.

Another way to study recognition memory may be to examine how the familiarity or novelty of a stimulus may affect a subject’s response to that stimulus. Such a method may be to employ a familiarity generalisation procedure (Honey, 1990) as described below. Familiarity generalisation is

a variation on stimulus generalisation. Stimulus generalisation occurs when a conditioned response (CR), which was established to a conditioned stimulus (CS), is elicited by another stimulus. For example, Blough (1975) trained pigeons to respond to vertical lines of certain wavelengths. He presented a line of 587 nm followed by food reinforcement. This pairing resulted in raised responding to the 587 nm line, and to lines close to 587 nm. Responding reflected a Gaussian distribution, with peak responding to the trained stimulus. There was greater generalisation of conditioned response to stimuli that were close together on the wavelength continuum. The theoretical explanations relating to generalisation are discussed in section 1.2.2.

Best and Batson (1977) reported that generalisation may be due to novelty of stimuli. A group of rats (Cof/Cof-Li) were preexposed to coffee, another group (Vin/Cof-Li) were preexposed to vinegar and a third group (Cof-Li) were not preexposed to any flavour. In a conditioning stage, these groups were exposed to coffee followed by an injection of lithium chloride. There were two control groups that were not preexposed to any flavours; one control group (Li) were given lithium chloride injections during the conditioning stage, the second control group (H_2O) were given water with a saline injection in the conditioning stage. In the test with vinegar, the group that had no preexposure (Cof-Li) consumed less vinegar than the preexposed groups (Cof/Cof-Li and Vin/Cof-Li) and the H_2O control group, but similar consumption to the Li group. For group Cof-Li, the CR established to the novel stimulus generalised to the test stimulus, vinegar. However, generalisation was less apparent if the subjects were preexposed

to the conditioning stimulus or the test stimulus. Best and Batson explain the similarity of consumption between the Cof-Li group and the Li group as being due to the Li group's general unwillingness to consume fluid; they found that after conditioning but before the test, these rats consumed less water than any other groups. These results suggest that generalisation may be affected by the novelty of the stimulus: Group Cof-Li showed enhanced generalisation of conditioned response between stimuli that were novel, but preexposure to coffee reduced this generalisation in group Cof/Cof-Li.

Honey (1990) highlights a potential limitation of this study that relates to the measurement of response in the test stage. When exposed to a novel flavour rats will show a neophobic response (see Burn, 2008) and the UR to a novel flavour is avoidance. In group Cof/Cof-Li, this UR may combine with the CR to produce the results presented by Best and Batson (1977). Honey proposed that using a familiar stimulus in the test stage would overcome this problem as the UR would have habituated and would allow the CR in the test to reflect only generalisation from the conditioned stimulus.

Honey (1990) investigated the effect of preexposure to an auditory stimulus on generalisation of conditioned response. Auditory stimuli were a 2.0 kHz tone and a 20 Hz clicker; these were counterbalanced, so are represented as A and B. One group of rats (B/A) were preexposed to B/A, a second group (A) were exposed to only A, a third group (B) were preexposed to B, a fourth group (App) were not preexposed to any stimuli but were placed in the apparatus for the session duration. All rats were then conditioned with A, which was paired with delivery of food pellets. In a

test session, rats were presented with stimulus B. Group B/A and group App made more responses during the test than groups A and B. Honey interpreted this difference on the basis of stimulus familiarity. Due to the preexposure sessions, the conditioned stimulus (A) and the test stimulus (B) were both familiar to group B/A, and B and A were both novel to group App. Group B/A and group App responded more to stimulus B because the conditioned response established to B generalised to A. This generalisation was more apparent when the stimuli were matched in terms of familiarity or novelty.

1.2 An associative account of recognition memory

1.2.1 The SOP model.

In this thesis, I propose that processes seen in recognition memory tasks, such as object recognition, may involve associative processes. Associative accounts are able to explain many findings from conditioning and learning procedures. Wagner (1976, 1978, 1981) developed a model of memory over several publications; know as 'standard operating procedures' (SOP) of memory. The SOP model is able to predict and explain many of the findings in associative learning literature. As with other models of memory (e.g. Atkinson & Shiffrin, 1968), SOP provides a framework for long-term and short-term memory processes. According to SOP, when a subject is exposed to a stimulus, certain elements or groups of elements (called nodes) in the brain are activated, and the activation of these nodes may result in associations.

There are three states of activation that these elements or nodes can be in: inactive, in a primary state of activity (A1), or in a secondary state

(A2) (see Figure 1). The A1 state of activity is similar to working memory and the A2 state is similar to peripheral working memory. When a stimulus is first presented, the elements of its representation will be activated to a primary (A1) state of activity. The A1 state has a limited capacity for elements of representations, so the activation quickly decays to a secondary state of activity (A2). Elements decay from the A2 state to an inactive state. When stimulus representations are in A1, they generally elicit more behaviour than when elements are in A2. Elemental decay is promoted by competition for nodal activation due to additional stimulation. Elements decay from A1 to A2 in an exponential fashion and this is always faster than the decay from A2 to I. In simulations of SOP, Wagner (1981; Brandon, Vogel & Wagner, 2003) states that the decay rate from A1 to A2 is five times that of decay from A2 to I. Elements can be activated from inactive to A2, but not from A2 to A1.

Associations occur when elements for stimuli are in the same, or different, states of activation. Excitatory connections will be formed between stimulus elements when, for example, both the conditioned stimulus (CS) and unconditioned stimulus (US) are in A1. Excitatory connections allow A1 activity in the CS to produce A2 activity in the US. Inhibitory connections will be formed when the CS is in A1 but the US in A2. Inhibitory connections result in a decrease in the A2 activation of the US when the CS is in A1. The overall strength of the CS – US association is a product of both the excitatory and inhibitory connections.

In short-term memory, there are two priming rules: self-generated and retrieval-generated (Wagner, 1976); both of these alter how subsequent

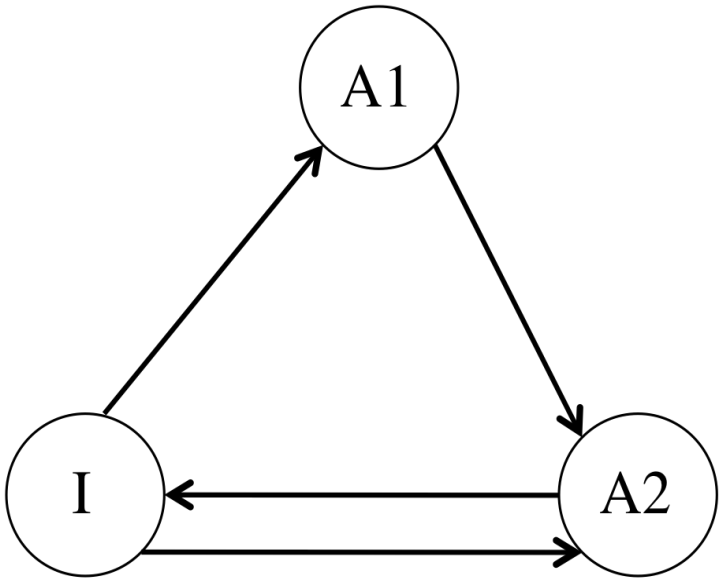


Figure 1. The SOP model, based on Wagner (1981). A1 represents the primary active state, A2 represents the secondary active state, I represents the inactive state, and arrows indicate the change in activation levels of elements. Elements activate from the inactive state (I) to A1, they decay into A2, and decay from A2 to inactive. Elements can be activated from I to A2, but not from A2 to A1.

stimulus presentations are processed. When a stimulus is primed it is less likely to evoke a response. These two priming rules outline how a representation of a stimulus can be activated into A2. Self-generated priming (SGP) is activation of elements by means of recent presentation of that stimulus. On presentation of the stimulus, elements are activated from inactive to A1, and then decay to A2. Short-term habituation is a result of SGP. If the stimulus is presented again while elements are in A2, there will be fewer elements activated to A1, and thus less A1 processing. Retrieval generated priming (RGP) refers to associative activation of elements. When two stimuli are first presented together, they are both activated to the A1 state, and thus associated together. When the representation of one of these stimuli is later reactivated to A1, the associated stimulus is activated to A2. This A2 activation limits the A1 activation of the associated stimulus. Associative activation to A2 is a result of the proportion of the cue's elements that are in an active state, and the overall strength of the CS - US association. Retrieval to A2 of the associated stimulus is greater when the cue is in A1 than A2.

SOP and recognition memory.

The SOP model predicts discrimination between a familiar and a novel object, as seen in an object recognition experiment (Figure 2A). This can be explained using self-generated priming. In the sample stage, when the rat encounters an object, the elements for the object are activated into A1; then elements begin to decay rapidly to A2. In the test stage, these elements may still be in A2 and so cannot be activated to A1 when the stimulus is presented again. When the rat encounters the novel object its

elements activate to A1 and so attracts more of the animal's attention. This can be seen in the rat's behaviour through greater exploration of the novel object. If enough time passes for the sample objects' elements to decay from A2 to inactive, then, in the test, the rat will explore both familiar and novel objects equally. The bias between exploration of novel and familiar objects could also be a retrieval-generated process. In the sample stage, the context and the object elements are both activated into an A1 state, and so become associated. In the test stage, the context activates a representation of the familiar object, so elements relating to the familiar object move to an A2 state of activation. The novel object will be activated to A1 and so the rat explores the novel object more. This process is less subject to effects of time because stimulus associations are formed which persist over time. Results of variations of object recognition experiments can be explained using the SOP model as outlined below (Figure 2).

Duration between sample and test stage.

The duration of time between the sample stage and the test stage in a standard object recognition experiment (Figure 2A) can be manipulated. With a short delay between sample and test, rats show good discrimination between novel and familiar objects (e.g., Ennaceur & Delacour, 1999). Longer delays generally diminish recognition so that there is less discrimination between novel and familiar items (e.g., Mumby, Glenn, Nesbitt, & Kyriazis, 2002). This is likely to be a self-generated process. After the sample stage, the elements relating to the object decay to A2, and eventually back into inactive if the delay is long. If the elements for the familiar stimulus are then re-activated from inactive to A1 in the test, it

may result in equal exploration of the novel and familiar object, as the novel stimulus too will elicit A1 activation. A retrieval-generated explanation is also possible, but would not be susceptible to the delay. In the sample stage, the context would become associated with the object. The next presentation of the context would prime the object into A2, thus leading to more interest in the novel object. This process should endure across even a long delay because associations persist, providing there has been no exposure to the context. After a long delay, there would still be good discrimination between novel and familiar objects.

Object and context associations.

Dix and Aggleton (1999) conducted a variant of the object recognition task using context-stimulus pairings. There were four sample stages; two different context-object pairings were used and each of these was shown twice (Figure 2B). In sample stages 1 and 4, rats were shown context X with two copies of object A, in stages 2 and 3 they were shown context Y with two copies of object B. The test stage was conducted in context X, plus a copy of object A and object B. A second session was conducted in the same way as the first, except the sample stages were reversed, so context X and object A were exposed in stages 2 and 3, and context Y and object B were exposed in stages 1 and 4. The test stage was conducted in context Y, with copies of objects A and B. Results from this experiment showed that rats explored the object that had not been previously paired with the test context. This indicated that rats might be sensitive to associations between objects and contexts.

The findings can be explained in terms of retrieval-generated priming. In the sample stages, the contexts and objects' elements were activated into an A1 state (and so would form an excitatory association). In the test, the rat was exposed to one context, with a congruent (expected) object and an incongruent (unexpected) object. The context primes the expected object, and so limits A1 activation of that object. The unexpected object has not been primed, so is able to activate to A1. This has the result that the rat explores the unexpected object more. This seems to be purely a retrieval-generated process; self-generated priming cannot account for this.

Relative recency.

Relative recency tasks compare rats' discrimination between objects that were presented at different time points, such that one object was presented more recently than another. Mitchell and Laiacona (1998) used a three-stage procedure, which included two sample stages, and a test stage (Figure 2C). In the first sample stage, rats were shown two objects for five minutes. After an hour, they received the second sample stage; in this they were shown two novel objects. After a delay, which varied from 1-168 hours, the test stage was run. In this stage, the rat was shown one object from each of the sample stages. The objects only differed in the time since they were first presented, one was presented an hour before the other. Rats explored the old object more than the recent object in delays of up to, and including, 24 hours. SOP can explain this difference. This recency effect seems to be mainly consistent with a self-generated process. In the test stage, the elements that relate to the second object may still be in A2, but the elements that relate to the object that was seen first will have

decayed to A2, and become inactive. When this ‘older’ object is presented in test, more of its elements will be activated to an A1 state, and so will be explored more.

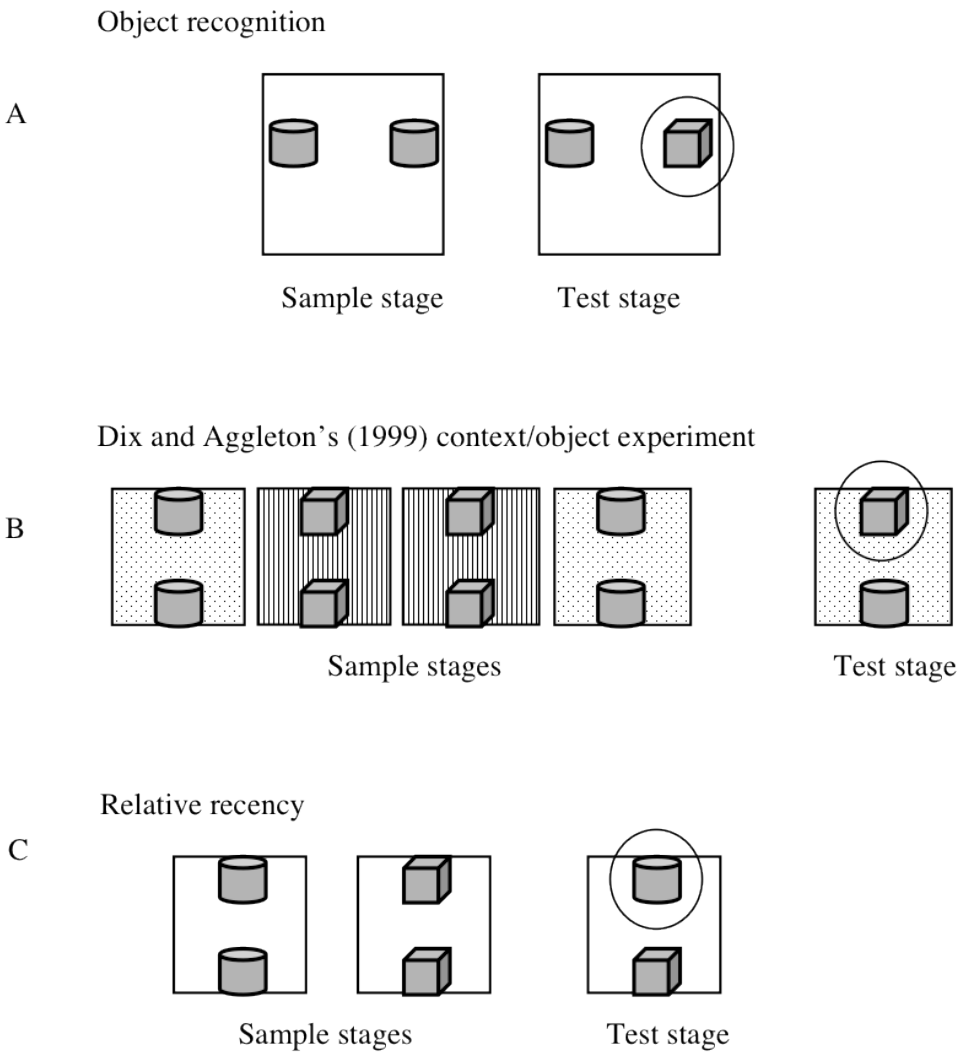


Figure 2. Diagrams of variants of object recognition experiments. A. Object recognition, circle indicates the object that is generally explored the most. B. Context and object recognition, as reported by Dix and Aggleton (1999). C. Relative recency discrimination.

1.2.2 Common elements model.

The SOP model cannot explain familiarity generalisation because it would not predict a response in the test stage, unless an association was formed between the stimuli in the preexposure stage. An elemental associative model (McLaren & Mackintosh, 2000) can explain stimulus generalisation. The model makes three assumptions: 1. Stimuli may be represented by a graded pattern of activation over a set of elements. 2. Similar stimuli may have overlapping elements, and their similarity is based on the proportion of common elements. In terms of the wavelength continuum, different values along the dimension will have overlapping sets of elements. 3. Sampling of stimulus elements is selective. During presentation of a stimulus, not all elements will be sampled, and thus not all corresponding units will be activated. Conditioned responding may generalise between stimuli on the basis of the elements they share.

In experiments that test generalisation, common elements can be added to make stimuli more similar, e.g., A and B become AX and BX, so there would be more generalisation between AX and BX compared to that between A and B (McLaren & Mackintosh, 2002). This common element is often an extra physical stimulus, such as a light or another flavour. Generalisation among stimuli that share similar physical attributes is a well-established phenomenon (for review see, Honig & Urcuioli, 1981). However, generalisation may also occur on the basis of stimulus elements that are not physical, but psychological. Familiarity could be one such dimension (Best & Batson, 1977; Honey, 1990). Familiarity could, in principle, be represented by these stimulus elements in a way consistent

with McLaren and Mackintosh's (2000) theory. Thus, there could be greater generalisation between stimuli that shared common elements of familiarity/novelty.

Bennett, Wills, Wells, and Mackintosh (1994) reported no evidence of generalisation on the basis of novelty/familiarity. They conducted an experiment with four groups: group W received preexposure to water, group Suc-L received preexposure to sucrose-lemon, group Suc-Q received sucrose-quinine preexposure, and group Lem received preexposure to lemon. All groups were conditioned with saline-lemon solution and lithium chloride; and tested with sucrose-lemon. If generalisation was enhanced by novelty, group Lem should show more generalisation (they should consume less fluid) than group Suc-Q. This would occur because for group Lem, the conditioning and test stimulus share a novelty element (saline in the conditioning stage and sucrose in the test stage), but there are no shared novelty elements between the conditioning and test stimulus for group Suc-Q. However, results were conflicting with this hypothesis: the groups preexposed to lemon (Suc-L and Lem) drank more than groups that were not preexposed to lemon (W and Suc-Q). According to Bennett et al. (1994) exposure to lemon in group SucL resulted in latent inhibition which retarded the acquisition of conditioned response in the conditioning stage, resulting in less generalisation than group W, who would not have suffered latent inhibition in the conditioning stage. These findings provide no support for Best and Batson's (1977) findings; but, as Bennett et al. (1994) highlight, few exposures and a single conditioning trial may not have allowed these novelty/familiarity cues to become apparent.

Honey's (1990) experiment cannot be subject to these arguments concerning latent inhibition. Whilst it is possible that rats in group B/A were subject to latent inhibition (see, Lubow & Moore, 1959) due to the 48 presentations of A, this would result in group B showing greater generalisation than group B/A. This pattern is the opposite of that reported by Honey (1990), indicating that latent inhibition is not an explanation of this effect (Hall, 2001).

Hall (2001) has provided an alternative explanation of Honey's (1990) results. He suggested that the high level of responding by group A/B to stimulus B may have been due to sensory preconditioning. Because stimulus A and B were presented in the same session, with short (280 s) ITIs, an excitatory association may have formed between the stimuli. Responding to B then could be due to a B - A - US - CR associative chain. According to this account, a short ITI would allow a stronger association to form between the two preexposed stimuli than a long ITI. This in turn would mean that a group given preexposure to A/B with a short ITI might show greater responding in a test with B than a group given preexposure to A/B with a long ITI.

Thus, if it were accepted that familiarity elements might form in relation to a stimulus, the common elements model (McLaren & Mackintosh, 2000, 2002) would be a good explanation of familiarity generalisation. This explanation assumes that there may be a mechanism to detect familiarity/novelty (Honey, 1990). One explanation, provided by Honey, is that this may be a comparator that enables subjects to compare a stored representation with incoming stimulation.

1.3 The perirhinal cortex and recognition memory

1.3.1 Location of perirhinal cortex.

In rats, the perirhinal cortex is located in the medial temporal lobe (MTL). Burwell (2001) defines it as comprising Brodmann's areas 35 and 36 and is positioned dorsally and ventrally adjacent to the third quarter of the rhinal sulcus. The area rostral to perirhinal cortex consists of agranular insular cortex and granular insular cortex. Perirhinal cortex begins close to -2.80 mm relative to bregma. The area caudal to perirhinal cortex is the postrhinal cortex at approximately -7.64 mm relative to bregma. The perirhinal cortex receives input from the piriform cortex, frontal cortical areas, insular areas, temporal regions, entorhinal areas, parietal areas, occipital areas, and a small amount from cingulate areas. The perirhinal cortex is a multi-modal structure; it receives olfactory, auditory, visual and visuospatial information (Burwell & Amaral, 1998).

1.3.2 Perirhinal cortex is important for recognition.

Encounters with novel stimuli lead to higher neuronal activity in perirhinal cortex than familiar stimuli (VanElzakker, Fevurly, Breindel, & Spencer, 2008; Wan, Aggleton, & Brown, 1999; Zhu, Brown, McCabe, & Aggleton, 1995). In one experiment, rats were exposed to familiar and novel pictures, one in each visual field. Neuronal activation was imaged using immunohistochemistry for the protein products (Fos) of the immediate early gene c-fos. Fos expression indicates recent increases in neuronal activity (VanElzakker et al., 2008). There was a higher level of activated neurons for novel pictures than for familiar pictures (Wan et al.,

1999). This indicates that perirhinal cortex is sensitive to novelty, and so may be involved in discrimination between novel and familiar stimuli.

Animals with lesions to the perirhinal cortex sometimes exhibit deficits in discrimination between novel and familiar stimuli (e.g., Albasser, Davies, Futter, & Aggleton, 2009; Barker, Bird, Alexander, Warburton, 2007; Baxter & Murray, 2001; Buckley & Gaffan, 1997, 1998; Gaffan, Eacott, & Simpson, 2000; Mumby, Piterkin, Lecluse, & Lehmann, 2007; Norman & Eacott, 2005). With short delays between sample and test stages in an object recognition experiment, rats with perirhinal lesions are less impaired in their discrimination between novel and familiar items than at longer delays. Norman and Eacott (2005) compared performance of control rats with that of rats with perirhinal lesions in an object recognition task. They found that rats with lesions were not impaired, compared to controls, at short delays of two, five, and ten minutes. Similarly, with a one-minute delay, control rats and rats with lesions showed discrimination between novel and familiar objects. However, at a 15-minute delay, control rats retained this discrimination, but rats with perirhinal lesions showed no discrimination (Ennaceur, Neave, & Aggleton, 1996). Using a delay of one hour also impaired discrimination of rats with perirhinal lesions compared to controls (Winters & Reid, 2010). In a delayed-nonmatch-to-sample task, discrimination of rats with rhinal lesions was normal after a four-second delay, but impaired compared to controls after 15 seconds (Mumby & Pinel, 1994). In all these experiments, the same animals are often tested at a short and a long delay and are often impaired only after a long delay. The delay-dependent impairment cannot be due to a general problem with

discrimination, but is more likely to reflect memory impairment (Mumby & Pinel, 1994).

Performance in object recognition is correlated with the size of lesions in perirhinal cortex (Albasser et al., 2009). A significant negative correlation was found between performance in the object recognition task and the size of the lesion: the bigger the lesion, the worse the discrimination between familiar and novel objects. Increasing the time the rats had to sample the object did not help discrimination. When multiple exposures were used in a separate experiment, rats' discrimination did improve (Mumby et al., 2007). Overall, this evidence suggests that the perirhinal cortex is important in discriminating between familiar and novel items.

1.4 The representational-hierarchical view of recognition memory

The representational-hierarchical (RH) model (Cowell, Bussey, & Saksida, 2010a, 2010b; Saksida, 2009) incorporates the evidence that perirhinal cortex is important for recognition memory, and suggests that it contains complex stimulus representations. This model was developed from Bussey, Saksida and Murray's (2002) perceptual-mnenomic feature-conjunction (PMFC) model. The PMFC model began by proposing that the MTL does not just control memory functions, but that the perceptual system (from the ventral visual stream) is also involved (Bussey & Saksida, 2005, 2007). The RH model provides an account for visual memory, as used in recognition memory tasks such as object recognition. Psychological functions, such as memory, should not be thought of as separable processes that relate to separate structures, but that brain regions

contain certain representations that relate to stimuli (Cowell et al., 2010a, 2010b; Saksida, 2009). The entire processing stream from the visual cortex to the MTL is important for memory and perception. This processing is based on a hierarchical organisation continuum, where simple features are represented at the visual cortex end, and complex representations are represented at the perirhinal end. If one brain area were lesioned, this would impact on the representations held there, but would not mean that the subject no longer has a particular psychological function.

This was demonstrated by McTighe, Cowell, Winters, Bussey, and Saksida (2010). Control rats and rats with lesions to the perirhinal cortex were tested in an object recognition experiment. In the first stage, rats were shown two identical objects. During a one-hour delay, rats were either placed in a holding cage or in a visually restricted box. They were then exposed to either the familiar objects they had seen in the first stage, or to an identical pair of novel objects. In the condition in which rats were returned to the holding cage, rats with perirhinal lesions were impaired, compared to controls, in exploring the novel object, but similar to controls when exploring repeated objects. Rats with perirhinal lesions treated the novel object as though it were familiar. Furthermore, in the condition in which the rats spent the interval in the visually restricted box, rats in both groups performed similarly, those with perirhinal lesions now explored the novel object the same as the controls. This could be due to the representations held at particular levels. Without visual restriction in the delay, the rat was exposed to other features that would have interfered with simple representations, i.e., some of these extra features might be the same

as those the novel object has, for example, a straight line. This means that in the test, the features of the novel object seemed familiar. The conjunctive (complete) representation of the familiar object will be represented in anterior regions, such as perirhinal cortex, and would prevent interference. With damage to those areas, recognition would have to be based on the simple features (which have been interfered with). With visual restriction, these features were not affected and so even with damage to perirhinal cortex, discrimination was comparable to controls. Damage to perirhinal cortex does not result in a loss of memory, rather that the subject has to rely on representations from non-damaged regions.

1.5 Conclusions

The SOP model is useful because it can explain normal performance in most recognition tasks as it can explain why rats have a bias towards novel stimuli. Other models have not explained this bias. SOP is not restricted to visual memory; its procedures and rules can be applied to stimuli in various modalities, e.g., visual, tactual. However, SOP has some limitations. It is not clear how it would account for results such as those reported by McTighe et al., (2010) in rats with perirhinal lesions. After a delay with visual restriction, lesioned rats' discrimination of novel and familiar objects was similar to that of control animals. SOP cannot explain how visual restriction may restore recognition in rats with perirhinal lesions. However, the findings from that report have been challenged (Albasser et al., 2011). When familiar or novel objects were presented separately, rats with perirhinal lesions explored those objects

similarly to controls. According to this measure, perirhinal lesions did not make novel stimuli seem familiar.

Models of recognition memory (e.g., Brown & Aggleton, 2001; Cowell et al., 2010a, 2010b) include the function of the perirhinal cortex in explaining subjects' performance in various tasks. SOP does not make references to any brain regions, so some assumptions are required to explain lesion deficits. The elements and processes described in SOP may relate to neurons, such that the group of elements that respond to a stimulus relate to a collection of neurons (Sanderson et al., 2010). In a recognition memory task, elements that activate in response to the stimuli may be in perirhinal cortex, because that may be where objects are represented (Cowell et al., 2010a, 2010b) or because neurons in perirhinal cortex respond to novelty (Wan et al., 1999).

SOP cannot explain familiarity generalisation; however an associative model of common elements (McLaren & Mackintosh, 2000, 2002) may if familiarity was considered as an additional stimulus element. McLaren and Mackintosh (2002) state that familiarity could not be considered as a stimulus element, because it is subject to latent inhibition and so has reduced saliency. This means that familiar stimuli lose their ability to enter into associations. In a test with another stimulus, a familiar stimulus will not acquire as much associative strength as the other stimulus and this results in discrimination between the stimuli. However, the evidence from Honey (1990) suggests that latent inhibition may not explain familiarity generalisation, and so in this thesis, I aimed to further test familiarity generalisation.

Familiarity generalisation cannot be explained by the representational-hierarchical account because it does not explain familiarity/novelty processes. According to the RH account, memory for different aspects of a stimulus is represented in a hierarchical manner through the cortex; however, this does not explain exactly how recognition occurs. This means that the RH account may be limited when making predictions concerning performance of normal animals in tasks that manipulate object pairings and possible associations.

Many theories of memory consider that there are separable processes or stores for long-term and short-term retention (e.g., Atkinson & Shiffrin, 1968). The RH account predicts that performance will decline (due to interfering stimuli) with a long delay between the sample stage and the test stage in an object recognition experiment, but otherwise, long-term memory processes are ignored. This seems to be a concern when other models of memory have placed emphasis on defining short-term or long-term processes.

The RH model focuses on visual memory, from representations in the visual cortex, through the medial temporal lobe. Concentrating on visual memory may limit the model. The perirhinal cortex receives input from other modalities, including auditory and olfactory areas (Burwell & Amaral, 1998). The RH model could be altered slightly to include other types of sensory memory. However, there have been no reports of a deficit in recognition of animals with perirhinal lesions with auditory stimuli (Kowalska, Kuśmierek, Kosmal, & Mishkin, 2001; also see, Wan et al., 2001) or tactile stimuli (Winters & Reid, 2010). However, considering that

perirhinal cortex has more input from auditory areas than from visual areas (Burwell & Amaral, 1998), it would be reasonable to encourage more research with auditory stimuli.

1.6 Application to thesis

My aim in this thesis was to examine associative processes that may be present in recognition memory. SOP is able to predict a number of findings concerning recognition, e.g., performance in object recognition tasks, so it was reasonable to use the model to predict performance in other types of recognition task. I used the SOP model to predict performance in recognition memory tasks in regards to object recognition through priming (Chapter 2; see Appendix 4 for experiment designs) and the spacing effect (Chapter 3). I also used a stimulus generalisation task, similar to Honey's (1990), to examine recognition (novelty/familiarity discrimination) and to test if performance in this task was affected by manipulations that affect object recognition, such as perirhinal lesions (Chapter 4). Unlike the SOP model, the RH model (Cowell et al., 2010a, 2010b) does not make predictions concerning priming or spacing in object recognition tasks. Currently, it could not make any predictions concerning the stimulus generalisation task, particularly because I used auditory stimuli. It does not explain responding in terms of familiarity/novelty, and does not consider associations between stimuli. Findings from my experiments may highlight the associative nature of recognition memory and promote extensions to current models.

Chapter 2. Associations in Object Recognition

In Chapter 2, I present experiments that tested predictions from the standard operating procedures (SOP) of memory model (Wagner e.g., 1976, 1981), concerning the formation of associations (object-object or object-context) in object recognition. In object recognition tasks, rats discriminate between novel and familiar stimuli. Theories of object recognition (e.g., Brown & Aggleton, 2001; Cowell et al., 2010) do not provide a full account of how or why this discrimination occurs, and how experimental manipulations affect performance in object recognition tasks. The SOP model (Wagner e.g., 1976; 1981) can be used to interpret the results of published work, and can be used to make novel predictions. Some of these novel predictions are tested in the present chapter. One such prediction was that rats' discrimination between stimuli might be seen after associative activation of a stimulus representation.

Honey and Good (2000; Honey, Good & Manser, 1998) provided evidence of retrieval generated priming of stimuli. In an experiment, rats were given two auditory-visual pairings, A-X and B-Y. After exposure, rats were given a test of A followed by X and Y. Honey and Good found that the rats oriented toward Y more than X. According to SOP, stimulus A primes a representation of X, and so when the rat hears A, it will be expecting X; in contrast, Y will be unexpected, and so will elicit more exploratory behaviour. This indicates that associative pairings reduced the unconditioned response (the orienting response) when presentation of one of the paired stimuli primed its associate.

Honey and Good's (2000) experiment contained many training trials to ensure good learning about the stimulus pairs. In the experiments presented in this chapter, I used an object recognition task, in which the rats were only exposed to each stimulus pair once. I aimed to test rats' learning about object pairings in a one-trial exposure session so that the experiment was similar to other object recognition experiments (e.g., Ennaceur & Delacour, 1988).

My aim here was to test predictions of the SOP model using object recognition tasks. The experiments consisted of three stages (Table 1); in the first stage, rats were presented with two pairs of stimuli; in the second stage, one stimulus was presented to prime the rats' memory for the third (test) stage that followed. The prime given in the second stage was designed to affect the activation states of the representations of the stimuli. The prime in the second stage was important, as all stimuli were exposed for an equal duration of time in stage one, so any differences at test were likely to be due to the stimulus exposed in the second stage.

Table 1.
Design of main priming experiment (3 and 4).

Stage 1	Stage 2	Test
PX	XX	PQ
QY		

Note, P, Q, X, and Y refer to stimuli.

2.1 Experiment 1

A pilot experiment was conducted to establish whether standard object recognition effects could be obtained using my apparatus, stimuli and measurements. This was necessary due to the range of apparatus and measurements reported in published experiments. There were two types of stimuli used: objects, that rats could explore visually and tactilely; and context stimuli, which may generate more visual than tactile exploration. It was important to establish that rats were able to discriminate in these classes of stimuli, objects and contexts. This experiment also aimed to determine whether the length of exposure (5 minutes) in the sample stage and the duration between the sample and the test (10 minutes) was sufficient for successful discrimination between novel and familiar stimuli.

2.1.1 Method.

Subjects.

Sixteen male Lister-hooded rats (*Rattus norvegicus*), supplied by Charles River (UK), served as subjects. Rats were pair-housed in identical cages that had plastic bases and steel bars. Cages contained sawdust, paper bedding, and a cardboard cylinder for environmental enrichment. Rats were kept in a lightproof room with lights on a 12-hr light cycle with an 0700 onset. The temperature in the holding room and the experimental room was 20°C ± 2°C, with a humidity of 50 %.

On the day prior to the test, rats weighed between 440 and 530 g, with a mean of 483.44 g, and had free access to food and water throughout the experiment. The rats had previously been exposed to auditory stimuli in conditioning chambers, but were naïve to the current apparatus and stimuli.

Apparatus.

The apparatus used comprised four identical white rectangular walled high-density polyethylene boxes (Mini Mobile, supplied by Slingsby, Shipley, UK; Appendix 3A). Each arena measured 60.0 cm (h) x 40.0 cm x 45.0 cm. A sheet of white acrylic was placed in each box to provide a flat floor. A black wooden frame supported a FireWire camera (Fire-I, Unibrain, Athens, Greece), which was fixed 90.0 cm over the centre point of the floor of the arena. The view of each camera included the entire floor of its corresponding arena, and the lower portion of each wall. The camera was connected to a computer that ran AnyMaze video tracking software (Stoelting Co., Illinois, USA). This tracked the position of the rats' heads in the arena, so was used to record time spent in pre-specified zones where the objects were placed. Two lights were also positioned on the wooden frame (90.0 cm above the arena floor), each consisting of a circle of six light-emitting diodes (LEDs). These arena lights were on throughout the experiment. As well as the arena lighting, ceiling-mounted fluorescent strip lamps lit the room where the apparatus was held.

Objects and context inserts were used as stimuli. The objects used were a green plastic toilet cleaner bottle (25.5 x 6.5 x 4.5 cm) and a spherical brown ornament (7.5 x 8.0 cm). Each object was secured to the floor of the arena with Blu-Tack (Bostik, Stafford, UK). One context insert was made from three wooden boards that were hinged together, and covered with linoleum. The largest of the boards was 32.0 x 45.0 cm and had one board attached along each 45.0 cm side; these two boards were 21.0 x 45.0 cm. Two of these inserts were positioned to cover the arena's

inner white walls. The largest board was positioned in front of the arena's smallest wall, with the smaller boards covering the arena's longest side, so when two inserts were placed in the arena they joined at the centre point of the longest wall. There were two patterns of lino used: a white and black tile, and a blue tile. The white tiles were large squares (16.0 x 16.0 cm) interspersed with smaller black diamond-shape tiles (4.0 x 4.0 cm) so the overall pattern was quite blank and sparse, whereas the blue tiles were small (1.5 x 1.5 cm), so created a quite dense pattern.

Procedure.

Rats were first given exposure to an arena over three days, spending ten minutes in the arena per day in order to familiarise them to the environment. No stimuli were placed within the arena during these days.

Half (n = 8) of the rats were then assigned to group Object, and the other half (n = 8) to group Context. During the sample stage, group Object was shown two identical objects (Figure 3; Appendix 3D). One object was placed in the top left corner of the arena, and the second object was placed in the lower right corner of the arena. After five minutes in the arena, rats were returned to their home cage. The arena and objects were cleaned with an ethanol solution and paper towels between each trial. After ten minutes, rats were placed back into the arena, which now contained a copy of the sample object, and a novel object. The objects were positioned in the same places as in the sample stage, but the position of the novel object was counterbalanced, so for half the rats (n = 4) it was on the right side and for the other half (n = 4) it was on the left side. Two types of object were used,

and each was used as the sample and as the novel test object for half of the trials. The test stage lasted for five minutes. For group Context the procedure was the same, except that context inserts were used instead of objects.

Data collation and analyses methods.

The measurement used was the duration of time that rats spent in a pre-specified zone that was placed around each object. Each zone for group Object was rectangular (12.5 x 13.5 cm) and covered an area 168.75 cm^2. For group Context the zone was made of rectangles that covered the short wall of the arena and an area on the adjacent top and lower wall of the arena (21 cm down the long wall), and the floor adjacent to these three walls. The total area was 1221.98 cm^2; see Appendix 5 for diagrams of zones. The zones were sized as such due to the placement of objects in arenas; rectangular zones were used when objects were in the corners of the arena, to capture most of the rats' movement around the object, and circular zones were used when objects were placed away from the corners, again to capture most of the rats' movement around the objects. Results are reported in percentage of time in the zone and in a discrimination ratio. The time spent in the zone with the object was similar to the object exploration that is reported in other studies (e.g., Dix & Aggleton, 1999; Ennaceur & Delacour, 1988) of object recognition. Object exploration is often defined as "directing the nose at a distance of ≤ 2 cm to the object and/or touching it with the nose" (Ennaceur & Delacour, 1988, p. 49). In the present studies, time in the zone measures when the rat is near the object so it may not be as specific a measure as observation scoring.

However, studies have shown that results from automated procedures match results from human observers (Rutten et al., 2008; Silvers, Harrod, Mactutus, & Booze, 2007).

The time in zones measurement gives a good indication as to where rats spend time in the arena; however, the data may not be completely independent because the rat is in the zone of the familiar or novel object or in the rest of the arena. The measurement of time in one zone is dependent on the time spent in the other zones. This problem does not apply to the discrimination ratios. The discrimination ratio was calculated by subtracting the time spent in the zone with the familiar stimulus (P) from the time spent in the zone containing the novel stimulus (Q), divided by the time spent in P and Q summed together. This gives a ratio that can range between one and minus one, where zero indicates chance level, or no discrimination between stimuli. Data from the sample stage and test stage were reported. Data were analysed with ANOVA and SMEs with a pooled error term were performed to further analyse interactions.

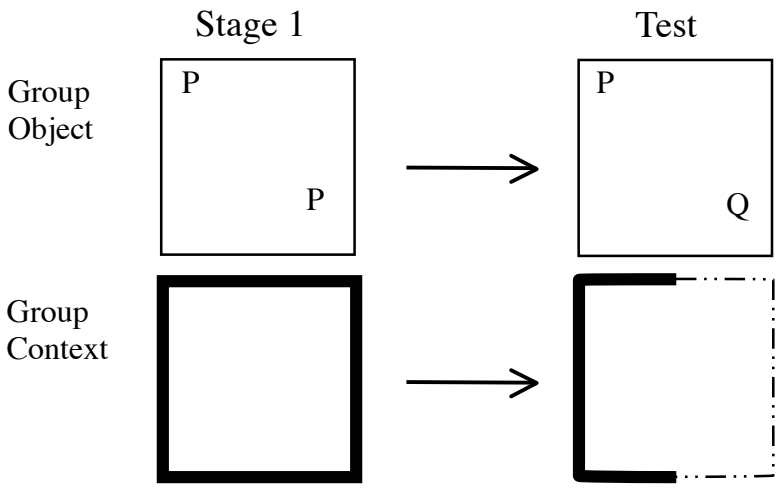


Figure 3. Arrangement of object and context inserts in arenas in Experiment 1. P and Q refer to objects, thick and dotted borders refer to contexts. In the sample stage, two identical stimuli were placed in the arena; in the test stage a familiar stimulus was presented with a novel stimulus.

2.1.2 Results and discussion.

Time.

During the sample stage, rats in group Object spent similar amounts of time in each zone, left side M = 18.91 % (SE = 2.31), right side M = 19.14 % (SE = 2.12). Group Context showed similar results, left side M = 17.89 % (SE = 2.31), right side M = 18.15 % (SE = 2.12). Analysis of variance (ANOVA) was conducted with side (left or right) and group (Object or Context) to check for any side preference and whether the type of stimulus used had any effect. There was no effect of side, no effect of group, and no interaction between these factors, all Fs < 1, η_P^2s < .02. In the test stage (Figure 4), time in the zone of the familiar (P) and novel (Q) object was analysed. Only the first two minutes of the test were included. In both groups, rats spent more time in the zone that contained Q (the novel stimulus) than the zone that contained P (the familiar stimulus). An ANOVA with time in the zone (P or Q) and group (Object or Context) supported this description. Rats spent more time in the zone that contained the novel object (Q) than the zone that contained the familiar object (P), $F(1, 14) = 29.84$, $p < .001$, $\eta_P^2 = .68$. There was no effect of group, $F(1, 14) = 2.38$, $p = .145$, $\eta_P^2 = .15$, showing that the pattern of results was similar with both types of stimulus used. There was a significant interaction between Time In The Zone and Group, $F(1, 14) = 5.60$, $p = .033$, $\eta_P^2 = .29$. To examine this interaction, simple main effects analyses with a pooled error term were conducted. Both groups spent more time in the zone of the novel stimulus (Q) than the familiar stimulus (P), for group Object, $F(1, 7) = 17.76$, $p = .004$, $\eta_P^2 = .72$, and for group Context, $F(1, 7)$

= 17.45, p = .004, η_P^2 = .71. Groups spent a similar amount of time in the zones containing familiar (P) stimuli, $F(1, 14) = 1.53$, p = .236, η_P^2 = .10, but different amounts of time spent in the zones that contained novel (Q) stimuli, $F(1, 14) = 5.46$, p = .035, η_P^2 = .28. Group Object spent more time in the novel (Q) zone than group Context. Overall both groups spent more time in the zone with the novel stimulus (Q) than the familiar stimulus (P).

Ratios.

Results were similar to those that used the percentage of time spent in each zone. During the sample stage, rats spent similar amounts of time in the left and right zone, mean ratio for group Context = .001 (*SE* = .075), and the mean ratio for group Object = .033, (*SE* = .075). An ANOVA with a factor of group (object or context) showed that there was no difference between groups, $F < 1$, η_P^2 = .01, and scores were not different from zero, as shown by the intercept, $F < 1$, η_P^2 = .01.

In the test stage (Figure 4), the discrimination ratio for group Object was higher than that for group Context; however, this difference was not significant as shown by an ANOVA, containing the factor of group (Object or Context), $F(1, 14) = 4.48$, p = .053, η_P^2 = .24. The intercept was significant, $F(1, 14) = 36.84$, $p < .001$, η_P^2 = .73, showing that across both groups, the discrimination ratio was higher than zero, indicating that rats spent more time in zone containing stimulus Q (the novel stimulus).

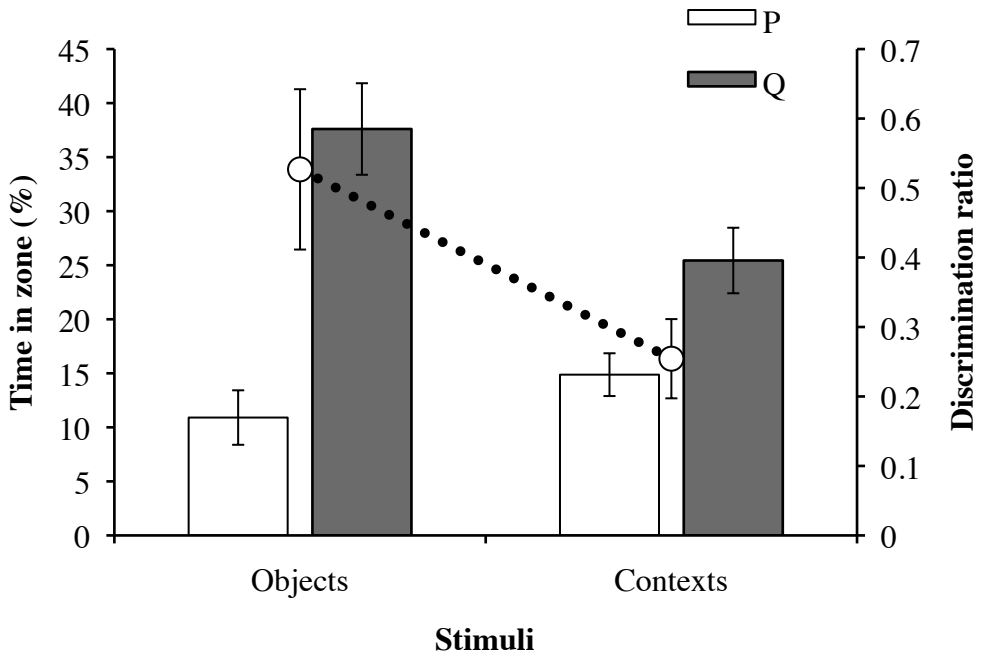


Figure 4. Data from test stage of Experiment 1. Percentage of time spent in zone P and zone Q is represented by bars corresponding to the left Y-axis. Ratio (Q-P)/(Q+P) is represented by line graph corresponding to the right Y-axis. Error bars represent one standard error of the mean. Stimulus P was the familiar stimulus that was exposed in stage one; stimulus Q was the novel stimulus.

This experiment was conducted to test rats' discrimination between novel and familiar stimuli (Dix & Aggleton, 1999; Ennaceur & Delacour, 1998; Norman & Eacott, 2005) using junk objects and context inserts. Results did confirm the prediction; using percent of time spent in the zone containing the object, and discrimination ratios, rats spent more time in the zone that contained the novel stimulus than the zone that contained the familiar stimulus.

2.2 Experiment 2

Experiment 1 supported a widely demonstrated recognition effect. It was important to demonstrate this effect with our apparatus and stimuli, as these vary across research labs. Stimuli tested in Experiment 1 were used in the following experiments.

Experiment 2 was designed to test the prediction made by the SOP model that behaviour toward stimuli would be affected by priming (self-generated or retrieval-generated) of the representation of the stimulus. Experiment 2 comprised three stages (Figure 5); in the first stage, rats were shown two pairs of stimuli (PX and QY). Using these pairs in this stage was necessary to test whether exposure to four different stimuli affected exploration of stimuli in later stages. An increase in the number of stimuli presented may diminish recognition (Cowell et al., 2006). It was important to test this as these pairs were to be used in stage one of Experiment 3. In stage two they were exposed to P, and in the test they were shown PQ. The exposure to P in the second stage would activate the representation of P into A1, which would decay into A2 so that in the test stage, P would have many elements in the A2 state, and Q would have more elements than P in

the A1 state. This would result in the rat showing more behaviour (exploration) of Q. This is self-generated priming: P primes itself by its own presentation. This may also be a retrieval-generated process; in the second stage, exposure to P may lead to P being associated with the context, and so in the test, when the rat is placed back in the context, P would be expected (and its elements primed to A2), but Q would not, so there would be more of Q's elements in A1. Both priming processes predict that rats should show more behaviour towards Q.

Experiment 2 was also important to test whether prefamiliarisation of stimuli would affect discrimination. If stimuli in a test stage were already familiar, discrimination may be affected. Relative recency experiments (e.g., Mitchell & Laiacona, 1998) demonstrate that rats explore a stimulus that was presented least recently. This would result in more exploration of Q than P in the present experiment. Counterbalancing of PX and QY in stage 1 may attenuate this; however, the presentation of P in stage 2 means that Q will always be the least recent stimulus. The number of stimuli used in the present experiment may affect these recency processes. Exposure to extraneous stimuli may interfere with learning (e.g., McTighe et al., 2010). In the present experiment, discrimination may be effected because stimuli are presented repeatedly. Cowell et al. (2006) reported that a network model of recognition memory in normal subjects cannot discriminate between stimuli that have been repeatedly presented. I conducted Experiment 2 to test that priming processes in object recognition were still active after trials in which stimuli were preexposed.

2.2.1 Method.

Subjects.

Thirty-two rats were used as subjects; they were of the same sex and strain as Experiment 1. They weighed between 340 g and 455 g, with a mean weight of 384.06 g. They were kept and housed as described in Experiment 1. Subjects had been exposed to auditory stimuli in conditioning chambers, but were naïve to the stimuli that were used in the present experiment.

Apparatus.

The apparatus used was the same as in Experiment 1. Objects used were: a yellow rubber duck (9.0 x 9.0 x 7.0 cm), an hourglass-shaped bottle containing black and red peppercorns (18.0 x 6.5 cm), a plastic bottle in the form of penguin figurines (20.5 x 10.0 x 9.0 cm), a plastic blue bottle with a black sports cap (20.0 x 7.0 cm), and a glass mineral water bottle (23.0 x 8.5 cm). The contexts used were those described in Experiment 1 - a white context, and a blue context.

The LED lights always provided illumination in the arena for all subjects. The ceiling lights were turned off when contexts were presented.

Procedure.

There were two groups, each of which was exposed to a different set of stimuli. Group Object (n = 16) was exposed to pairs of objects, and group Context (n = 16) was exposed to objects and contexts. Rats were given exposure to an empty arena over three days, spending ten minutes in the arena per day. The experiment was conducted the day following the last of these sessions.

The experiment was conducted in three stages (Figure 5). In stage 1, half of the rats in group Object were given exposure to stimuli P and X, then exposure to Q and Y. For these subjects, stimulus P was in the lower right of the arena and stimulus X was in the upper left of the arena. Stimulus Y was in the lower right of the arena and stimulus Q was in the upper left of the arena. For the other half of the subjects, QY was exposed first, then PX. Y was placed in the top left corner of the arena, and Q in the lower right corner. X was in the lower right corner, and P was in the top left corner. Four objects were used, these were counterbalanced in a pair, so objects used as stimuli P and Q were counterbalanced, and objects used as X and Y were counterbalanced. In stage 2, rats were shown two identical objects: PP. Objects were again positioned in the top left and lower right corners of the arena. In the test stage, rats were shown P and Q. The position of P and Q were the same as in stage 1, so for half the animals, P was in the top left corner, and for the other half it was in the lower right, and the same for Q.

For group Context, the procedure was the same, except context stimuli were used instead of objects X and Y in stage 1, and so identical objects (P or Q) were placed in the top left and lower right corners of the arena (Figure 5). Stage 2 was the same as for group Object; rats were exposed to two identical objects (P). The test stage was the same as for group Object; objects P and Q were placed in the arena.

Data collation and analysis methods.

The measurement used was the percentage of time that rats spent in the zones. For group Object the zones were circular and had a radius of

10.5 cm and an area of 330.06 cm^2. Zones were rectangular (12.5 x 13.5 cm) with an area of 168.75 cm^2 for group Context. Results are reported in percentage of time in the zone, and also in ratios for the test stage.

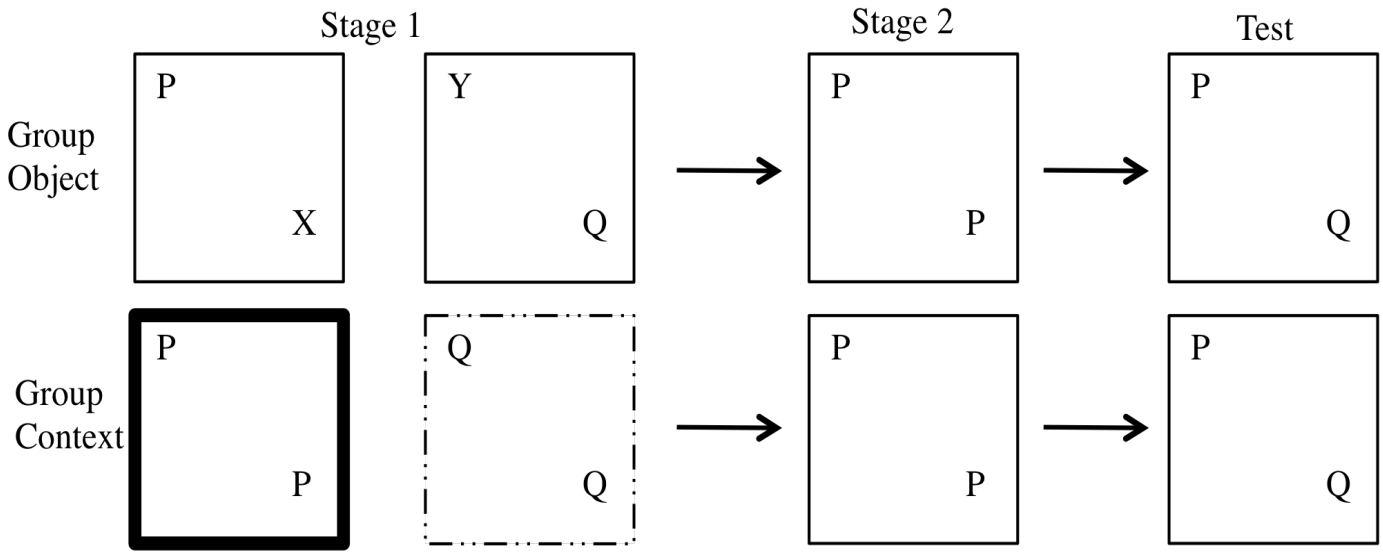


Figure 5. Experiment stages and the arrangement of stimuli and contexts in Experiment 2. Letters P, Q, X and Y refer to objects; thick and dotted lines refer to context inserts. In stage 1, rats were presented with pairs PX and QY; in stage 2, two identical copies of P were shown. In the test stage objects P and Q were shown to both groups.

2.2.2 Results and discussion.

Data from one rat in group Context were excluded due to experimenter error during the procedure; a second rat's data were deleted due to a marked side preference throughout the experiment. The following data are from group Context (n = 14) and two Object groups (n = 8 each, so a total of n =16). The apparatus and procedures used for the Object groups were identical, the only difference between the groups being that they that were run on different days.

Stage 1 and 2.

Results from Stage 1 (Figure 6) suggested that rats in each group spent similar amounts of time in the zones in each stage. An ANOVA performed on data from on stage 1, with group (Context or Object) and stimulus pair (PX or QY) as factors, revealed an effect of group, $F(1, 28) = 39.39$, $p < .001$, $\eta_P^2 = .59$, but no effect of stimulus pair, $F < 1$, $\eta_P^2 < .01$, and no interaction between these factors, $F < 1$, $\eta_P^2 < .01$. The significant difference between the groups was due to the overall levels of time in the zones. Group Context overall spent less time (M = 45.61 %, SE = 2.33) in the zones than group Object (M = 65.66 %, SE = 2.18). This could be because group Context may have spent time exploring the context walls, as well as the objects, whereas group Object may have spent more time in the zones with the objects because there was nothing else to explore. The zone size programmed for group Object was larger than for group Context, so this may also have contributed to the overall levels of time in the zones.

ANOVA on stage 2 (PP) with group as a factor revealed a group difference in overall exploration in this stage, $F(1, 28) = 35.76$, $p < .001$,

$\eta_P^2 = .56$. Again, group Object spent more time in the zones ($M = 67.58$ %, $SE = 2.91$) than group Context ($M = 42.13$ %, $SE = 3.11$).

Test.

Time.

In the test (Figure 7), rats in both groups spent more time in the zone that contained stimulus Q, than in the zone with stimulus P. This was confirmed by an ANOVA, with object (P or Q) and group (Context or Object) as factors. Rats spent more time in the zone containing Q than the zone containing P, $F(1, 28) = 6.08$, $p = .020$, $\eta_P^2 = .18$; there was also an effect of group, $F(1, 28) = 54.90$, $p <. 001$, $\eta_P^2 = .66$, but no interaction between these factors, $F < 1$, $\eta_P^2 = .02$. Group Object spent nearly twice as much time in the zones ($M = 40.11$ %, $SE = 1.78$) than did group Context ($M = 20.79$ %, $SE = 1.91$).

Ratio.

Groups had a similar discrimination ratio score in the test stage (Figure 7); these ratios were above zero, indicating that rats spent more time in the zone that contained stimulus Q than the zone that contained stimulus P. An ANOVA confirmed these descriptions. There was no effect of group, $F < 1$, $\eta_P^2 < .01$, and the intercept was significant, $F(1, 28) = 7.25$, $p = .012$, $\eta_P^2 = .21$.

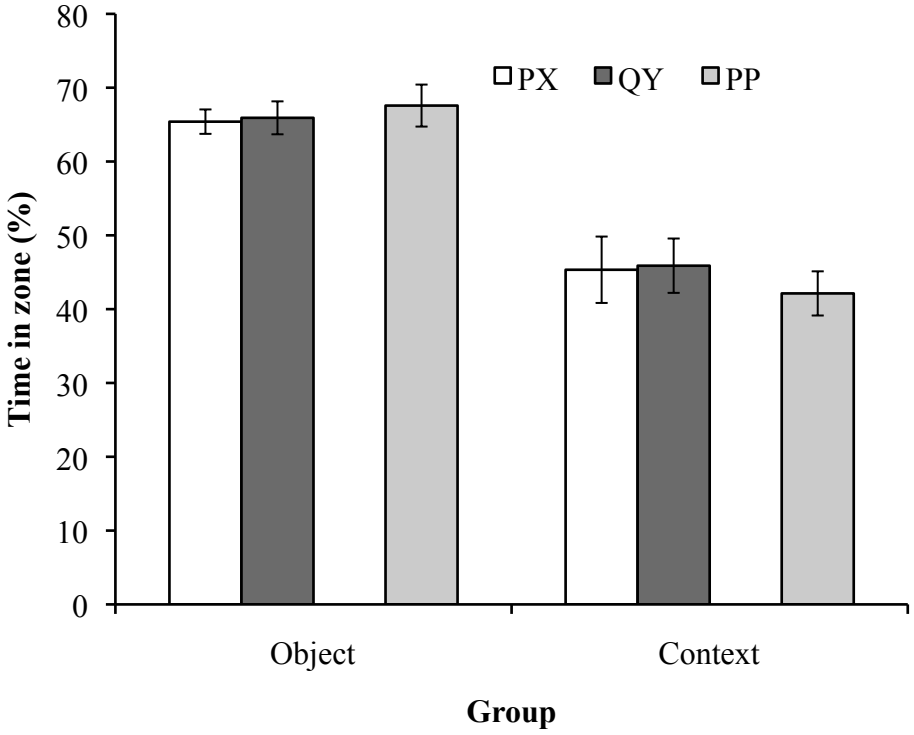


Figure 6. Percentage of time spent in Stage 1 (PX and QY) and in Stage 2 (PP) in each group Object and group Context. Error bars show one standard error of the mean.

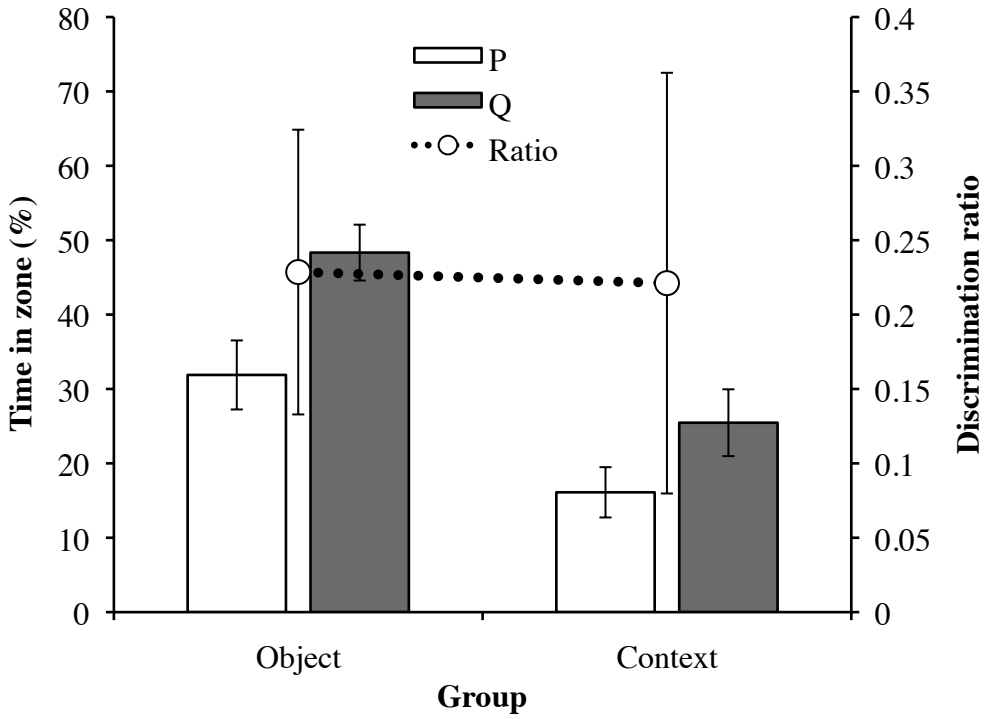


Figure 7. Percentage of time in zones (P or Q) in the test stage, represented by bars corresponding to the left Y-axis, and discrimination ratios, represented by the line corresponding to the right Y-axis. Error bars represent one standard error of the mean.

The results showed that rats spent more time in the zone that contained stimulus Q than the zone that contained stimulus P and this was true for both the group that was exposed to objects and the group that was exposed to contexts and objects. This shows that this effect is robust using different sets of stimuli, suggesting that it is a general effect, and not specific to the stimuli used.

The aim of this experiment was to test the prediction from the SOP model that rats would favour one stimulus in the test stage due to the priming given in the stage before and this is what was found. The presentation of PP in stage 2 meant that on test, elements relating to P were in an A2 state of activation, which meant that more of stimulus Q's elements were in A1, so exploration was biased towards Q. This seems to be the result of a self-generated priming process; however, retrieval-generated priming may have also been involved. In stage 1, the pairs of objects may have become associated; they may also have formed an association with the context. In stage 2, the association of the context with PP would have been strengthened, so that in the test stage, the context primes more of P's elements to A2 because of the stronger association. More of Q's elements would be activated to A1, so resulting in more exploration of Q.

This experiment also served to test whether prior familiarisation of stimuli affected recognition performance. This experiment made the rats familiar to all stimuli in stage 1. This was followed by a standard object recognition experiment (PP, followed by PQ). In the test stage, rats still explored the less familiar stimulus more. P had been made even more

familiar in stage 2, so in the test, in comparison to P, Q was less familiar and so the rats explored it more. The effect seems slightly reduced in comparison to Experiment 1, but was still present. This meant that rats' novelty preference would survive a familiarisation stage. This was important for Experiments 3 and 4.

2.3 Experiment 3

Experiments 1 and 2 demonstrated that self-generated priming is a central mechanism in object recognition. When a stimulus is presented, it reduces the tendency that it will be explored at a later time. This could be due to the changes in activation states of the representations of the stimuli. As well as being a self-generated priming effect, it could be a retrieval-generated priming effect. In the priming stage the stimulus becomes associated with the context it was presented in, so the context activates an expectation of the stimulus. In Experiment 2, the context – P association was strengthened in stage 2. In the test stage, P's elements would be activated to A2, whereas more of Q's elements would be activated to A1.

Retrieval-generated priming may also occur from stimulus-stimulus associations (Honey & Good, 2000). Experiment 3 was designed to test whether associations made between objects would affect later discrimination (or preference) of two objects that had both been encountered previously for the same duration of time. The design of the experiment was the same as Experiment 2, the only difference was in stage 2, XX was presented instead of PP. I predicted that if a retrieval-generated mechanism were used in recognition, then in the test stage rats would explore object Q more than object P.

2.3.1 Method.

Subjects.

Sixteen rats (Harlan, UK) of the same sex and strain as Experiment 1 were used. They were housed and kept as in Experiment 1. Rats weighed between 320 and 380 g, with a mean of 351.25 g. Rats had previously been exposed to auditory stimuli in conditioning chambers, but were naïve to the current apparatus and stimuli.

Apparatus.

The apparatus used was described in Experiment 1. Only objects were used in this experiment. Objects used were: plastic penguin figurines, a blue drinks bottle, a glass mineral water bottle, and an hourglass-shaped glass bottle. Counterbalancing and positioning of objects was the same as in Experiment 2. The arena LED lights and the ceiling-mounted lights were both in use throughout this experiment.

Procedure.

The experiment was conducted in the same way as for group Object in Experiment 2 (Figure 8; Appendix 3E). The only difference was in stage 2; in this experiment, object X was presented instead of P. The durations of stages were also tested in this experiment, to see whether there was an optimal time for forming associations and retrieving associated representations. For half of the animals, the duration of exposure during stages 1 and 2 was five minutes (group 5). For the remaining animals, stages 1 and 2 were ten minutes per exposure (group 10). The test stage was five minutes for all subjects.

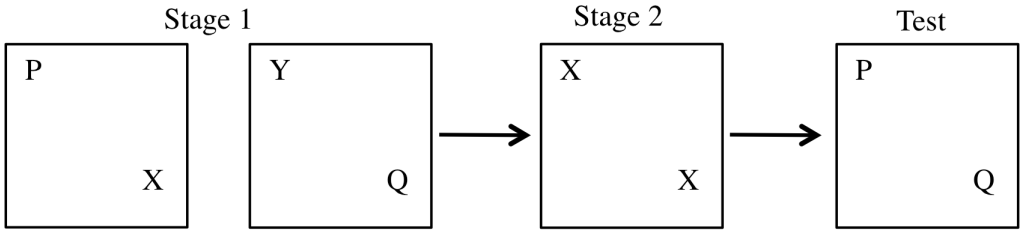


Figure 8. Arrangement of objects (represented by letters P, Q, X and Y) in Experiment 3.

Data collation and analysis methods.

The measurement used was the duration of time that rats spent in the zones. For both groups 5 and 10 the zones were circular and had a radius of 10.5 cm and an area of 330.06 cm^2. Results are reported as percentage of time in the zone, and as ratios in the test stage.

2.3.2 Results and discussion.

Stage 1 and 2.

In stage 1, the time that rats spent in the zones when being exposed to PX and QY was compared. When exposed to objects for 10 minutes, rats accordingly show more time in the zone than rats exposed to objects for 5 minutes. For P, group 5 $M = 103.66$, $SD = 23.38$, group 10 $M = 201.26$, $SD = 53.87$; for X, group 5 $M = 92.75$, $SD = 22.00$, group 10 $M = 155.68$, $SD = 56.90$. For Q, group 5 $M = 112.73$, $SD = 26.31$, group 10 $M = 200.15$, $SD = 41.80$; for Y, group 5 $M = 81.49$, $SD = 17.28$, group 10 $M = 175.39$, $SD = 23.27$. During stage 1 (Figure 9), rats explored the pairs of stimuli for a similar percentage of time, and group 5 and 10 spent similar percentages of time in the zones. This was confirmed by ANOVA performed on data from stage 1 with stimulus pair (PX or QY) and group (5 or 10) as factors. There was no effect of stimulus pair, $F < 1$, $\eta_P^2 = .02$, no effect of group, $F(1, 14) = 3.52$, $p = .082$, $\eta_P^2 = .20$, and no interaction between these factors, $F < 1$, $\eta_P^2 = .04$.

During stage 2, when exposed to objects for 10 minutes, rats accordingly spend more time in the zones than when rats were exposed for 5 minutes. For ‘old’ X (i.e., X that was positioned in the same corner as X in stage 1), group 5 $M = 117.28$, $SD = 26.14$, group 10 $M = 202.03$, $SD =$

48.61. For ‘new’ X (i.e. X that was positioned where P had been in stage 1), group 5 $M = 92.28$, $SD = 31.70$, group 10 $M = 195.65$, $SD = 73.35$. Rats in both groups spent similar percentages of time in the two zones containing samples of object X (Figure 9). This was confirmed by a *t*-test, $t(14) = 1.23$, $p = .238$, $\eta_P^2 = .10$.

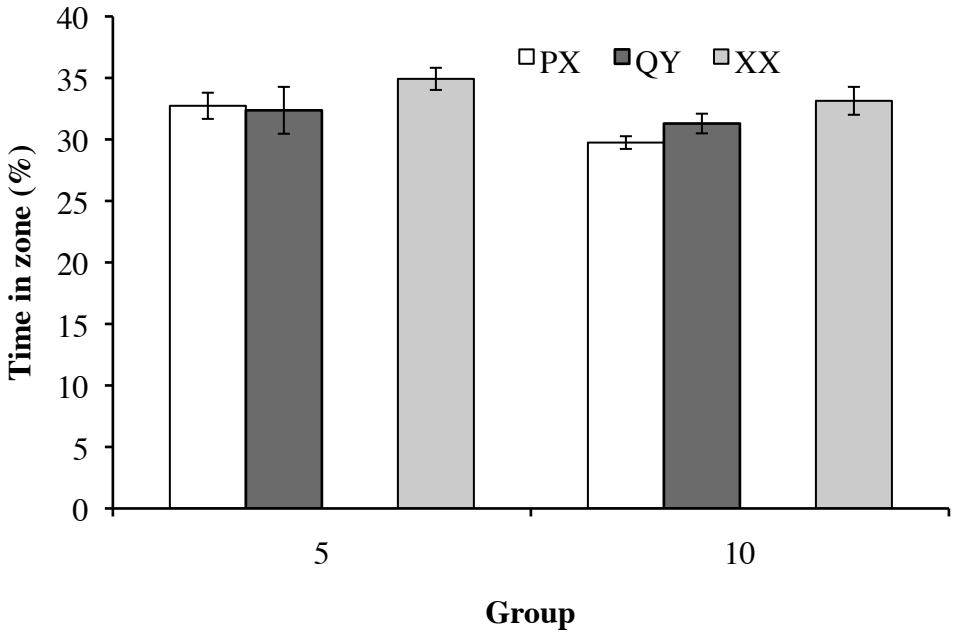


Figure 9. Time in zones in stage 1, in each PX and QY trial, and in stage 2, XX trial. Error bars represent one standard error of the mean.

Test.

Time.

The mean time that group 5 spent in the zone with P was 79.33 seconds (*SD* = 15.79), group 10 *M* = 83.85 (*SD* = 14.10). In the Q zone, group 5 *M* = 103.30 (*SD* = 22.21), group 10 *M* = 99.63 (*SD* = 18.54). Figure 10 shows the percentage of time that rats in groups 5 and 10 spent in the zones surrounding objects P and Q. Throughout the duration of the test stage, rats in both groups spent more time in the zone that contained stimulus Q than in the zone that contained stimulus P. This observation was confirmed by ANOVA of stimulus (P or Q) with group (5 or 10) as a between-subjects factor. There was a significant effect of stimulus, $F(1, 14) = 6.00$, $p = .028$, $\eta_P^2 = .30$, but no effect of group, $F < 1$, $\eta_P^2 < .01$, and no interaction between these factors, $F < 1$, $\eta_P^2 = .02$. These results indicate that rats spent more time in the zone with stimulus Q than stimulus P, and the duration of the sample stages given previous to the test did not affect this discrimination.

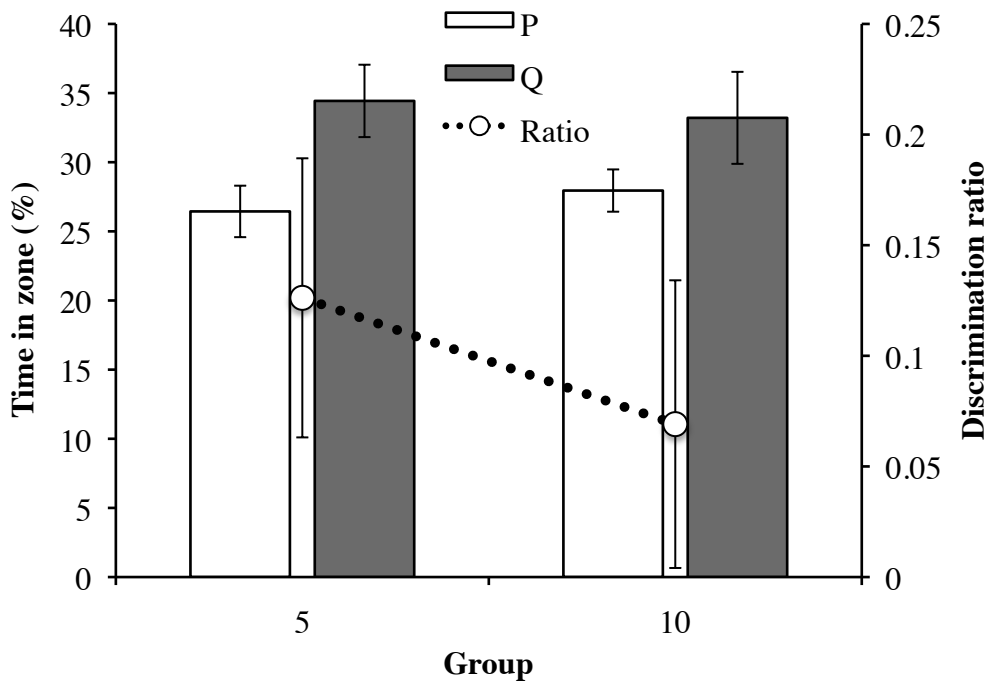


Figure 10. Percentage of time rats spent in each zone that contained stimulus P or Q, represented by bars corresponding to the left Y-axis, and discrimination ratios represented by the line graph, corresponding to the right Y-axis. Error bars represent one standard error of the mean.

Ratio.

The discrimination ratios in the test stage were similar for both groups and were higher than zero, indicating more time spent exploring Q. An ANOVA confirmed there was no difference between the groups, $F < 1$, $\eta_P^2 = .03$, but that the intercept was significant, $F(1, 14) = 4.65$, $p = .049$, $\eta_P^2 = .25$, indicating that rats spent more time in the zone that contained stimulus Q.

The bias towards Q seems to have been the result of pairing the stimuli in stage 1 and the priming in stage 2. At test, P and Q were equally familiar, so the association between P and X have must have been activated in stage 2 when X was presented. Exposure to X caused P to be 'remembered' (activated into A2) so that in the test, Q was more novel (more elements were in A1) than P, and thus explored more. Only the SOP model makes this prediction, other theories of recognition memory, such as the representational-hierarchical account, may not predict this finding.

2.4 Experiment 4

There were some concerns about whether the effects seen in Experiment 3 could be attributed to the positioning of the stimuli in the arena. In stage 2, rats may have explored the copy of X that was positioned where P was positioned in stage 1 (Eacott & Norman, 2004), because it was incongruent with the memory they had. This means they would have spent more time in the arena (in stage 2) in the place where Q was to be positioned in the test, meaning this area would have become familiar, so in the test the rat would have explored Q just because that area of the arena was more novel. Experiment 4 dealt with this issue by using context inserts

as X and Y, and objects as P and Q. Thus, in stage 1 context X was presented with two copies of object P and context Y was presented with two copies of Q. In stage 2, only the context was exposed, and in the test, only objects were exposed. The stimuli that were presented in the test had been presented for an equal amount of time in stage 1, so any bias in discrimination in the test stage may be due to priming in stage 2. The use of context inserts and objects promotes equal exploration of each side of the arenas, so discrimination in the test cannot be attributable to biases concerning exploration of different areas of the arena.

2.4.1 Method.

Subjects.

Sixteen rats, of the same sex and strain as in Experiment 1, were used. They were housed and kept as in Experiment 1. The rats weighed between 360 and 440 g, with a mean weight of 393.75 g. The rats had previously been exposed to auditory stimuli in conditioning chambers, but were naive to the current apparatus and stimuli.

Apparatus.

The apparatus used was described in Experiment 1. In this experiment, objects - a yellow rubber duck and an hourglass-shaped glass bottle - were P and Q; and contexts - white walls and blue walls - were X and Y. Stimuli were counterbalanced as in Experiment 3. The LED lights provided illumination in the arena for all subjects. The ceiling lights were turned off in this experiment.

Procedure.

The procedure was similar to those employed in Experiments 2 and 3, except context inserts and objects were used (Figure 11). As Experiment 3 showed that there was no difference according to whether a five or ten-minute exposure duration was employed, the duration of each stage was five minutes. During stage 1, context walls (X or Y) were placed in front of the white arena walls and two identical objects (P or Q) were placed in the arena, one in the top left, and one in the lower right corner. During stage 2, only the context inserts (X) were placed in the arena. In the test stage, there were no context inserts and rats were presented with the two objects, P and Q. Cleaning and counterbalancing procedures were the same as previous experiments.

Data collation and analysis methods.

The measurement used was the duration of time that rats spent in the zones. In this experiment, the zones were rectangular (12.5 x 13.5 cm) and had an area of 168.75 cm^2. Results are reported as percentage of time in the zone, and also as a discrimination ratio in the test stage.

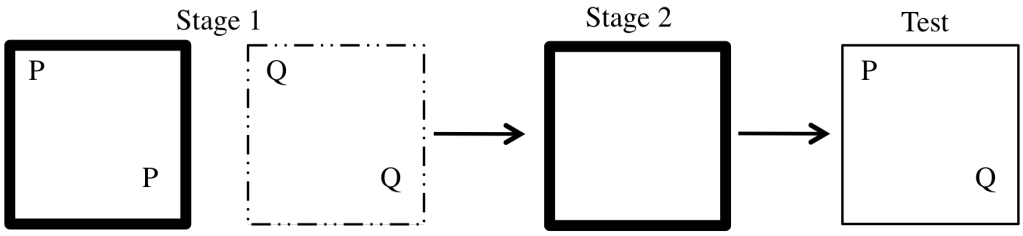


Figure 11. Arrangement of objects (P and Q) and context inserts (thick and dotted lines) in Experiment 4.

2.4.2 Results and discussion.

Data from one rat were excluded from the analyses because it failed to investigate one of the stimuli during the test stage.

Stage 1 and 2.

In stage 1 (Figure 12), rats explored the two stimuli in each trial to a similar extent. This was confirmed by a *t*-test on the data from the PX and QY tests, $t(14) = 1.01$, $p = .297$, $\eta_P^2 = .07$. Stage 2 was examined for any side preferences. There were no objects placed in the arena in this stage, only contexts. The analysis focussed on time spent in the place that had contained objects in stage 1. Rats spent similar amounts of time in each side: left side $M = 19.73$ % (SEM = 1.75); right side $M = 15.03$ % (SEM = 1.87); $t(14) = 1.62$, $p = .128$, $\eta_P^2 = .16$.

Test.

Time.

During the test, the context was, for the first time, removed, and the rats spent the first period of the test examining the 'new' walls. Consequently, data from the first minute and a half were excluded from analysis, and only data from the subsequent minute were analysed. Rats spent more time in the zone that contained Q than in the zone that contained P (Figure 13). This description was confirmed by a *t*-test, $t(14) = 2.76$, $p = .015$, $\eta_P^2 = .35$.

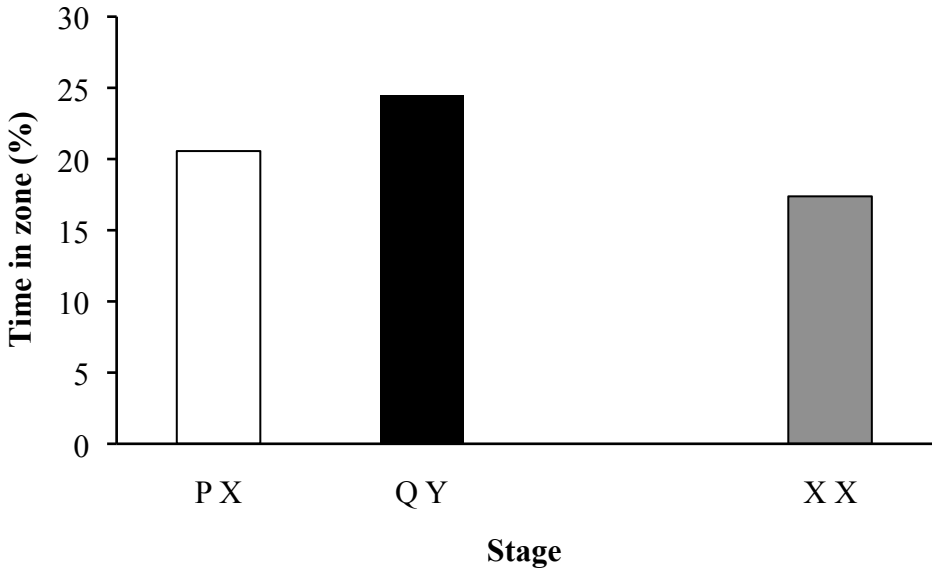


Figure 12. Percentage of time in the zones in Stage 1 (PX and QY) and stage 2 (XX).

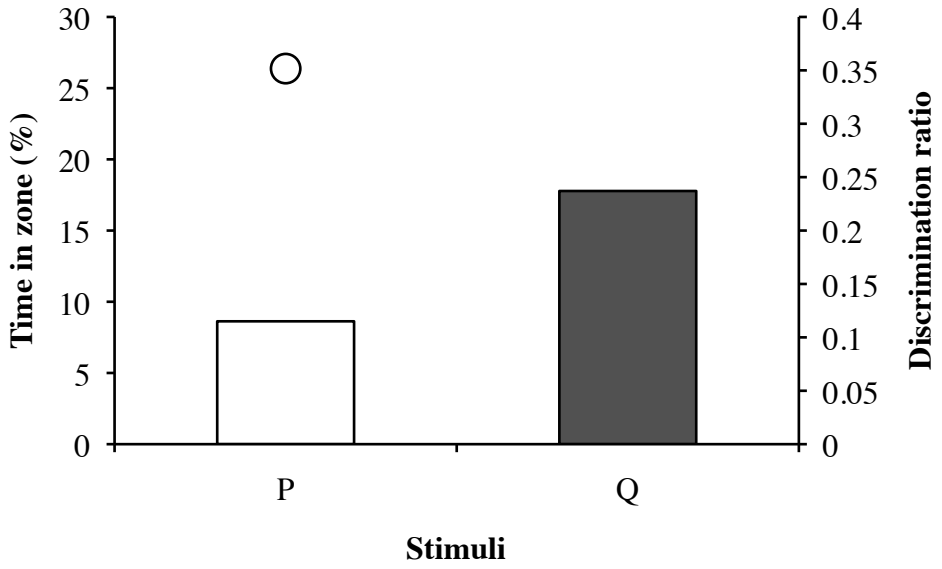


Figure 13. Percentage of time rats spent in zones containing stimulus P and Q, represented by the bars and corresponding to the left Y-axis. The discrimination ratio is represented by the circle and corresponds to the right Y-axis.

Ratio.

The discrimination ratio was quite high (Figure 13); a *t*-test confirmed that it was significantly different from zero, $t(14) = 2.78$, $p = .015$, $\eta_P^2 = .36$. Experiment 4 supported the findings from Experiment 3; priming a stimulus using an associate affected behaviour in the test stage. Experiment 4 showed that the effect could still be obtained when the potential confound seen in Experiment 3 was eliminated. Experiment 4 also contributed to the generality of the effect, in that it was still seen using different stimuli.

2.5 General Discussion

The aim of the experiments presented in this chapter was to test predictions concerning associations in memory that were made using the SOP model. Results showed that recognition memory, as measured by an object recognition task, may involve an associative process. Results from Experiments 1 and 2 are consistent with the possibility that preference for stimulus Q could be due to self-generated priming. Presentation of stimulus P could have activated its representation to A2, through the decay process from initial A1 activation. However, retrieval-generated priming could also be involved in this. In stage 2, stimulus P may have formed a strong association with the context, so that in the test stage, P's elements were primed to A2 and Q's elements were activated to A1. Experiment 2 also confirmed that this effect was not altered by prior familiarisation of the stimuli; even though the stimuli were all familiar, the priming stage still had an effect.

Experiments 3 and 4 were performed to determine whether retrieval-generated priming might operate in this object recognition task. In both experiments, rats spent less time in the zone that contained the stimulus that was associated with the stimulus presented in stage 2, and more time in the zone that contained the non-associated stimulus. This could have been due to the activation states of the stimuli. In stage 2, the presentation of the stimulus (X) could have primed the associated stimulus' (P) representation into an A2 state, so that in the test stage (PQ), P would still have had lots of elements in A2 while the other stimulus (Q) would have had more elements in A1, and so elicited more behaviour as indicated by the rats spending more time in the zone that contained Q. A similar account was used to explain Honey and Good's (2000) results. In their experiment (A → X, B → Y; A → XY) they argued that the presentation of A activated the X elements to A2, so that when XY were presented, only Y was able to activate its elements to the A1 state.

Associative activation was also reported to be the cause of preference for a particular location in an experiment by Sanderson and Bannerman (2011). They used a cross maze to test whether long-term spatial habituation in mice was a result of an association between pairs of arms of the maze or between a location and a body turn response. Mice were given two training trials; these each consisted of being given trials in two arms of a cross-shaped maze, e.g., AB and DC. After this mice were given trials in which three arms of the maze were open, e.g., ABD. Mice showed a preference for the arm that was un-primed, in this example, arm D. Sanderson and Bannerman attribute this finding to an associative

process using locations to retrieve representations, rather than a body turn response. Arm A primed the representation of B (elements were activated into A2); arm D was unexpected (elements were in A1), so mice explored D more. Results from Sanderson and Bannerman support those presented in this chapter, despite some differences in experimental procedure; for example, their mice were given eight test trials, and responses were rewarded with food.

Experiment 2 suffers from a small effect size; this is possibly due to the familiarisation of stimuli in stage 1, as it is difficult to make a subject more familiar with a stimulus, when it is familiar with that stimulus already. Gaskin, Tardif, Piterkin, Kayello, and Mumby (2010) reported that beyond a minimal sample duration (60 – 90 s) additional time during a sample phase did not increase rats' performance in the test stage. Rats' discrimination ratios were similar across sample durations. This suggests that there is an upper limit to familiarity or time needed to build a representation, so that once the subject is familiar with the stimulus, no more exposure to it will help to increase later recognition.

Results from Experiment 4 suggest that contexts and objects may form particularly strong associations, so when one of these is presented as a cue lots of its associate's elements are activated to A2, resulting in successful discrimination of a novel stimulus. The effect size of Experiment 4 was similar to that of the standard OR task in Experiment 1, and the discrimination ratio was fairly high. This suggests that context stimuli may be particularly salient to rats, and particularly able to evoke an associated stimulus. For example, Iordanova, Good, and Honey (2008)

presented rats with an auditory stimulus, X, in context A in the morning, and stimulus Y in context B. In the afternoon, stimulus X was presented in context B, and stimulus Y was presented in context A. Following this, at midday rats were given presentations of X with a shock, and Y with no shock. In a test that took place in the morning, rats showed more fear in context A than B. In a test that took place in the afternoon, rats showed more fear in context B than A. This demonstrates that the rats learnt to expect a certain auditory cue in a particular context.

The data in the test stages of these experiments are based on different time periods because the biased exploration of the novel object is only a short brief effect. As the object becomes familiar (elements decay into A2), the object becomes less attractive, and so exploration decreases. This often occurs within the first two minutes of the test stage and differs between types of object experiment (Dix & Aggleton, 1999). Dix and Aggleton found most exploration of the novel stimulus in the first two minutes of the test in a standard object recognition experiment. However, in a context and object experiment, three minutes was the most sensitive measure. Using different time periods may mean that it is difficult to compare results across experiments.

The results of the experiments presented in this chapter demonstrated that recognition memory might involve associations made between stimuli, an idea that is not explicit in other models of recognition memory. The representational-hierarchical model (Cowell et al., 2010a, 2010b) does not make any specific predictions concerning associations between stimuli, or between objects and stimuli, and so it could not

interpret the results I reported in this chapter. Based on the present and previous research (Honey & Good, 2000; Sanderson & Bannerman, 2011), the associative activation account is perhaps the best candidate for explaining these results.

Chapter 3. Spacing Of Stimulus Presentations

In Chapter 3, I present experiments that tested predictions from the SOP model (Wagner, 1976, 1978, 1981) concerning trial spacing. Trial spacing is a widely researched effect in human and animal studies, yet there is no agreement of why spaced stimulus exposure leads to better learning than massed exposure. The experiments presented in this chapter manipulated the spacing of exposure to a stimulus in the sample stage of an object recognition experiment, and tested how this affected rats' recognition.

Effects of massed and spaced training are widely documented in many areas of human learning and memory research. The earliest report of the benefit of spaced training was from Ebbinghaus (1885/1964). He, himself, learnt series of syllables and found distribution of repetitions over time to be advantageous relative to massing them at one time. Research involving human participants has also focussed on verbal memory tasks, which reported the beneficial effect of spaced training (for review see, Cepeda, Pashler, Vul, Wixted, & Rohrer, 2006). As well as affecting human memory, trial spacing effects are also reported in animal studies. Spaced stimulus exposures generally lead to better learning or memory. Davis (1970) measured habituation to startle when rats were given exposure to tones, with either a 2-second or a 16-second interval between tone presentations. Over trials, those rats given massed exposures (2-second intervals) displayed a significantly lower startle response than those given spaced exposures (16-second intervals). After a 1-minute delay rats received a test stage. Davis found that the startle frequency of animals that

had received spaced training was lower than that for those that had received massed training. Massed exposures produced strong short-term habituation, but spaced exposure produced durable long-term habituation. Davis concluded that habituation was more durable following training with long rather than short intervals between stimulus presentations.

The superiority of spaced training has been reported in habituation of crabs' escape reactions (Tomsic, Berón de Astrada, Sztarker, & Maldonado, 2009), flavour conditioning in rats (Domjan, 1980), contextual conditioning in rats (Barela, 1999; Fanselow & Tighe, 1988), appetitive conditioning in rats, (Sunsay & Bouton, 2008; Sunsay, Stetson, & Bouton, 2004) and bumblebees (Menzel, Manz, Menzel, & Greggers, 2001), and spatial habituation in rats (Sanderson & Bannerman, 2011).

There have been few experiments investigating the spacing effect in object recognition. As far as I am aware, there is only one published paper that reported a beneficial effect of spacing in the sample stage of an object recognition experiment (Anderson, Jablonski, & Klimas, 2008). During the sample stage, rats were given either massed (9 minutes) or spaced (three x 3 minutes with a 1-hour ITI) presentations of objects. Those rats given spaced training showed a greater novelty preference than those given massed training, indicating that they had a better memory for the familiar object. The spaced group also showed a novelty preference that was significantly above chance, whereas the massed group did not.

Rats in the spaced group were taken out of the apparatus for the duration of the ITI, meaning that they had more handling. A second experiment showed that this did not affect results. However, since handling

may be a salient experience for rats, and something that is difficult to standardize (Reed & Adams, 1996), using an automated method of controlling exposures would be advantageous, as all animals would experience equal, minimal handling. This was an aim for the experiments presented in this chapter.

Further evidence on this issue comes from studies that investigated the effect of removing the animal from the context during the inter-trial interval. For example, Sunsay and Bouton (2008) reported an experiment in which they compared performance between rats that were given spaced trials, which either remained in the context or were removed from the context. Results showed that scores from those animals that were removed from the context were lower than those that remained in the context. Sunsay and Bouton concluded that exposure to the context is of vital importance in contributing to the spacing effect. However, Anderson et al. (2008) did see a spacing effect without exposure to the context. This discrepancy seems to be difficult to resolve; however, leaving the animal in the context for the ITI solves issues relating to handling and the time the animal spends in the apparatus (leaving the animal in the context equates experience in the context for those that have massed and those that have spaced exposures).

There have been some attempts to outline the theoretical mechanisms that are responsible for the spacing effect. Barela (1999) used tested various explanations concerning the spacing effect in Pavlovian conditioning, including the Rescorla-Wagner (1972) model, the comparator hypothesis, and Wagner's SOP (1981) but concluded that none of the

theories he tested sufficiently explained the effect. Barela dismissed the SOP model because evidence from Fanselow et al. (1993) showed that rats CR (activity burst) to footshock was greater with four second intervals between trials than 60 second intervals. This is opposite to that predicted by the SOP model. Barela judged that there were two mechanisms used in spacing, one that occurs for ITIs below 60 seconds, and a second for ITIs above 60 seconds. This idea was supported by Sunsay and Bouton (2008), who altered this 60 seconds benchmark to 240 seconds; however, they did not dismiss Wagner's SOP model as Barela did, and found it to be the model most consistent with their results. Sanderson and Bannerman (2011) also considered the SOP model to be the best fitting model.

This is the view taken in this chapter; I suggest that it is the priming mechanisms described by Wagner (1976, 1978) that contribute to the spacing effect. Self-generated priming can explain effects seen with short ITIs and effects seen with short delays, whereas retrieval-generated priming can explain effects seen with longer ITIs and effects seen at long delays; this is explained below. The SOP model (Wagner, 1976, 1978, 1981) predicts that spaced presentations of stimuli would be learnt more effectively than massed presentations of stimuli. This can be done in a self-generated or a retrieval-generated way. Self-generated priming is the mechanism in SOP that explains short-term habituation. Once a stimulus has been presented, its representation becomes primed in memory (becomes activated in the A2 state); this limits reactivation of the stimulus's representational elements when the stimulus is next presented. When stimulus presentations are close together in time, this limited

reactivation becomes apparent, and there is less behaviour (habituation) towards the stimulus.

Long-term habituation depends on associative activation (retrieval-generated priming) and occurs more readily when stimulus exposures are spaced. With longer intervals between presentations of stimuli, the association between stimuli will be stronger than with shorter intervals. The longer interval allows more elements of each of the stimulus representations to decay to an inactive state, so allowing both stimulus elements to be reactivated to the A1 state, resulting in an association. Associations may form between the context and the stimulus. This has been demonstrated by studies that involve a change of context. Jordan, Strasser and McHale (2000) measured rats' licking while they were exposed to tones in a particular context. Rats' lick suppression decreased over training, indicating that they became habituated to the context and tone presentations. When the context was changed, rats' lick suppression increased. This demonstrated that habituation was disrupted by a change of context, indicating that associations are context specific.

Self-generated priming processes in short-term habituation can compete with retrieval-generated priming processes because massed presentations may mean that some elements relating to stimuli may still be in A1 or A2 on the next stimulus presentation, leading to fewer elements being reactivated. Because elements remain in A2, associations are less likely to occur. With weak associations, long-term habituation is reduced.

With regards to the train-test delay, the more recently a stimulus has been presented the more likely it will still be in an active state. The

shorter the delay between training and test, the lesser responding (more habituation) there will be compared to a long delay. This is because stimulus elements will reside in A2 so with little (or no) A1 activation, there will be greater short-term habituation. After a longer interval, responding will increase. This may be because more elements have decayed, and so are able to reactivate to A1. If elements have built associations, the delay between sample and test will not affect long-term habituation because the association will persist.

Hintzman (1974) proposed a similar theory, that spacing stimulus exposure was effective because it allowed complete recovery from habituation. When a stimulus is presented, an internal process begins to store a memory of the stimulus. This (which Hintzman refers to as habituation or adaptation) continues until the stimulus is no longer presented, or attention is directed away from the stimulus. When this happens, recovery from habituation begins. If the stimulus is repeated before recovery is complete, encoding of the stimulus will be less effective than if there is a delay before the second stimulus.

The representational-hierarchical account of recognition memory (see, Cowell et al., 2010a, 2010b; Saksida, 2009) does not allow predictions to be made concerning trial spacing, but does predict that with a longer delay between the sample and test stage of an object recognition experiment, performance will decline (Cowell et al., 2006). In the delay between sample and test, the subject may view other stimuli, and the simple features of these interfering stimuli (represented in the caudal layer) overlap those of the sample object. This means the perirhinal layer is

needed to hold the complete representation of the object. At a short delay, both the caudal and the perirhinal layer may contribute to the representation of the stimulus, resulting in successful recognition. However, at increasing delays, only the representation in the perirhinal layer will be reliable, as all features in the caudal layer will seem familiar. This model states that at a long delay there will be decreased recognition and exploration of novel and familiar objects will be similar.

The experiments presented in this chapter used a visual object recognition procedure to examine effects of massed and spaced presentations on rats' exploration of familiar and novel stimuli. Based on predictions from the SOP model, the hypotheses were that rats given spaced presentations of stimuli would show better discrimination between novel and familiar stimuli in the test stage than when given massed presentations. In Experiment 8, the delay between sample and test was manipulated; I predicted that discrimination between novel and familiar stimuli would be better at a shorter delay than a longer delay. A secondary hypothesis was made based on results from Davis (1970) and Sanderson and Bannerman (2011), that exploration in the sample stage may decline faster when stimulus presentations are massed, and decline more slowly when presentations are spaced.

3.1 Experiment 5

It was necessary to control for handling and context exposure to ensure that treatments were identical across both a spaced and a massed sample stage (see, Anderson et al., 2008, Sunsay & Bouton, 2008). In order to do this in object recognition, an experiment that presented stimuli in a

visual manner was used. Because rats were to stay in the context during the ITI, the ITI had to be conducted in darkness. Winters and Reid (2010) reported that rats were able to discriminate stimuli based on tactile properties. This meant that in my experiments, objects or stimuli had to be screened from the rats so that they could not touch them during the intervals; to this end, objects or stimuli were placed in glass vases.

It was first necessary to test which objects were best to use; rats' performance may be less than that in a standard object recognition experiment in which the rat is also able to employ tactile cues in recognition. There is evidence from Forwood et al., (2007) that rats could display recognition using visual stimuli. In that experiment they used picture stimuli of photographs, shapes and patterns. Rats showed good memory for all the stimuli and they explored the novel stimulus more than the familiar stimulus.

Experiment 5 was a pilot experiment to test whether rats were able to perform successfully in an object recognition experiment that used visual stimuli, using apparatus and stimuli in our laboratory. Three pairs of stimuli was used, two object pairs and a pair of shade (black and white) stimuli. If results were positive, experiments that manipulated trial spacing were possible.

3.1.1 Method.

Subjects.

Eight male Lister-hooded rats (*Rattus norvegicus*), supplied by Charles River (UK), served as subjects. Rats were pair-housed in identical cages that had plastic bases and steel bars. Cages contained sawdust, paper

bedding, and a cardboard cylinder for environmental enrichment. Rats were kept in a room with lights on a 12-hr light cycle with an 0700 onset. The temperature in the holding room and the experimental room was 20°C ± 2°C with a humidity of 50 %.

The rats had free access to food and water throughout the experiment. The rats had previously taken part in an experiment in which they were exposed to trains of clicks, each followed by a food reward. The current experiment used neither food reinforcement nor auditory stimuli and so these previous experiences should not have interfered with their current performance.

Apparatus.

The apparatus used was that used in Chapter 2, Experiment 1. Additional apparatus used included two cylindrical glass vases (35cm tall with a diameter of 13.5cm) were placed in each arena, one in the top left corner and the other in the bottom right. There were two pairs of objects: pair 1 consisted of a green toilet cleaner bottle (25.5 cm x 6.5 cm) and a brown clay elephant ornament (7.5 cm x 10.0 cm); pair 2 were a silver aluminium flask (19.5 cm high with a base diameter of 7.0 cm) and a vinegar bottle (12.0 cm high with a base diameter of 6.5 cm). One pair of shades was used; these consisted of white paper and black card, both in size A3 (29.7 × 42.0 cm).

Procedure.

Before the experiment began, rats received 10 minutes of exposure to the arena over three days. This was to ensure that the rats were familiar

with the arena and vases, so that when the experiment began their attention would be fully captured by the stimuli.

One session consisted of two stages: a sample stage and a test stage (Figure 14; Appendix 3C). For the sample stage, identical objects were placed in each vase, and rats were placed in the arena for ten minutes. The computer tracking began automatically when rats were placed in the arena and the experimenter's hand was out of view of the camera. The objects used in the sample stage were counterbalanced: half the rats were shown one object, e.g., the green bottle and the other half were shown a second object, e.g., the elephant ornament.

At the end of sample stage, rats were removed from the arena and placed back in their home cages, whilst the arena and vases were wiped down with an ethanol solution. Objects were repositioned, so the old (familiar) object was presented, along with a novel object. After this ten-minute interval, rats were placed back into the arena for five minutes. Positioning of the novel object was counterbalanced: for half the rats it was on the left side of the arena, and for the other half it was on the right side. Rats received three of these sample and test sessions; each session used a different pair of stimuli. In the first session, all rats were exposed to the first pair of objects, the green bottle and the brown elephant. In the second session, the flask and vinegar bottle were used. In the third session the shade stimuli were used.

Sample stage	Test stage
AA	AB

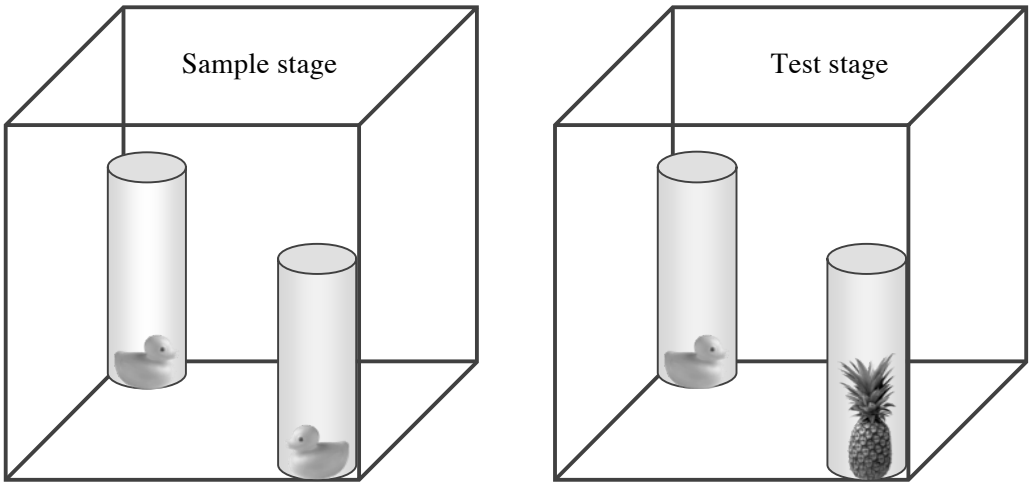


Figure 14. Design of experiment (top box). Lower diagram represents placement of vases and objects in the arena. On the left: the sample stage; each vase contains a copy of one object. On the right: the test stage; one vase contains the object from the sample stage (top left) and the other contains a novel object (lower right).

Data collation and analyses methods.

The time rats spent in each zone that contained the stimuli were recorded. The zones placed around each jar were a right-angled triangle (26.0 x 26.0 x 36.8 cm); the right angle was positioned in the corner of the arena, and the minimum distance between the zone and the vase was 2.0 cm. Measurements and analyses were the same as those performed for the experiments in Chapter 2.

3.1.2 Results and discussion.

Sample.

All rats received a sample and a test session with each stimulus type: both pairs of objects, and shades. For the sample stage, when objects were presented, rats spent a similar duration in each zone (pair 1: 23.30 %, *SE* = 2.74, for the left zone and 19.08 %, *SE* = 2.70, for the right zone. Pair 2: 26.07 %, *SE* = 2.61, for the left zone and 18.43 %, *SE* = 1.21, for the right zone). When the shades were presented, rats again spent a similar duration in each zone, (left zone *M* = 21.81 %, *SE* = 5.34, right zone *M* = 21.55 %, *SE* = 3.22). These descriptions were confirmed by an ANOVA with factors of stimulus type (objects pair 1, pair 2, or shades) and side of stimulus (left or right). There was no effect of stimulus type, $F < 1$, $\eta_P^2 = .02$, or of side, $F(1, 7) = 1.211$, $p = .308$, $\eta_P^2 = .15$, and no interaction, $F < 1$, $\eta_P^2 = .07$. This indicated that the rats sampled the stimuli equally.

Test.

Time.

The first three minutes of the test stage were included in the analyses. In all tests, rats spent more time in the zone that contained the

novel stimulus than the zone that contained the familiar stimulus (Figure 15). This was confirmed by an ANOVA with factors of stimulus type (objects pair 1, pair 2, or shades), and the novelty of the stimulus (familiar or novel). Only the novelty of the stimulus produced a significant effect, $F(1, 7) = 12.29$, $p = .010$, $\eta_P^2 = .64$. There was no effect of which stimulus was used, and no interaction, both Fs < 1, $\eta_P^2 = .04$ and .01 respectively.

Ratios.

In the test stage, the discrimination ratio of each object pair was similar (Figure 15). This was confirmed by an ANOVA, which revealed no difference between ratios for the three pairs of stimuli, $F < 1$, $\eta_P^2 = .02$. The intercept was significant, $F(1, 7) = 13.39$, $p = .008$, $\eta_P^2 = .66$, indicating that the discrimination overall was higher than indifference (zero).

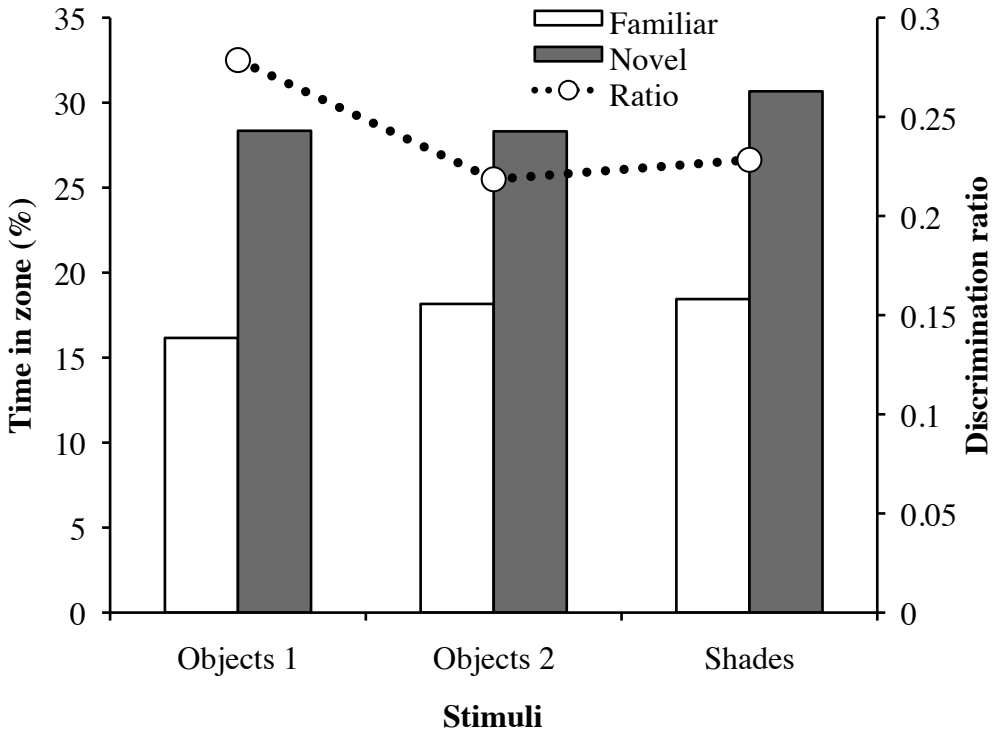


Figure 15. The bar graph represents the duration of time (in percentage) spent by rats in the zone containing the familiar (white bars) or novel (gray bars) stimuli in the test stage, corresponding to the left axis. Discrimination ratios are represented by the line graph, corresponding to the right axis. 'Objects' refer to the test in which rats were shown objects; 'shades' refer to the test in which rats were shown shades.

This experiment demonstrated that using only visual cues, rats spent more time in the zone with the novel stimulus, indicating that they discriminated between the stimuli. These results support those reported by Forwood et al. (2007) that rats are able to recognise visual stimuli. The present results also parallel reports from Berlyne (1950) and Dember (1956) that rats are sensitive to brightness stimuli.

The positive findings from Experiment 5 meant that it was possible to proceed with experiments to test spacing of stimuli. Any changes in the pattern of the familiar/novel discrimination could be interpreted as being due to the manipulation of the stimulus spacing, rather than any factors to do with the stimuli, such as the rats not being able to distinguish between them.

3.2 Experiment 6

Experiment 6 was designed to test the effects of spaced or massed exposure on object recognition. In this experiment, one group of rats was given spaced exposures to stimuli in the sample stage and another group was given massed exposures. This experiment was designed to control for several factors that may have affected performance in other studies (e.g., Anderson et al, 2008). This included ensuring equal handling of all rats, whether in a massed or a spaced group. Time in the apparatus was also equated between groups. The number of exposure trials was also controlled. Hintzmann, Summers and Block (1975) reported stimuli that were interrupted, i.e., stimuli that were presented in multiple trials, were better recognised in a later test than stimuli that were not interrupted, i.e., presented in a continuous trial. This could have contributed to the results

reported by Anderson et al. (2008), as their massed group experienced one, non-interrupted, exposure to the stimuli but the spaced group had separate trials. Experiment 6 controlled for this by having repeated trials in both the spaced and the massed condition.

In terms of SOP, spaced exposures allow more time for elements to decay, thus leading to more reactivation on the next stimulus presentation, and perhaps building stronger associations between the context and stimuli. Rats given spaced exposures to stimuli would show better or longer lasting discrimination than those given massed exposures. The representational-hierarchical model does not specify what effect spaced exposures might have on recognition. A long delay between train and test results in reduced recognition due to other stimuli being sampled during the delay. This could not apply to the spaced ITIs in this experiment because the rats would not be able to view any extraneous stimuli during the ITI.

3.2.1 Method.

Subjects.

Subjects were 16 rats of the same sex and strain as those used in Experiment 5. Rats were kept and housed as in Experiment 5.

Apparatus.

The arenas, cameras and lights used were the same as those used in Experiment 5. The stimuli used were the wall inserts that were used in Experiment 1.

Two types of exposure session were given, one that gave spaced exposures to stimuli (long ITI), and a second that gave massed exposures to stimuli (short ITI, Figure 16). Both types of session were 36 minutes in

duration, and had a total of eight exposures to stimuli (four minutes). In the spaced condition, lights were programmed to switch on every 240 seconds (4 minutes) for 30 seconds. In the massed condition, lights were programmed to switch on for 30 seconds, then off for 30 seconds; moreover, in order to equate session length, and to ensure that the last stimulus exposure was always separated by the same interval from the test in both conditions, there was 1710 seconds (28.5 minutes) before the first light switched on.

Procedure.

In order to make the rats familiar with the arena and the changes in light, they were given exposure to the arena over four days, one session per day. They received two sessions of short ITI exposure, and two sessions of long ITI exposure. No stimuli were placed in the arena over these days.

The animals were then divided into two groups; one group (n = 8) was given spaced exposures (long ITI) and the second group (n = 8) was given massed exposures (short ITI).

Walls were placed in the arena at the start of the experiment; in the sample stage, the whole arena had walls of the same pattern (Appendix 3B). Both wall patterns were used as sample stimuli; half the rats had the white and black pattern, and the other half had the blue pattern. At the end of the sample stage, the rats were removed from the arena and placed back in their home cages whilst the walls were repositioned for the test stage, and the arena and walls were cleaned with an ethanol solution. After this 10-minute interval, the rats were placed back in the arena. In the test stage, half of the arena had a familiar wall pattern, and half had a novel wall

pattern. Positioning of the novel stimuli was counterbalanced, so that for half the rats the novel stimulus was on the left side of the arena and for the other half it was on the right side.

Sample stage	Test stage
AA	AB
Spaced or massed	

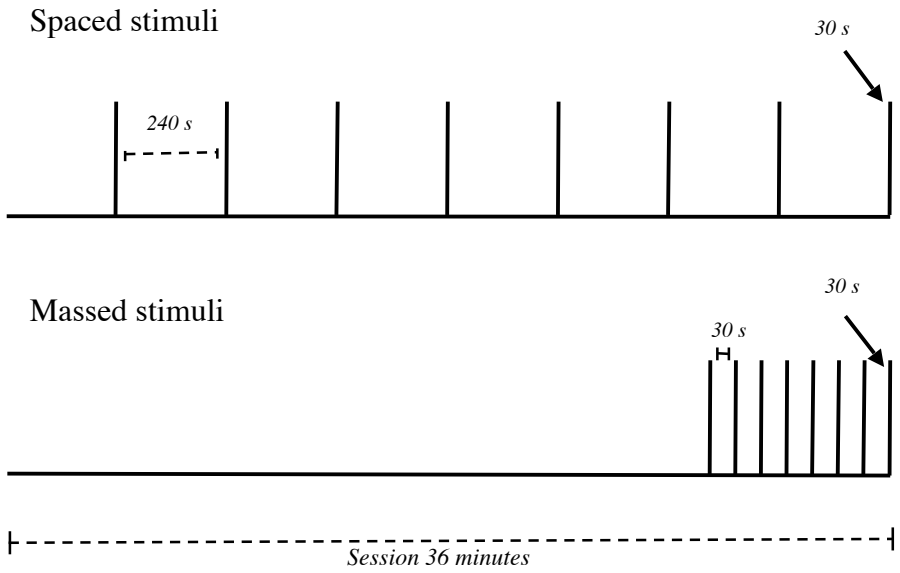


Figure 16. Experiment design (top box). Diagram represents the sessions with spaced trials (top) and massed trials (lower). Each vertical line indicates the illumination of the stimulus (30 seconds). The intervals of the spaced condition were 240 seconds, the intervals of the massed condition were 30 seconds.

Data collation and analyses methods.

The percentage of time that rats spent in each zone was recorded. Exploration in the sample stage was recorded only when the lights were on, as this was when the rats could see and explore the stimuli, and also because the tracking system could not work in the dark. This gave eight time periods of 30 seconds each. ANOVA on the percentage of time spent in the stimulus zones from the sample stage were conducted to assess if there were any differences between the spaced and massed groups.

The first minute of the test stage was examined in two 30-second bins to examine whether discrimination varied over time, for example if it was longer lasting in one condition than the other. A discrimination ratio was also used to analyse data from the test stage. This was calculated as described in Experiment 5. An ANOVA was conducted to check for differences between massed and spaced exposure. Simple main effects analyses with a pooled error term were conducted where relevant. One sample *t*-tests with the Bonferroni-Holm correction were conducted to compare the novelty preference to that of chance.

3.2.2 Results and discussion.

Sample.

Rats in both groups spent a similar duration of time in the zones that contained the stimuli (Figure 17), and the time spent in the zones stayed at a constant level across the eight light trials.

An ANOVA with factors of trial and group revealed that rats in both groups showed no differences across trials, $F < 1$, $\eta_P^2 = .05$, and time

in zones did not differ between groups, $F < 1$, $\eta_P^2 = .87$; these factors did not interact, $F(7, 98) = 2.01$, $p = .06$, $\eta_P^2 = .13$.

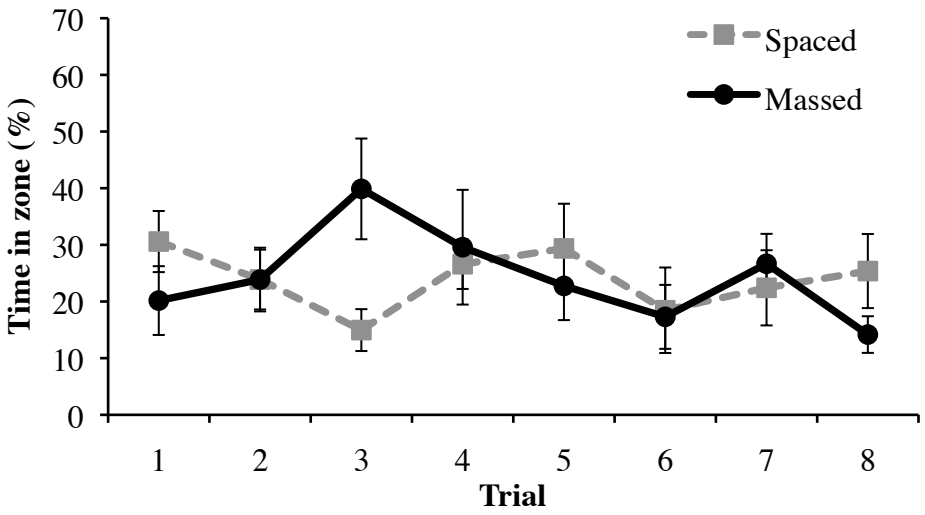


Figure 17. Time in zone in the sample stage for the group that had spaced exposures and the group that had massed exposures. Error bars represent one standard error of the mean.

Test.

Time.

Rats spent more time in the zone with the novel stimulus (B) than with the familiar stimulus (A; Figure 18). The group given spaced exposures to stimuli seemed to show a greater discrepancy between time in the zones of the familiar and of the novel stimulus than the group given the massed stimulus exposure. However, an ANOVA using factors of time period (0 – 30 s or 31 – 60 s), novelty (familiar or novel stimulus), and group (massed or spaced) did not confirm this. Results showed that rats spent more time in the zone that contained the novel stimulus, $F(1, 14) = 35.35$, $p < .001$, $\eta_P^2 = .72$. There was a significant interaction between novelty and time period of the test, $F(1, 14) = 5.95$, $p = .029$, $\eta_P^2 = .30$. There was no effect of group, $F < 1$, $\eta_P^2 < .01$, or time period, $F < 1$, $\eta_P^2 = .04$, and the Group x Time period interaction was not significant, $F < 1$, $\eta_P^2 = .03$. Novelty did not interact with group, $F(1, 14) = 1.89$, $p = .191$, $\eta_P^2 = .12$. There was no three-way interaction between the factors, $F(1, 14) = 2.58$, $p = .120$, $\eta_P^2 = .16$.

The significant interaction (Novelty x Time in test) was explored using simple main effects (SME) analyses with a pooled error term. There was no difference in time spent in the familiar stimulus zone from 0 – 30 s to 31- 60 s, $F(1, 14) = 2.12$, $p = .167$, $\eta_P^2 = .132$.

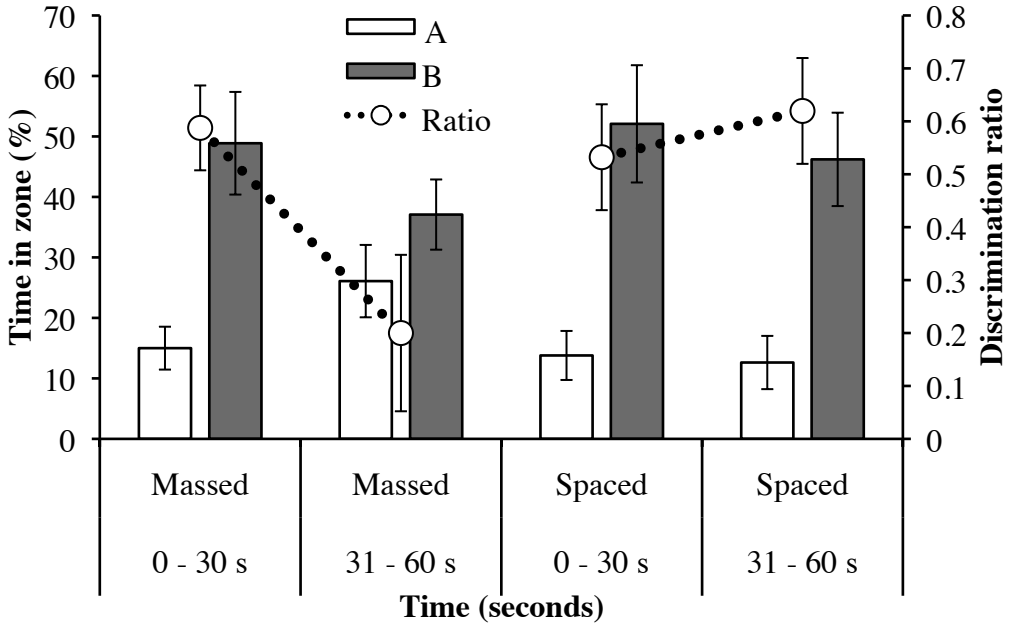


Figure 18. Percentage of time spent in the zone containing the familiar object (A) or the novel object (B), represented by bars corresponding to the left Y-axis. Discrimination ratios for each group are shown by the line graph, which corresponds to the right Y-axis. Error bars represent one standard error of the mean.

Time spent in the novel stimulus zone decreased at 31 – 60 s, $F(1, 14) = 4.69$, $p = .048$, $\eta_P^2 = .25$. Bonferroni Holm corrected *t*-tests were conducted to test differences between groups at different time points during the test. The group given spaced exposures showed greater time in the zone of the novel stimulus than the familiar stimulus at both 0 – 30 s, $t(7) = 3.79$, ($M = 38.29$, $SE = 10.10$), $p = .007$, $\eta_P^2 = .67$, and 31 – 60 s, $t(7) = 5.37$, ($M = 33.58$, $SE = 6.26$), $p = .001$, $\eta_P^2 = .80$. The group given massed exposures showed greater time in the novel stimulus zone at 0 – 30 s, $t(7) = 5.01$, ($M = 33.88$, $SE = 19.12$), $p = .002$, $\eta_P^2 = .78$, but not at 31 – 60 s, $t(7) = 1.32$, ($M = 11.00$, $SE = 23.65$), $p = .230$, $\eta_P^2 = .20$.

Ratios.

Overall, discrimination ratios were quite high; however, were enduring for only the group given spaced training (Figure 18). Groups had similar discrimination scores at 0 – 30 seconds, but at 31– 60 seconds the group given spaced exposures showed a higher discrimination ratio than the group given massed exposures.

An ANOVA with time period (0 – 30 s or 31 – 60 s) and group (massed or spaced) revealed no differences. There was no effect of time period, $F(1,14) = 1.28$, $p = .278$, $\eta_P^2 = .08$, no effect of group $F(1, 14) = 1.94$, $p = .186$, $\eta_P^2 = .12$, there was no significant interaction between these factors, $F(1, 14) = 3.20$, $p = .095$, $\eta_P^2 = .19$.

SME analyses were conducted due to the approaching significance of the interaction. At 31 – 60 seconds, the spaced group had a larger ratio than the massed group, $F(1, 14) = 5.01$, $p = .042$, $\eta_P^2 = .26$. This was not apparent at 0 – 30 seconds, $F < 1$, $\eta_P^2 < .01$.

One-sample *t*-tests were conducted to test whether discrimination ratios differed from chance. Discrimination was significant in the massed group at 0 - 30 s, $t(7) = 7.33$, $p < .001$ ($M = 0.59$, $SE = 0.08$), $\eta_P^2 = .88$, and for the spaced group at 0 - 30 s, $t(7) = 3.18$, $p = .015$ ($M = 0.53$, $SE = 0.17$), $\eta_P^2 = .59$. At 31 - 60 seconds, discrimination was significant for the spaced group at 31 - 60 s, $t(7) = 5.36$, $p = .001$ ($M = 0.62$, $SE = 0.12$), $\eta_P^2 = .80$, but not in the massed group, $t(7) = 1.35$, $p = .22$, ($M = .20$, $SE = .42$), $\eta_P^2 = .21$. The spaced group's ratios were significant at both time points, but the massed group's ratios was only significant from chance at the first time point.

Spaced exposures in the sample stage led to rats spending more time in the zone containing the novel stimulus in the test stage than those given massed exposures. Experiment 6 was designed as a visual task, as the wall stimuli were most likely to encourage a memory based on visual features; however, rats may have been able to use the textures of the walls to help discriminate between them (see, Guić-Robles, Valdivieso & Guajardo, 1989; Hughes, 2007). Experiment 7 was designed to replicate Experiment 6, but instead of wall stimuli, objects were used to make it more similar to other object recognition experiments. To ensure that rats could not explore the objects during the dark phases of the sample stage, the objects were placed in glass vases (see Experiment 5), so that the rats could only see the objects when the lights were switched on. This meant that the spacing of the exposures in the sample stage was now vital to building a memory/representation of the stimulus.

3.3 Experiment 7

Experiment 7 was a replication of Experiment 6; however, the stimuli were changed from wall stimuli to objects and shades that were placed in vases (as used in Experiment 5). This change in stimuli should not alter the overall effect, so I predicted that discrimination would be better when rats were given spaced exposures in the sample stage. Experiment 7 was also within-subjects (all rats had a massed and a spaced task) as opposed to between-subjects, as in Experiment 6.

3.3.1 Method.

Subjects.

Subjects were 16 rats of the same sex and strain as Experiment 5. They were kept and housed as in Experiment 5.

The rats had previously taken part in an experiment, in which they were exposed to clicks and tones. They had also received conditioning with electric shocks. They were, however, naïve to the current apparatus and stimuli.

Apparatus.

The apparatus (arenas, cameras, lights, and vases) was the same as employed in Experiment 5. The stimuli were objects (an aluminium flask and a vinegar shaker, see Experiment 5 for details) and shades (black and white). The two preexposure conditions (massed or spaced exposure) were the same as those used in Experiment 6.

Procedure.

All rats were given one 10-minute familiarisation session to the arena and vases in a dark room, followed by two sessions of each

preexposure condition (massed and spaced) prior to the experiment, so that the arena, the vases, and the switching on-and-off of the lights, became familiar. There were no stimuli placed in the vases during these sessions.

The experiment was conducted the day following the last familiarisation session. This experiment was within-subjects, so all rats received two sessions: one session with spaced exposures and one session with massed exposures. The exposure condition was counterbalanced, so in the first session half (n = 8) the rats had massed exposures, and the other half (n = 8) had spaced exposures. Each subgroup of animals received the opposite exposure condition in the second session.

Each of these sessions used a different set of stimuli: the first session used shades, and the second session used objects. Stimuli were counterbalanced so that half the rats in each subgroup was shown two identical white vases in the sample stage, and the other half was shown two identical black vases.

At the end of the sample stage, rats were removed from the arena and placed back in their home cages whilst the objects were repositioned for the test stage, and the arena and vases were cleaned with an ethanol solution. After this short interval (10 minutes) the rats were placed back in the arena and were exposed to the familiar stimulus, and a novel stimulus. Positioning of novel shades was counterbalanced, so for half the rats it was on the left side, and for the other half it was on the right side. The arena lights were on throughout the duration of this stage. The time that rats spent exploring the stimuli was recorded.

After three days, the second session took place. By the end of the two sessions, all rats had been tested with the massed (short ITI) condition and the spaced (long ITI) condition.

Data collation and analyses methods.

These were the same as Experiment 6.

3.3.2 Results and discussion.

Sample.

Rats spent similar amounts of time in the zones containing the stimuli in both conditions and across trials (Figure 19). This was confirmed by an ANOVA. In both conditions, rats spent similar percentages of time in the zones, $F < 1$, $\eta_P^2 < .01$, and this did not differ over the trials, $F(7, 105) = 1.76$, $p = .10$, $\eta_P^2 = .11$; the interaction between these factors was not significant, $F < 1$, $\eta_P^2 = .04$.

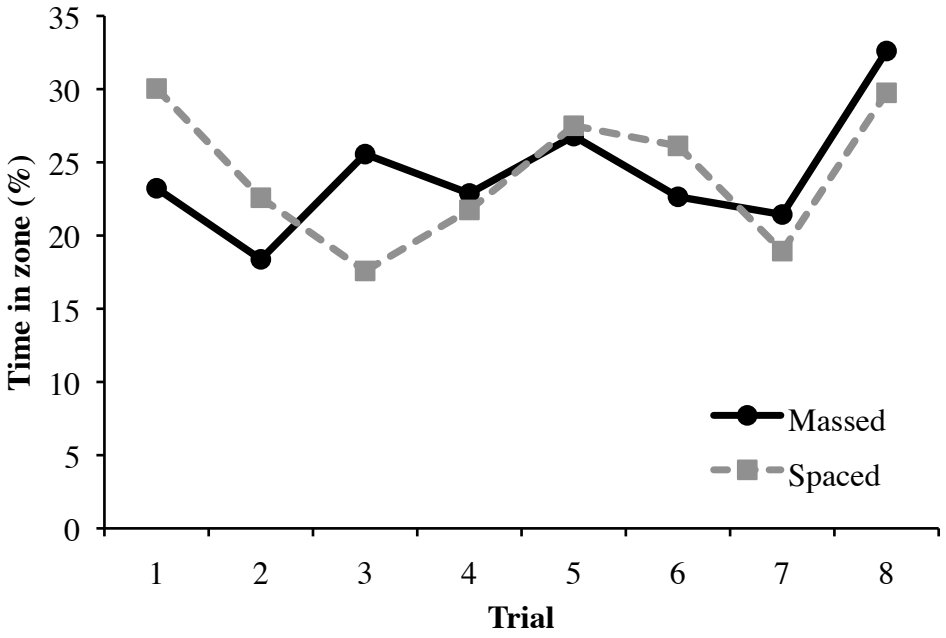


Figure 19. Percentage of time that rats spent in the zones containing the vases in the sample stage for both conditions.

Test.

Time.

Results are presented as percentage of time spent in a zone with stimulus A (the familiar stimulus) or stimulus B (the novel stimulus). In both conditions, rats spent more time in the zone with the novel stimulus than in the zone with the familiar stimulus during the first minute of the test; this preference continued into the second minute during the spaced condition, but not during the massed condition (Figure 20).

Nonetheless, an ANOVA with factors of condition (spaced or massed), time (first or second minute) and novelty (time spent in familiar or novel zones) did not confirm this description. There was greater exploration in the massed condition than in the spaced condition, $F(1, 15) = 4.95$, $p = .042$, $\eta_P^2 = .25$, and more time spent in zones in the first minute than the second minute, $F(1, 15) = 12.55$, $p = .003$, $\eta_P^2 = .46$. There were no other significant effects or interactions; there was no effect of novelty, $F(1,15) = 2.28$, $p = .152$, $\eta_P^2 = .13$, no Condition x Time interaction, $F < 1$, $\eta_P^2 = .04$, Condition x Novelty interaction, $F < 1$, $\eta_P^2 = .01$, Time x Novelty interaction, $F(1, 15) = 2.27$, $p = .152$, $\eta_P^2 = .13$. There was no three-way interaction between all these factors, $F < 1$, $\eta_P^2 = .05$.

Ratios.

In both conditions, rats showed discrimination ratios higher than zero in the first minute of the test (Figure 20). However, in the second minute of the test, discrimination in the massed condition had declined, whereas discrimination in the spaced condition had increased. An ANOVA partially confirmed this description. There was no effect of condition, $F(1,$

15) = 1.86, p =.193, η_P^2 = .11, or time, F < 1, η_P^2 = .03, but there was an interaction between condition and time, $F(1, 15) = 5.80$, p = .029, η_P^2 = .28. The intercept was not significant, $F(1, 15) = 2.80$, p =.12, η_P^2 = .16. SMEs analyses were conducted on the Condition x Time interaction, and these showed that discrimination ratios were significantly different, between conditions in the second minute, $F(1, 15) = 4.91$, p = .043, η_P^2 = .25, but not in the first, F < 1, η_P^2 < .01. Bonferroni-Holm corrected t-tests were conducted to test whether discrimination ratios were difference from chance level (zero). When given spaced training, during the second minute of test rats showed significant discrimination, $t(15) = 2.91$, p = .011, (M = 0.29, SE = 0.10), η_P^2 = .36. In the first minute of the test for the spaced condition, discrimination was not significant, $t(15) = 1.33$, p = .21, (M = 0.15, SE = 0.11), η_P^2 = .11. When given massed exposures, ratios did not reach significance, in either the first, $t(15) = 1.19$, p = .25, (M = 0.13, SE = 0.11), η_P^2 = .09, or the second minute of the test, t < 1, (M = 0.13, SE = 0.15), η_P^2 = .04.

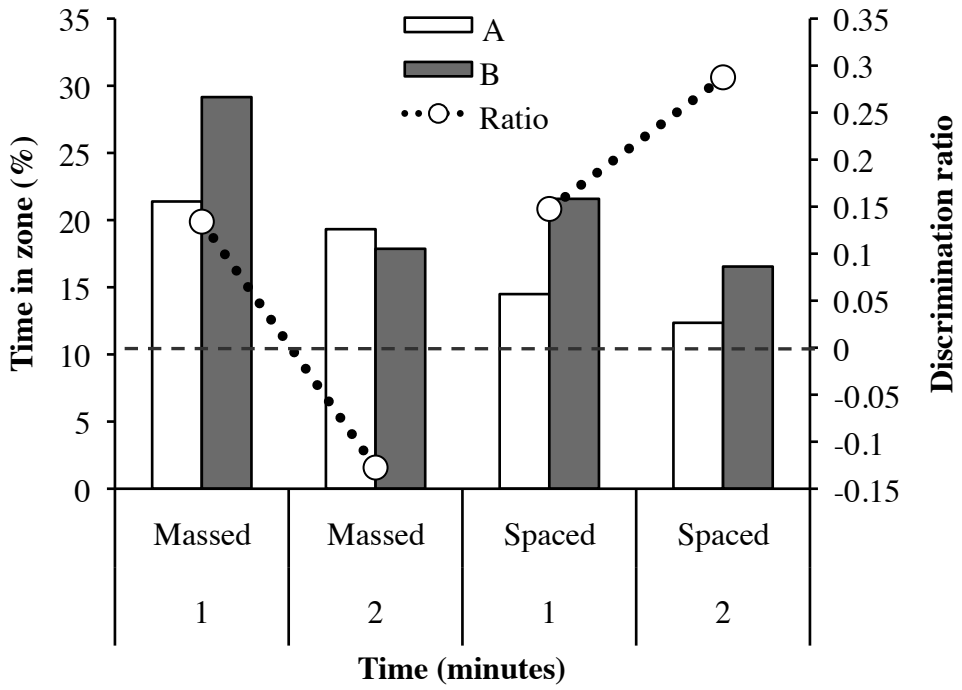


Figure 20. Time in zone during the test stage in the zone containing the familiar stimulus (A) and the novel stimulus (B). Percentage of time in zone represented by the bar chart, corresponding to the left Y-axis. Discrimination ratios are illustrated by the line graph, which corresponds to the right Y-axis. Horizontal dashed lined represents no discrimination (zero).

There was some indication, when considering ratio scores, that results from Experiment 7 support those from Experiment 6: rats' recognition of familiar stimuli, and subsequent selective exploration of the novel stimulus, was better when exposures in the sample stage were spaced (long ITI) rather than when they were massed (short ITI). These results support those reported by Anderson et al. (2008), that in an object recognition experiment, spacing stimuli in the sample stage led to better or longer lasting discrimination in the test stage. The experiments presented in this chapter have eliminated confounds relating to that experiment. Both conditions in the present experiments had an equal number of trials; in Anderson et al.'s experiment, the massed group had one trial whereas the spaced group had three trials. Using the lighting to control stimulus exposures in the present experiment meant that all the subjects in the present experiment were handled equally, and that rats spent the same amount of time in the apparatus in both conditions.

These results support the predictions from the SOP model that spaced exposures allow for better associations to form between the context and the stimulus. These associations led to better discrimination in the test stage. Changing the delay between sample and test may highlight short-term and long-term habituation further; a short interval may lead to greater short-term habituation, so lead to greater discrimination than a long interval. At long delays, only associative (RGP) processes may be used, and so discrimination may be lower than that at short delays.

3.4 Experiment 8

Experiment 8 was conducted to test whether a short or long delay between the sample and test stage would affect rats' recognition, and confirm that the spacing of the exposure of the stimuli in the sample stage influenced recognition. A model of object recognition by Cowell et al. (2006) simulated that longer delays lead to a decline in recognition. Experiment 8 tested this prediction, but also whether the spacing of the stimuli in the sample stage may enhance or depress performance.

A second purpose of Experiment 8 was to further test predictions from SOP, in particular to try and specify whether priming was likely to be involved. Using a short delay (2.5 minutes) between the sample and test stage may increase short-term habituation (self-generated priming) compared to a long delay. After a short delay, the elements relating to the sample stimulus would still be in A2 during the test stage, and so behaviour towards it should be minimal. This delay is much shorter than that in Experiments 6 and 7 (there it was 10 minutes) so it could be that short-term habituation will result in good discrimination between stimuli with both massed and spaced exposure. Experiment 8 used a long delay of 24 hours to test whether discrimination was worse than with a short delay. With this long delay, spaced exposure may support greater recognition of the familiar stimulus than massed exposure. At a long delay, contextual cues (retrieval-generated priming) may be used, as these have formed a good association with the stimulus. With massed presentations, there would not be such a good context-stimulus association and so discrimination may not be as apparent. There would be limited self-

generated priming, as activation would have decayed over the 24-hour interval.

Sanderson and Bannerman (2011) reported this effect in a spatial task. Mice were exposed to two arms of a spatial maze, either with a long or short inter-trial interval (ITI) and long or short delays between training and test. In the test, mice were allowed to explore a novel arm. Mice that were given a long training ITI showed greater preference for the novel arm than the groups that were given a short training ITI. Mice that were given a short delay (1 minute) between sample and test showed significantly higher difference scores than those given a long delay (24 hours). These results suggest that long-term and short-term memory processes may be competitive. The short interval between training trials resulted in weaker long-term habituation in test, suggesting that long-term habituation (RGP) is important in discriminating novelty. But also the effect of the short delay indicates that short-term habituation (SGP) is important in novelty discrimination.

Experiment 8 used the same exposure conditions as Experiment 7, a spaced and a massed condition. Two delays were also incorporated between the sample and test stage, 2.5 minutes and 24 hours. Based on previous findings (e.g., Sanderson and Bannerman, 2011) and the SOP model, I predicted that when the delay between the sample stage and the test stage was short, rats would show greater short-term habituation, and so greater discrimination that with a long delay. However, spaced exposures (allowing RGP) may result in greater habituation, and so result in greater discrimination in test than massed exposures.

3.4.1 Method.

Subjects.

Subjects were 32 rats of the same sex and strain as in Experiment 5. Rats were kept and housed as in Experiment 5.

Apparatus.

The apparatus used was that described in Experiment 5. The objects used were a green plastic bottle and a spherical brown ornament (7.5 cm x 8.0 cm). Shades used were black A4 (21.0 × 29.7 cm) card and white A4 paper. An up lighter lamp (B&Q, Hampshire, UK) with a 25-w red bulb was used during the delay in the short delay condition. The same exposure schedules used in Experiment 6 were used in Experiment 8 for spacing of the stimuli.

Procedure.

The procedure was the same as that used in Experiment 7, with the exception of an additional variable of the change in duration between the sample stage and the test stage (either 2.5 minutes or 24 hours). Rats were split into two groups (*n*s = 16); one group was assigned the long delay, and the other group was assigned the short delay. Each group had a session of each condition, spaced exposures and massed exposures.

In the first sample and test session, all rats received exposure to shade stimuli. One sample and test session was run over two days; on the first day the group assigned the 24-hr delay (*n* = 16) were given the sample stage (*n* = 8 had spaced exposures, *n* = 8 had massed exposures). Half of the rats (*n* = 8) in the group assigned the 2.5-min delay were given the sample and test stage, again half (*n* = 4) with spaced exposures and half (*n*

= 4) with massed exposures. On the second day, the rats in the 24-hr delay group (n =16) were given the test stage, and the remaining half of those rats in the 2.5-min delay group (n = 8) were given the sample stage, half (n = 4) with spaced exposures and half (n = 4) with massed exposures, and test stage.

The rats in the two delay groups spent the delay in different locations. Due to time constraints, rats given a 2.5-min delay were not taken out of the arena when the shades were changed for the test stage. Rats were left in the arena and the shades in each vase were switched for a copy of the sample shade and a novel shade. The lights were not operated during this interval, and only a lamp with a red bulb illuminated the room for the experimenter (rats are insensitive to red light, see, Jacobs, Fenwick, & Williams, 2001; Szél & Röhlich, 1992). Rats that were given a 24-hour delay were returned to the holding room during this time. After four days, a second sample and test session was run; rats were given the condition they had not had in the first session, e.g., if the first session was the spaced condition, the second session was the massed condition. In all other respects, the second session was identical to the first but used objects instead of shades.

Data collation and analyses methods.

Data were collated and analysed in the same way as Experiment 6. Data were collated from both sessions (like Experiment 7), so that the condition (massed or spaced) was a within-subject variable and the delay (2.5 minutes or 24 hours) was between-subjects.

3.4.2 Results and discussion.

Data from one rat were excluded from all analyses due to very low levels of exploration in the sample stage (it spent less than 10% of time in the zones). Another two rats' data were excluded from all analyses due to a computer error in the sample stage. $N = 29$ for each the sample and test stage. Results are taken from the first 30 seconds of the test stage.

Sample stage.

Rats' time in the zones during the sample stage (Figure 21) was fairly high in the first trial, and declined slightly across trials. In both the massed and spaced condition, time in the zones was similar. An ANOVA with the factors of trial (1 – 8) and ITI condition (spaced or massed) supported this description. This showed no effect of ITI condition, $F(1, 28) = 1.64$, $p = .211$, $\eta_P^2 = .06$, a significant effect of trial, $F(7, 196) = 6.94$, $p < .001$, $\eta_P^2 = .20$, but no interaction between these factors, $F < 1$, $\eta_P^2 = .02$. These results suggested that rats spent less time in the zones containing the stimuli toward the later trials of the session, and that rats' time in the zones was similar in both conditions. As the trials progressed the rats' response to the stimuli (time in zone) decreased; these results indicated that there might have been some habituation to the stimuli. This was not seen in any of the previous experiments (6 or 7), but is similar to results found by Davis (1970) and Sanderson and Bannerman (2011).

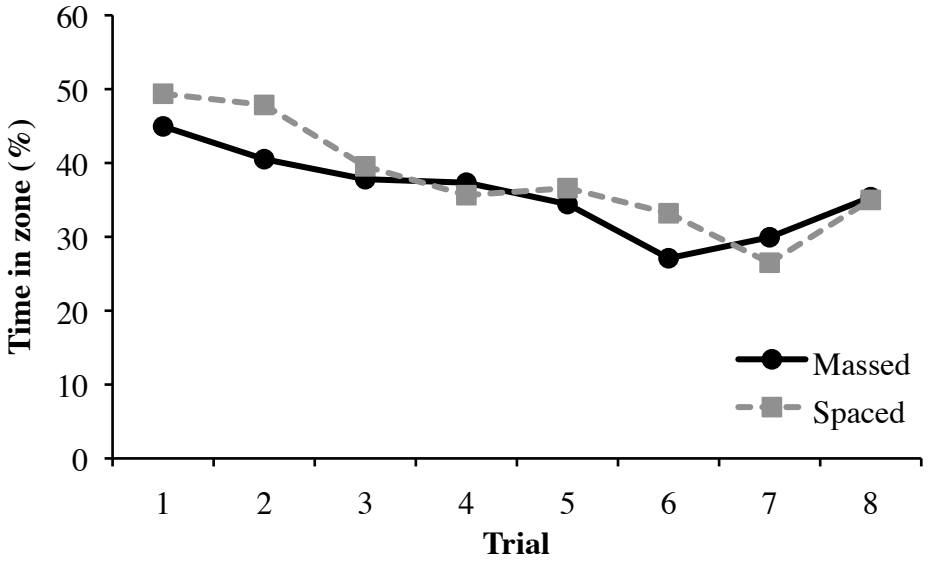


Figure 21. Percentage of time rats spent in the zones containing stimuli in each trial in the sample stage.

Test stage.

Time.

In all conditions and across both the short and long delay, rats spent more time in the novel zone than the familiar zone (Figure 22). This discrepancy was more apparent in the group given the 2.5-min delay, whether in the massed or spaced condition; accordingly the discrimination ratios for both conditions were fairly high. In the group given the 24-hour delay, the discrepancy between time spent in the zone with the familiar object and the time spent in the zone with the novel object was more perceptible when rats were given spaced exposures, rather than massed exposures, and accordingly the discrimination ratio for the spaced condition was higher than the massed condition.

An ANOVA with within-subjects factors of condition (spaced or massed) and novelty (time in the familiar or novel zone), and the between-subjects factor of delay (2.5 minutes or 24 hours) revealed only a significant effect of novelty, $F(1, 27) = 17.03$, $p < .001$, $\eta_P^2 = .39$; there was no effect of condition, $F < 1$, $\eta_P^2 = .02$, or of delay, $F < 1$, $\eta_P^2 < .01$. There were no interactions among any factors, Condition x Delay, $F < 1$, $\eta_P^2 = .02$, Novelty x Delay, $F(1, 29) = 3.80$, $p = .062$, $\eta_P^2 = .12$, Condition x Novelty, $F < 1$, $\eta_P^2 < .01$, and no three-way interaction between Condition x Novelty x Delay, $F < 1$, $\eta_P^2 = .02$. These analyses suggest that though rats spent more time in the zone containing the novel stimulus, the spacing of trials and the delay between the sample and test did not have an effect on the results.

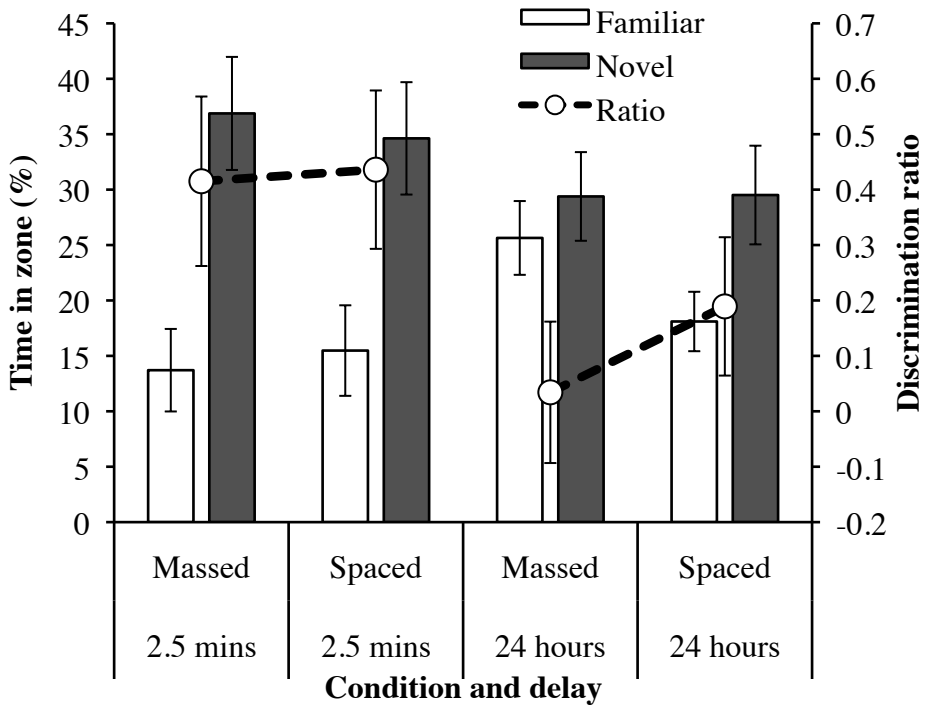


Figure 22. The time rats spent in each zone in the test stage are shown by the bar graph, relating to the left Y-axis. Discrimination ratios are represented by the line graphs, and relate to the right Y-axis.

T-tests with a Bonferroni-Holm correction were conducted on each of the four groups in order to test if any of the differences in time spent in the zones of the novel and familiar stimuli were significant. This was done because it was predicted that there would be a deficit in memory at a long (24 hour) delay, but no deficit at a short delay. Memory after massed stimuli would be worse than after spaced stimuli (as seen in Experiment 6 and 7). Rats spent more time in the novel stimulus zone in the massed condition with a short (2.5 min) delay, $t(15) = 2.89$, $p = .011$, ($M = 23.17$, $SE = 8.03$), $\eta_P^2 = .36$. The 2.5 min delay group with spaced exposures was not significant (αhe 2.5 min $t(15) = 2.45$, $p = .027$, ($M = 19.15$, $SE = 7.80$), $\eta_P^2 = .29$. Neither condition was significant in the 24-hour delay groups; for spaced condition, $t(12) = 2.00$, $p = .069$, ($M = 11.41$, $SE = 5.71$), $\eta_P^2 = .25$, for massed condition, $t < 1$, ($M = 3.74$, $SE = 7.23$), $\eta_P^2 = .02$.

These analyses suggest that discrimination is apparent when the delay is short (2.5 minutes) and the exposures are massed. A longer delay (24 hours) did not produce significant discrimination.

Ratios.

An ANOVA on discrimination ratios showed identical results to the time data. There was effect of delay, $F(1, 27) = 5.05$, $p = .033$, $\eta_P^2 = .16$, but no significant effect of condition, $F < 1$, $\eta_P^2 = .01$. There was no interaction between these factors, $F < 1$, $\eta_P^2 = .01$. The intercept was significant, $F(1, 27) = 14.79$, $p = .001$, $\eta_P^2 = .35$, indicating that the ratios were significantly higher than zero, showing that rats did spend more time in the zone that contained the novel stimulus.

To examine the significant effect of delay further, one-sample *t*-tests were conducted on the four ratios. These tests were again subject to the Bonferroni-Holm correction. The ratios of rats that had a short delay (2.5 minutes) and spaced exposure were significantly greater than zero, $t(15) = 3.05$, $p = .008$, ($M = .44$, $SE = .14$), $\eta_P^2 = .38$. The discrimination ratios of rats that had a short delay (2.5 minutes) and massed exposure were also significantly greater than zero, $t(15) = 2.72$, $p = .016$, ($M = .42$, $SE = .15$), $\eta_P^2 = .33$, while those of the groups that had a 24-hour delay were not (for spaced exposures: $t(14) = 1.41$, $p = .183$, ($M = .19$, $SE = .13$), $\eta_P^2 = .14$: for massed exposures, $t < 1$, ($M = .03$, $SE = .14$), $\eta_P^2 < .01$.)

These analyses suggest that the short delay (2.5 minutes) with either massed or spaced exposures allowed for discrimination between novel and familiar stimuli, this partially supports the significant results of the analyses with the time data. The groups that had the 24-hour delay with spaced or massed exposure did not show significant discrimination.

3.5 General discussion

The purpose of the experiments presented in the current chapter was to test predictions from the SOP model in regards to spacing of stimulus exposures in the sample stage of an object recognition experiment. Experiments 6 and 7 supported predictions that spaced exposures in the sample stage would lead to greater recognition in the test stage than massed exposures. This was demonstrated in a between-subject and a within-subject design.

Experiment 8 was conducted to test the effect of a short or long delay on recognition and whether spaced trials would still produce a

superior effect. The prediction made was that there might be less discrimination at a long delay than a short delay because short-term habituation will be greater than at a long delay. However, across all groups and conditions, rats spent more time in the novel zone than in the familiar zone. When the delay between sample and test was short (2.5 minutes), both spacing conditions produced good recognition (ratios significant above chance). With a 24-hour delay, rats did not show significant discrimination with either spaced or massed exposures. Experiments 6 and 7 did not support this: in those experiments, discrimination in the test was superior with long delay and spaced exposure compared to that seen after a massed exposure.

With regard to SOP, at short delays self-generated priming will be optimal, for whichever spacing condition is used so at the time of the test the representation is likely to be in an A2 state, so that the novel stimulus will elicit more A1 activity and thus more behaviour. At longer delays, the representation will have declined to an inactive state, meaning that only retrieval-generated priming is likely; the presentation of the context primes the stimulus representation to A2, so the novel stimulus is in A1 and so explored more. From the present results, it may be that only spaced stimuli build a good context-stimulus association that can be reactivated over long delays. Sunsay and Bouton (2008) reported that self-generated mechanisms were the cause of trial-spacing effects with ITIs below 240 seconds. Experiments 6 and 7 supported this idea; in the massed condition, exposures had an interval of 30 seconds. This may have resulted in short-term habituation, leading to a deficit in discrimination in the test. Self-

generated priming prevented any associations being formed, so there was little discrimination.

The patterns of results seen in the current chapter, particularly Experiment 8, do not completely replicate the results of Sanderson and Bannerman (2011). Results shown in their paper and the present chapter showed that recognition after a long delay was worse than after a short delay, and that spaced training produced better recognition than massed training. Results from the present chapter can only support findings relating to the training-test delay. However, the results from the current chapter may support the general idea proposed by Sanderson and Bannerman that there are separate processes governing short-term and long-term memory processes. A long delay between sample and test may indicate that a retrieval-generated process was at work. The spaced exposures in the sample stage allowed a good association to form between the object and the context, so even after a long delay rats were able to recognise the familiar object; it was primed into A2 when the rat was placed back into the apparatus, meaning that there was more behaviour elicited towards the novel object (its elements would have been activated to A1). This may show that self-generated priming underlies short-term processes, but retrieval-generated processes underlie long-term processes.

There was a prediction made concerning the sample stage, that habituation might occur more rapidly in a massed stimulus condition (Davis, 1970, Sanderson & Bannerman, 2011); however, most of the sample stages presented in this chapter showed no differences between conditions. Moreover, only Experiment 8 demonstrated any habituation to

stimuli in rats as indicated by a decline in time in the zones. This may lead to the conclusion that the exposure time given in the trials in the sample stage was not long enough for habituation to occur. Sanderson and Bannerman (2011) gave their mice ten 2-minute exposures in the sample stage, more time in each trial and overall than the experiments presented in this chapter. It is possible that rats did habituate to the stimuli; this is indicated in the test stages in rats' discrimination between familiar and novel stimuli. It could be that because the measurement is the time the rats spent in the zone that small changes in behaviour were not captured. Anderson et al. (2008) also used automated tracking and although not specifically reported, results seem to show that rats' exploration did not decline in the sample stage.

The results from the experiments presented in this chapter showed that there is reasonable justification to suggest the SOP model as a valid model to explain effects seen in recognition memory experiments. Other theories relating to recognition memory (e.g. the representational-hierarchical model, Cowell et al., 2010) do not provide any explanation of the effects of spaced stimulus exposures.

Future research would use longer exposures, and also perhaps more distinct ITIs. For example, increasing the long ITI may help ensure activation decay of elements. Sunsay and Bouton (2008) suggest 240 s is the maximum for a 'short' ITI; as this was the length of the long ITI in the experiments presented in this chapter, and this may be why some effects seen were not very strong. In the long ITI condition, primary activation is

assumed to decay mostly to an inactive state; a longer ITI would increase this further.

The aim of experiments in this chapter was to test the effects of massed and spaced exposures in the sample stage of an object recognition experiment on discrimination in a later test. This was done to replicate the spacing effect in an object recognition task. Results from the present chapter provided tentative support for the predictions made by SOP and were generally in line with those of similar studies. The results here, and from Chapter 2, both suggest that associative processes contribute to recognition memory.

Chapter 4. Familiarity Generalisation

In Chapter 4, I examine the possibility that generalisation may be enhanced between stimuli that share common elements of novelty/familiarity. This theory was proposed by Best and Batson (1977), and supported in experiments by Honey (1990), (see General Introduction (1.1.1) for further details). The common elements theory can be applied to generalisation, as formulised by McLaren and Mackintosh's (2000, 2002) model, as long as familiarity could be represented by stimulus elements. Experiments presented in this chapter used many preexposure trials (48 exposures to one stimulus) in order to ensure that the subject was familiar with the stimulus.

In recognition memory tasks such as delayed non-match to-sample and object recognition, subjects with lesions to the perirhinal cortex were unable to discriminate between novel and familiar items (e.g., Albasser et al., 2009; Barker et al., 2007; Buckley et al., 1997; Norman & Eacott, 2005; Meunier et al., 1993; Mumby et al., 2007). These results have been interpreted as suggesting that the perirhinal cortex is responsible for encoding a whole object representation (Bartko, Winters, Cowell, Saksida, & Bussey, 2007; Bussey et al., 2002). This account (see also Cowell et al., 2010a, 2010b) proposes that performance in a recognition task depends on having a complete representation (memory) of the object that was just encountered. Subjects with perirhinal lesions do not have a complete object representation, so perform poorly in discrimination tasks. Because this account is based on visual representations, it does not consider abstract

representations, such as familiarity and novelty, or how subjects respond to familiarity and novelty.

Other accounts (e.g., Aggleton & Brown, 2001) judge the perirhinal cortex as important for signalling novelty of stimuli. If this suggestion is correct, then lesions to perirhinal cortex should disrupt performance in any task that requires the subject to have a familiarity representation of stimuli.

I used the familiarity generalisation procedure (Honey, 1990) to test these accounts. If generalisation is mediated by familiarity (Best & Batson, 1977; Honey, 1990), then subjects with perirhinal lesions will be impaired in this task, relative to controls. Perirhinal lesions should diminish the subjects' ability to judge the familiarity/novelty of the stimulus. The representational accounts (e.g., Cowell et al., 2010a, 2010b) cannot make predictions concerning familiarity generalisation.

The familiarity generalisation procedure used in the experiments presented in this chapter involved three stages (Appendix 4, Table 3): preexposure, conditioning, and test. In the preexposure stage, rats were presented with either one stimulus (e.g., a tone) or two stimuli (e.g., a tone and a clicker) that were not reinforced. In the conditioning stage, rats were presented with one of these stimuli (e.g., the clicker) followed by an unconditioned stimulus (a shock) at the termination of the stimulus. In the test stage, the non-conditioned stimulus was presented (the tone) and conditioned responses to this stimulus were recorded. This procedure used aversive conditioning, unlike Honey's (1990) appetitive conditioning procedure. This was so that fewer trials could be given in the conditioning

stage, meaning that the conditioned stimulus would remain relatively novel for the group preexposed to one stimulus.

Experiment 9 tested the prediction that generalisation may be enhanced between stimuli that share elements of familiarity. A second aim was to test the effect of lengthening the inter-trial interval (ITI) in group T/C. This was tested because of suggestions concerning sensory preconditioning (Hall, 2001) that T and C may become associated in the preexposure stage. These associations may result in both T and C eliciting a CR. Experiment 10 examined the effect of perirhinal lesions on familiarity generalisation. Experiments 11, 12, and 13 aimed to clarify which stage of the generalisation procedure was important for familiarity representations. Scopolamine, an anticholinergic muscarinic antagonist was used to create perirhinal lesion-like impairments in recognition memory. Both perirhinal lesions and scopolamine produce deficits in recognition in object tasks, and so may have similar actions on familiarity generalisation.

4.1 Experiment 9. Familiarity Generalisation

In Honey's (1990) experiment, subjects that were preexposed to both A and B responded more in the test with B. For group A/B, A and B shared common elements of familiarity, which may have mediated generalisation. Hall (2001) suggested that the high level of responding by group A/B to stimulus B may have been due to sensory preconditioning. Because stimulus A and B were presented in the same session, with short (280 s) ITIs, an excitatory association may have formed between the stimuli, which may have resulted in B eliciting the CR that was established with A.

Experiment 9 was conducted to test this suggestion that sensory preconditioning might be an alternative explanation in this familiarity generalisation procedure (see Hall, 2001). Three ITI durations were used: 140 s, 280 s, and 420 s. The 280 s ITI replicates that used in Honey's (1990) experiment. An ITI half the duration (140 s) and one and a half times the duration (420 s) were included to test preconditioning with a smaller ITI and a longer ITI. If sensory preconditioning were responsible for any generalisation, a shorter ITI would lead to greater sensory preconditioning, which would result in greater generalisation. Groups given preexposure with the 140 s ITI may show greater responding than a group given 280 s ITIs, who may demonstrate greater responding than a group given 420 s ITIs.

4.1.1 Method.

Subjects.

Forty male Lister-hood rats (*Rattus norvegicus*) supplied by Charles River (UK) were used as subjects. Rats were kept and housed as in Experiment 1.

At the beginning of the experiment, the rats' food was removed, and they were fed a restricted amount each day so that their weights gradually reduced to > 80% of a baseline weight calculated from free-feeding rats' growth curves. These growth curves were measurements from a separate group of rats of the same strain. Before food restriction, the rats' weights ranged between 255 – 320 g (mean of 283.63 g). The rats' weights were recorded at the beginning of the experiment and monitored throughout. The rats were fed once a day after each experimental session. Water was freely available to the animals in their home cages throughout the experiment.

Apparatus.

The apparatus used were eight identical operant boxes (MED Associates Inc., VT, USA); each was housed within a larger chamber that attenuated light and sound. On one of the walls of the chamber there was an exhaust fan, which gave off background noise at 65 dB. The two end walls of the operant box were made of aluminium; the back wall, ceiling and door (30.0 x 24.0 x 20.5 cm) were made of transparent acrylic. The floor was made of nineteen stainless steel rods (4.8 mm diameter) through which a scrambled electric current (0.5 s at 1.0 mA) could be passed. These were positioned 1.6 cm apart. One of the aluminium walls contained a

recessed food tray where food pellets (45 mg, Noyes, Lancaster, NH) were delivered. The opposite aluminium wall contained a loudspeaker, which was used to produce a 2 kHz tone (measured 85 dBA); this served as stimulus T. A relay was fitted to the outside of the operant box; this was programmed to produce a train of clicks at 10 Hz, and 77 dBA, which served as stimulus C. T and C were 30 s in duration.

Two lights were positioned on the wall with the food tray; these were mounted on each side of the food tray (16.0 cm apart, centre to centre, and 10.5 cm above the floor). There was also a light in the centre of the opposite wall (17.5 cm above the floor). None of these lights was operated in this experiment.

A lever was fitted in the wall to the left of the food tray. The lever was 4.8 x 1.9 cm, and 6.0 cm above the rod floor. This was retractable, and so was only present during specific sessions. Lever pressing was recorded as a response.

A computer operating with Windows XP was used to run MED PC IV (MED Associates Inc.) software; this software controlled the presentation of stimuli, and recorded lever presses.

Procedure.

Rats were initially trained to retrieve pellets from the food tray. Pellets were delivered every 30 s for 10 minutes during a single session. No responding was recorded. Training intended to establish lever pressing as an instrumental response was given over the next six sessions. During the first session, which was 40 minutes in duration, every lever press the rat made was rewarded. The next five sessions were 60 minutes in duration.

In the second session, rats were rewarded for lever pressing after an interval drawn from a variable interval (VI) schedule with a mean of 20 s. The third session comprised three VI schedules; these had a mean of 20 s, 40 s, and 60 s and were presented in that order. Each schedule lasted for 20 minutes within the 60-minute session. In the next three sessions, rats' lever pressing was rewarded after an interval that had a mean of 60 s.

During all stages of the experiment, responding continued to be reinforced according to a VI60 schedule. After lever training, the preexposure stage began. There were six sessions of preexposure. There were five groups (*n*s = 8); group 0 received no preexposure to the auditory stimuli, but were placed in the apparatus and continued to press the lever; this session was 40 minutes in duration. Group T was given exposures to just the tone. They had eight trials in each session. The ITI was 280 s and the session was of 40 minutes duration. Groups T/C 140, T/C 280, and T/C 420 were all preexposed to the tone and the clicker. Groups differed in ITI and session duration. For group T/C 140 the ITI was 140 s and the session was of 40 minutes duration. For group T/C 280 the ITI was 280 s and the session was of 80 minutes duration. For group T/C 420 the ITI was 420 s and duration of the session was 120 minutes. ITIs were counted from the start of the session (or the termination of the CS) to the termination of the next CS. The order of the stimuli for the T/C groups was: C T T C C T T C C T T C C T T C or T C C T T C C T T C C T T C C T; each of these orders were used three times and neither sequence occurred consecutively more than twice. The group that received exposure to the tone received a sequence of eight tones.

After this stage, all rats received two conditioning sessions in which the clicker was paired co-terminally with a foot shock. These sessions were 80 minutes duration and contained two trials; the first trial occurred at 570 s, and the second at 2370 s from the beginning of the session.

After the conditioning sessions, all rats received one VI60 lever press session to recover lever pressing after conditioning. The day after this the test session was conducted. In the test session, all rats received four presentations of the tone and four presentations of the clicker. The test session used a VI60 schedule. The order was: T T T T C C C C, and the mean ITI was 280 s. All rats received one test session of 40-minute duration.

Data collation and analysis methods.

Responses were recorded during the time the stimulus was presented, and for 30 seconds preceding the stimulus. Results are reported in responses per minute and suppression ratios. Ratios were calculated by subtracting the responses made before the stimulus from responses made during the stimulus, divided by the total responses made before and during the stimulus ([stimulus - pre-stimulus]/[stimulus + pre-stimulus]). This gave a scale from one to minus one, where zero indicates no change in responding and minus one indicates complete suppression. Results for each stage were presented in responses per minute and in suppression ratios.

Responses during 30 seconds before the onset of the CS were examined to check for any group differences in baseline responding rates. This was important to confirm that any group differences during the CS were not due to differences in baseline responding. Data from only the first

session of pre-exposure were reported for that stage, because after the first session, responses generally remained high. Data from the conditioning stage were analysed over both sessions. Responding from both trials during each session was averaged. Ratios were calculated for each trial and a mean was then calculated for the session. Analysis of the test data was restricted to the first pair of trials because generalised suppression declined after this. All data were analysed using ANOVA with SME analysis with a pooled error term to examine sources of interactions.

4.1.2 Results and discussion.

Training.

All rats successfully learned to retrieve food pellets from the food well and to lever press to earn food pellets. The mean number of lever presses during the final session of VI60 was 10.99 per minute.

Preexposure.

Pre-CS responses per minute.

Pre-CS responses were steady across trials and groups for both the tone and the clicker trials (Figure 23). This description was supported with an ANOVA with trial and group as factors. Firstly, for sessions containing the tone, there was no effect of trial, $F(7, 245) = 1.40$, $p = .205$, $\eta_P^2 = .04$, no effect of group, $F < 1$, $\eta_P^2 = .06$, and no interaction between these factors, $F < 1$, $\eta_P^2 = .10$.

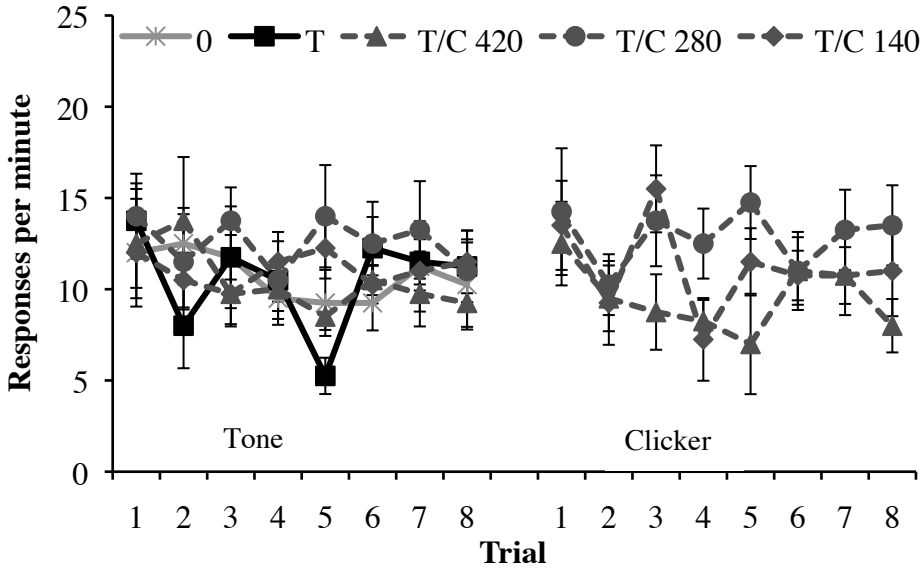


Figure 23. Mean responses per minute prior to the onset of the tone and clicker trials during the first session of preexposure. Error bars represent one standard error of the mean.

Secondly, for sessions containing the clicker, which were only given to the three T/C groups, responding also did not differ between groups. There was no effect of trial, $F(7, 147) = 1.48$, $p = .180$, $\eta_P^2 = .07$, no effect of group, $F(2, 21) = 2.38$, $p = .117$, $\eta_P^2 = .19$, and no interaction between these factors, $F < 1$, $\eta_P^2 = .07$.

CS responses per minute.

The data from the trials when the tone was presented showed a low level of responding on the first trial, which increased as the trials progressed, indicating a reduction in unconditioned suppression (Figure 24, upper left panel). An ANOVA with factors of trial and group supported this. There was an effect of trial, $F(7, 245) = 2.53$, $p = .016$, $\eta_P^2 = .07$, but no effect of group, $F(4, 35) = 1.41$, $p = .251$, $\eta_P^2 = .14$, and no interaction between these factors, $F(28, 245) = 1.26$, $p = .179$, $\eta_P^2 = .13$.

The data from the trials with the clicker only (Figure 24, upper right panel) included three groups because the group exposed to no stimuli and the group just exposed to tones did not receive clicker trials in this stage. Responding at the beginning of the session was low and gradually increased as the session progressed. An ANOVA with factors of trial and group confirmed this; there was an effect of trial, $F(7, 147) = 19.45$, $p < .001$, $\eta_P^2 = .48$, but no effect of group $F(2, 21) = 2.92$, $p = .076$, $\eta_P^2 = .22$, and no interaction, $F < 1$, $\eta_P^2 = .06$.

Ratios.

The suppression ratios reflect similar results to the response data; there was some depression towards the start of the session, but as trials went on, ratios increased, showing a decrease in suppression (Figure 24,

lower left panel). An ANOVA of ratios from the tone trials, with group as a between-subject variable, supported this description. There was a main effect of trial, $F(7, 28) = 3.81$, $p = .001$, $\eta_P^2 = .10$, no effect of group, $F < 1$, $\eta_P^2 = .10$, and no interaction between these variables, $F(28, 245) = 1.15$, $p = .278$, $\eta_P^2 = .12$. The intercept was significant, $F(1, 35) = 5.78$, $p = .022$, $\eta_P^2 = .14$, indicating that over the session, ratios were above zero. For the trials with the clicker (Figure 24, lower right panel), a second ANOVA also showed that there was an effect of trial, $F(7, 147) = 23.63$, $p < .001$, $\eta_P^2 = .53$, no effect of group, $F < 1$, $\eta_P^2 = .026$, and no interaction between these, $F(14, 147) = 1.52$, $p = .111$, $\eta_P^2 = .13$. The intercept was not significant, $F < 1$, $\eta_P^2 = .02$.

Overall, these results show that during the first preexposure session, responding was low at the beginning of the session, due to unconditioned suppression, but then responding increased over trials. This pattern was the same across groups.

Conditioning.

Pre-CS responses per minute.

Pre-CS responding was similar across trials and T/C groups showed higher responding than groups O and T (Figure 25). This description was supported with an ANOVA with trial and group as factors. There was an effect of group, $F(4, 35) = 2.79$, $p = .041$, $\eta_P^2 = .24$, but no effect of trial, $F(1, 35) = 2.71$, $p = .109$, $\eta_P^2 = .07$. There was no interaction between these factors, $F(4, 35) = 1.77$, $p = .158$, $\eta_P^2 = .17$. Pairwise comparisons with a Bonferroni correction showed that no groups differed, lowest $p = .058$ (group T/C 280 and group T).

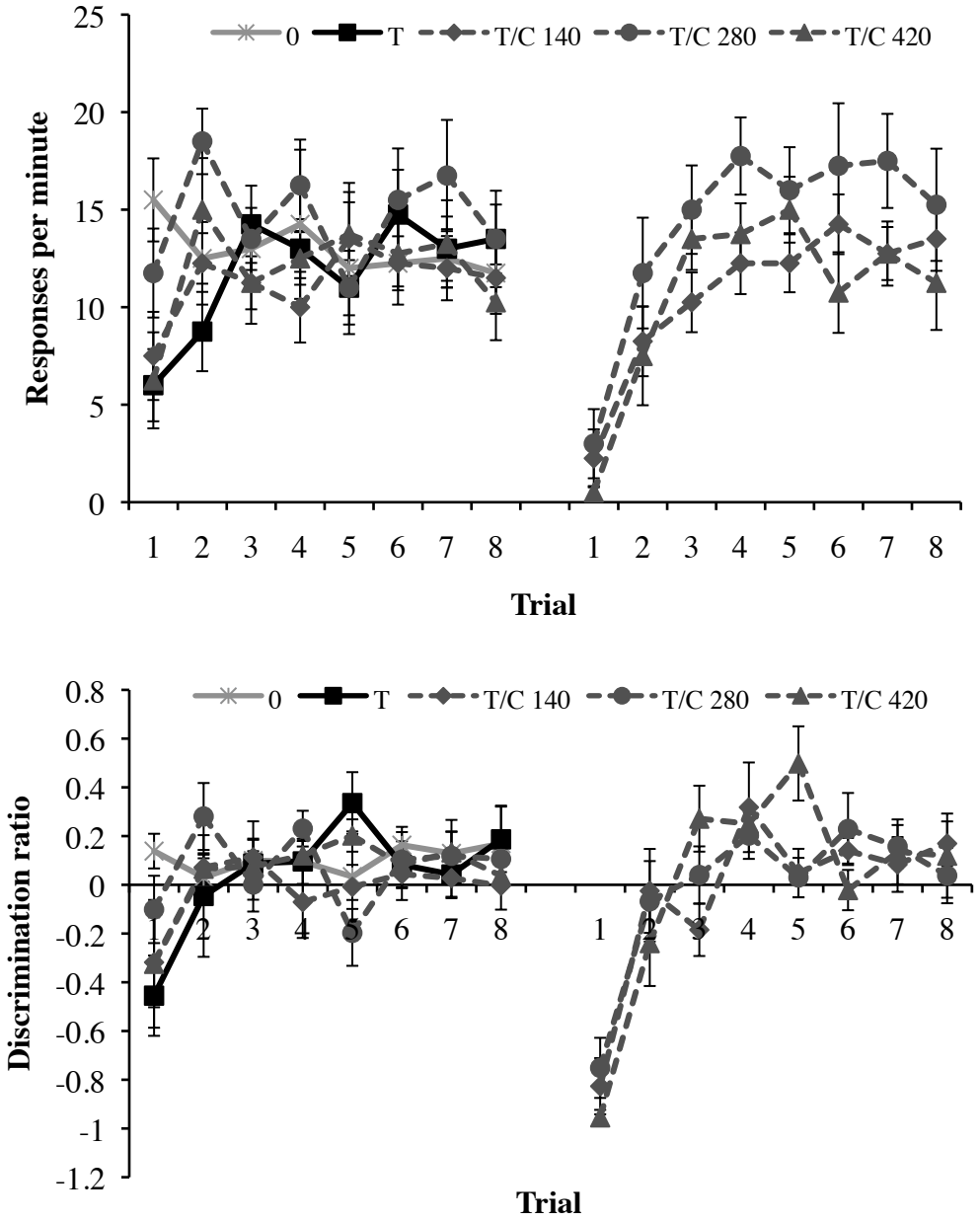


Figure 24. Mean responses per minute (upper graph) and suppression ratios (lower graph) for tone and clicker trials during the first session of preexposure. Error bars represent one standard error of the mean.

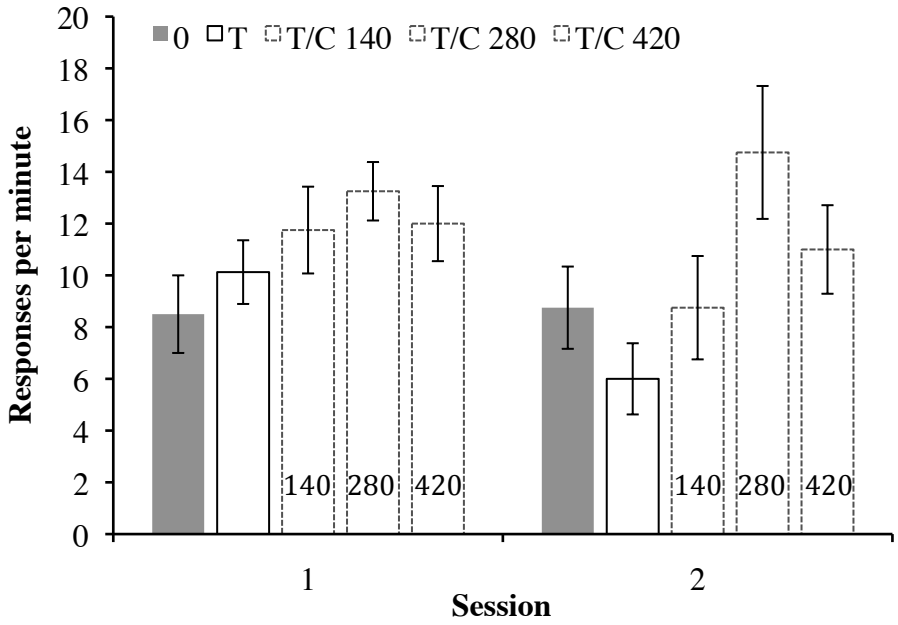


Figure 25. Mean responses per minute prior to the onset of the clicker trials during each conditioning session. Error bars represent one standard error of the mean.

CS responses per minute.

On the first session of conditioning the T/C groups did not show a decline in responding during the clicker; however, group T and group 0 showed lower levels of responding than the T/C groups (Figure 26). By the second session, all groups showed very low levels of responding. An ANOVA of responding from each conditioning session, with group as a between-subjects factor, confirmed those descriptions. There was a main effect of session, $F(1, 35) = 107.95$, $p < .001$, $\eta_P^2 = .76$, and also of group, $F(1, 35) = 19.85$, $p < .001$, $\eta_P^2 = .65$. There was also a significant interaction between these factors, $F(1, 35) = 16.48$, $p < .001$, $\eta_P^2 = .65$. This interaction was explored using simple main effects (SMEs) analyses with pooled error terms. These revealed a group difference in the first session, $F(4, 35) = 22.03$, $p < .001$, $\eta_P^2 = .72$, but not in the second, $F(4, 35) = 1.89$, $p = .134$, $\eta_P^2 = .18$. In the first session, group T and group 0 differed from each T/C group, all ps $< .001$, but did not differ from each other $p = .702$. The T/C groups did not differ, all ps $> .281$. This indicates that by the end of conditioning all groups were responding at a similar, low level. SMEs analysis also showed that group 0 and group T did not show differences in responding from session 1 to session 2, both Fs < 1, η_P^2s $< .01$. However, all the T/C groups did, their responding declining in the second session, group T/C 420, $F(1, 35) = 52.30$, $p < .001$, $\eta_P^2 = .60$, group T/C 280, $F(1, 35) = 52.30$, $p < .001$, $\eta_P^2 = .60$, and group T/C 140, $F(1, 35) = 69.17$, $p < .001$, $\eta_P^2 = .66$.

Ratios.

An ANOVA with session and group as factors showed identical results to response data. There was a main effect of session, $F(1, 35) = 31.83$, $p < .001$, $\eta_P^2 = .48$, of group, $F(4, 35) = 6.83$, $p < .001$, $\eta_P^2 = .44$, and a significant interaction between these two factors, $F(4, 35) = 9.57$, $p < .001$, $\eta_P^2 = .52$. The intercept was significant, $F(1, 35) = 241.53$, $p < .001$, $\eta_P^2 = .44$, indicating that over both trials, ratios were lower than zero.

SMEs analyses were conducted to investigate the Session x Group interaction. These revealed a significant difference between groups in the first session, $F(4, 35) = 18.12$, $p < .001$, $\eta_P^2 = .67$, but not in the second, $F < 1$, $\eta_P^2 = .07$. In the first session, group T and group 0 differed from each T/C group, all ps $< .001$, but did not differ from each other $p = .978$. The T/C groups did not differ, all ps $> .321$. As in the analyses for response rates, results showed that group 0 and group T did not show differences in their suppression ratios from session 1 to session 2 (for group 0, $F < 1$, $\eta_P^2 < .01$, and for group T, $F(1, 35) = 2.45$, $p = .127$, $\eta_P^2 = .07$.) However, all the T/C groups' suppression ratios increased in the second session (for group T/C 420, $F(1, 35) = 14.73$, $p < .001$, $\eta_P^2 = .30$, for group T/C 280, $F(1, 35) = 26.69$, $p < .001$, $\eta_P^2 = .43$, and for group T/C 140, $F(1, 35) = 26.26$, $p < .001$, $\eta_P^2 = .43$.)

These results indicate that even though the groups preexposed to the clicker showed slow learning at the beginning of the stage, by the end all groups showed good conditioning, as indicated by the low response level to the clicker. The suppression to the clicker is very low; this may also indicate a floor effect.

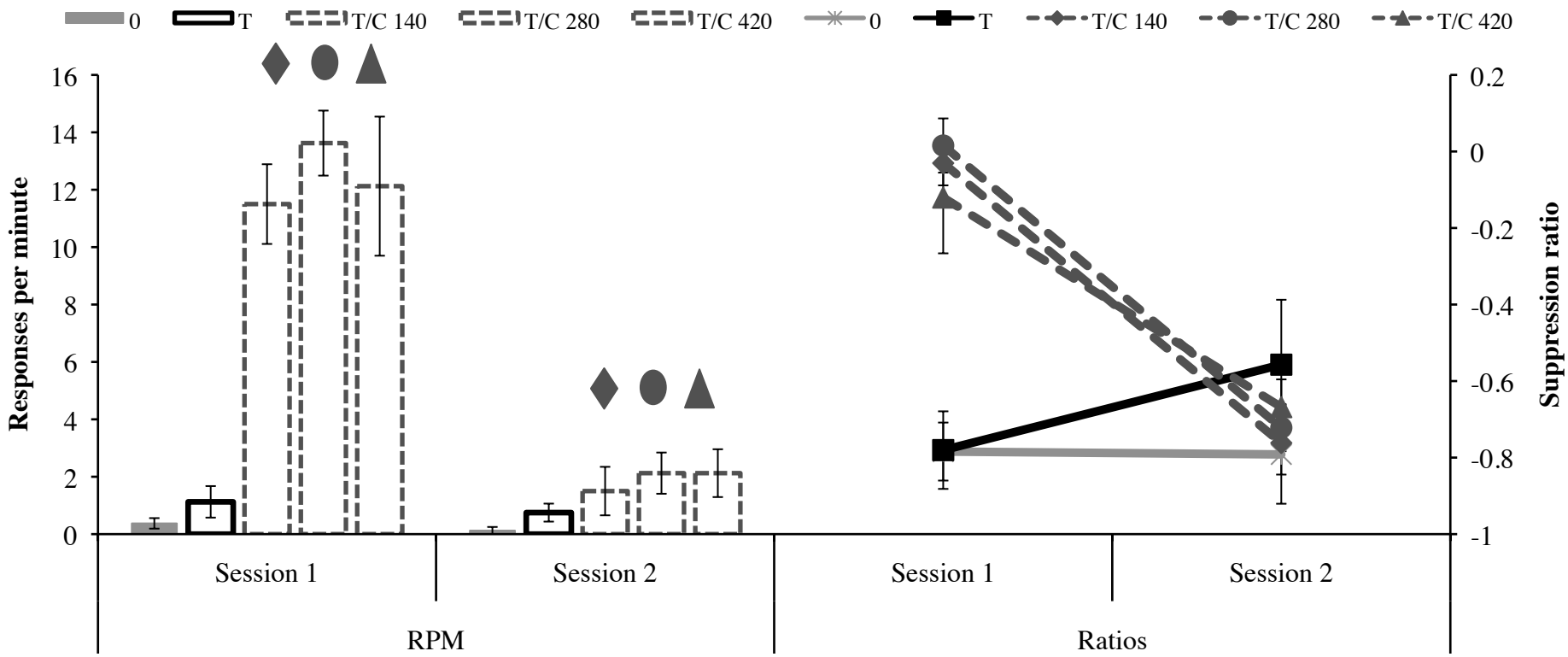


Figure 26. Responses per minute (left side) and suppression ratios (right side) during the two conditioning sessions. Each session contains two trials (C+). Error bars represent one standard error of the mean.

Test.

Pre-CS responses per minute.

Responses during the pre-CS period were similar across groups, and across trials (Figure 27). This was confirmed by an ANOVA with trial and group as factors. There was no effect of trial, $F < 1$, $\eta_P^2 = .02$, or group, $F < 1$, $\eta_P^2 = .04$, and no interaction between these, $F < 1$, $\eta_P^2 = .06$.

CS responses per minute.

The results of main interest are those from the test session (Figure 28). During the presentation of the tone, all but group T showed low levels of responding. To investigate the prediction that the shorter ITI in the T/C groups may induce a higher level of responding than a longer ITI, T/C groups' responding was analysed. An ANOVA revealed there was no difference between groups, $F < 1$, $\eta_P^2 = .02$. However there was an effect of trial, $F(1, 21) = 11.63$, $p = .003$, $\eta_P^2 = .36$, and an interaction of Group x Trial, $F(1, 21) = 3.62$, $p = .045$, $\eta_P^2 = .26$.

SME analysis revealed that groups T/C 420 and T/C 140 increased responding from trial 1 to 2, Fs$(1, 21) > 4.45$, ps $< .048$, η_P^2s $> .17$, whereas responding of group T/C 280 did not change, $F < 1$, $\eta_P^2 < .01$. There were no group differences at trial 1, $F < 1$, $\eta_P^2 = .01$, or at trial 2, $F(2, 21) = 1.00$, $p = .384$, $\eta_P^2 = .09$.

The prediction that T/C groups may be more suppressed than group T was analysed using an ANOVA. Because T/C groups had similar response rates, these were combined, and compared to group T and group 0. There was a significant group effect, $F(2, 37) = 16.90$, $p < .001$, $\eta_P^2 = .48$, an effect of trial, $F(1, 37) = 5.42$, $p = .025$, $\eta_P^2 = .13$, but no interaction

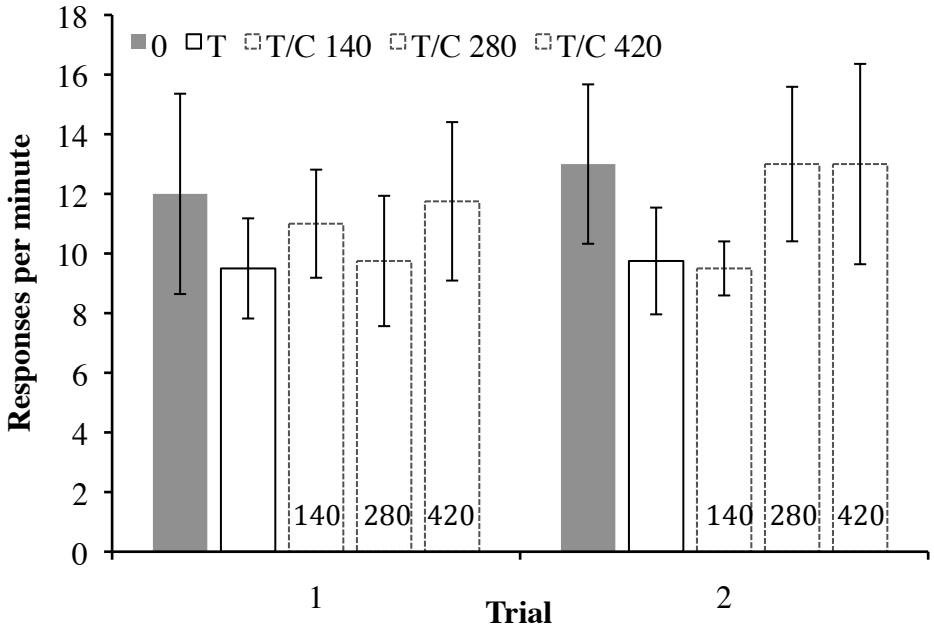


Figure 27. Mean responses per minute prior to the onset of the tone trials during the test session. Error bars represent one standard error of the mean.

between these factors, $F < 1$, $\eta_P^2 < .01$. Comparisons with a Bonferroni correction revealed that group T/C and group 0 responded less than group T, both *p*s < .001. Group 0 and group T/C did not differ, $p = .357$.

Ratios.

Suppression ratios indicated that group T showed less suppression than the T/C groups and group 0 (Figure 28). An ANOVA of the T/C groups' suppression ratios revealed no group effect, $F < 1$, $\eta_P^2 < .01$, no effect of trial, $F(1, 21) = 1.92$, $p = .180$, $\eta_P^2 = .08$, and a significant interaction between group and trial, $F(2, 21) = 3.77$, $p = .040$, $\eta_P^2 = .26$. SME analyses revealed that group T/C 140 decreased suppression from trial 1 to 2, $F(1, 21) = 8.41$, $p = .009$, $\eta_P^2 = .29$, whereas suppression of group T/C 280 and T/C 420 did not change, $F < 1$, $\eta_P^2 < .04$. There were no group differences at trial 1, $F < 1$, $\eta_P^2 = .02$, or at trial 2, $F(2, 21) = 1.09$, $p = .356$, $\eta_P^2 = .09$.

T/C groups were combined, and compared to group T and group 0. An ANOVA of group and trials revealed a significant group effect, $F(2, 37) = 10.39$, $p < .001$, $\eta_P^2 = .36$, a near significant effect of trial, $F(1, 37) = 3.92$, $p = .055$, $\eta_P^2 = .10$, but no interaction between these factors, $F < 1$, $\eta_P^2 = .01$. Comparisons with a Bonferroni correction revealed that group 0 showed less suppression than group T, $p < .001$; group T showed less suppression than group T/C, $p = .008$, but group T/C did not differ from group 0, $p = .080$.

These analyses show that responses rates of group T were higher than those of the T/C groups and group 0. Rats in group 0 may have shown relatively strong suppression because, for them, C and T shared additional

novelty elements that could act to mediate enhanced generalisation of the conditioned response from C to T. However, the effect may instead be due to unconditioned suppression to T on test, which was clearly evident in groups receiving T during preexposure. Rats in the T/C groups also showed suppression to the tone. For this group, both C and T shared familiarity elements, which may have mediated generalisation of the conditioned response. However, unlike group 0, the suppression in the T/C groups is unlikely to be due to unconditioned suppression.

The prediction made by Hall (2001) was that the shorter the ITI, the higher the conditioned responding would be (i.e., more suppression). This was not confirmed. There were no differences in responding or suppression during trial 1 or 2, though responding from trial 1 to trial 2 differed between groups.

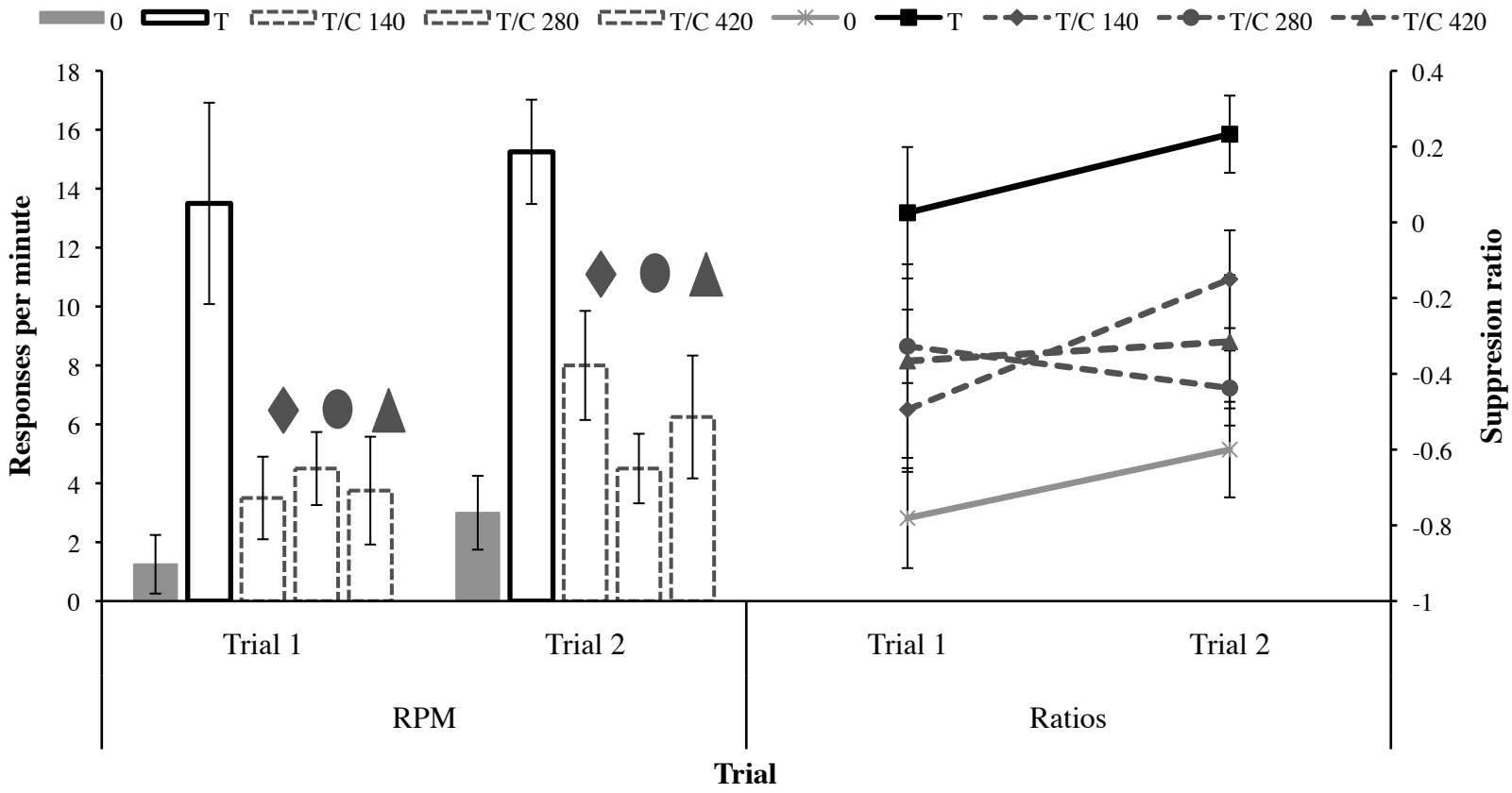


Figure 28. Responses per minute (left side) and suppression ratios (right side) during the first two tone trials in the test stage. Error bars represent one standard error of the mean.

Clicker test.

Pre-CS responses per minute.

Responding in the pre-CS period was consistent across groups and decreased across trials for most groups (Figure 29). This was confirmed by an ANOVA with trial and group as factors. There was an effect of trial, $F(1, 35) = 8.05$, $p = .008$, $\eta_P^2 = .19$, no effect of group, $F(4, 35) = 1.03$, $p = .408$, $\eta_P^2 = .11$, but an interaction between these factors, $F(4, 35) = 3.00$, $p = .031$, $\eta_P^2 = .26$.

SME analysis revealed there was no group effect at trial 1, $F < 1$, $\eta_P^2 = .05$, or trial 2, $F(4, 35) = 2.23$, $p = .085$, $\eta_P^2 = .20$. Group T/C 280 decreased responding in trial two from trial one, $F(1, 35) = 11.98$, $p = .001$, $\eta_P^2 = .26$, group 0 also showed decreased responses in trial two, $F(1, 35) = 5.87$, $p = .021$, $\eta_P^2 = .14$. There were no other differences across trials in the other groups, lowest $p = .306$.

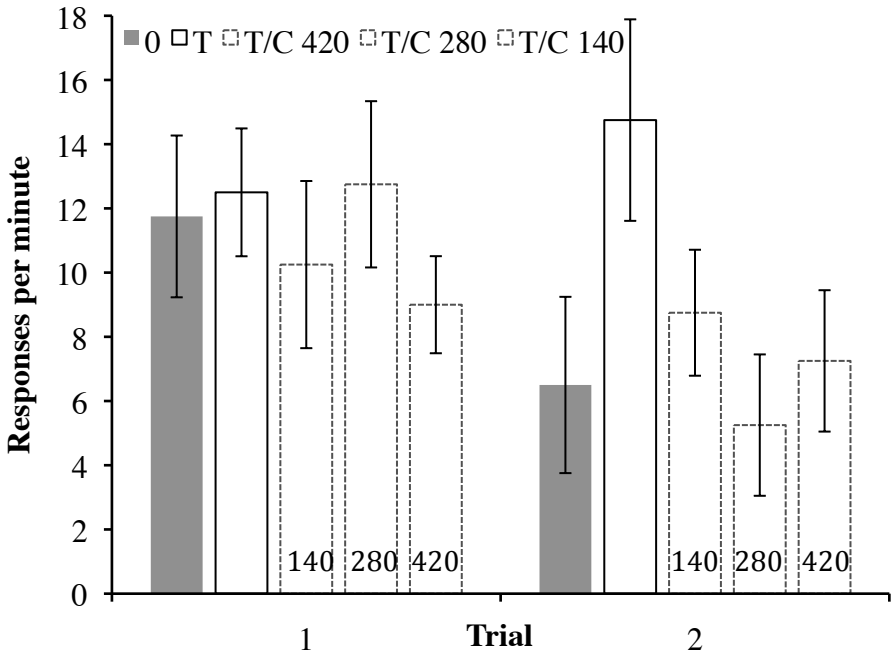


Figure 29. Mean responses per minute prior to the onset of the clicker trials during the test session. Error bars represent one standard error of the mean.

CS responses per minute.

Responding to the clicker (the conditioned stimulus) was also examined for group differences. Groups should all show low rates of responding to the clicker. Results from this test help to interpret whether responding during the tone test might indicate generalisation of conditioned response, or not. Generalisation would be restricted if there was no conditioned response established to the clicker. Responding was very low in all groups, but particularly in group T, which did not respond in the first or second trial. Group 40 T/C also demonstrated no responding in the second trial (Figure 30). An ANOVA revealed that all groups had low levels of responding that did not differ over the two trials. There was no effect of trial, $F(1, 35) = 1.30$, $p = .262$, $\eta_P^2 = .04$, or group, $F < 1$, $\eta_P^2 = .09$, and no interaction between these factors, $F < 1$, $\eta_P^2 = .07$.

Ratios.

Suppression ratios were low and, of central importance, did not differ among the groups (Figure 30). Group T showed complete suppression with ratios of minus one in both trials. An ANOVA supported this description. There was no effect of trial, $F < 1$, $\eta_P^2 = .01$, or group, $F(1, 35) = 1.11$, $p = .368$, $\eta_P^2 = .11$, or any interaction between these factors, $F(4, 35) = 1.18$, $p = .337$, $\eta_P^2 = .12$. The intercept was significant, $F(1, 35) = 440.46$, $p < .001$, $\eta_P^2 = .93$, indicating that overall, all groups had suppression ratios below zero.

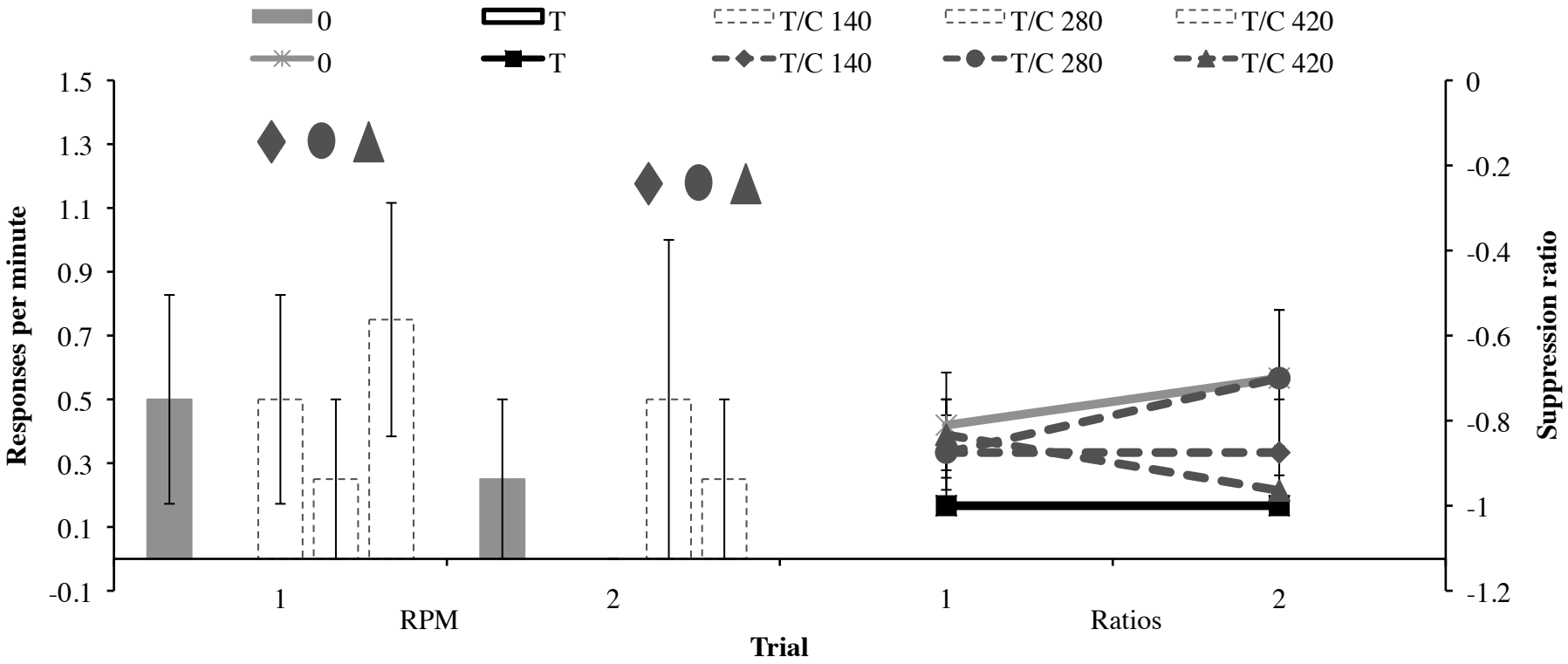


Figure 30. Responses per minute (left side) and suppression ratios (right side) during the first two clicker trials in the test stage. Error bars represent one standard error of the mean. Mean responses for group T (trials 1 and 2) and group T/C 140 (trial 2) were equal to zero and not shown in the graph.

4.1.3 General discussion.

My aim in Experiment 9 was to examine familiarity generalisation, and investigate effects of ITI duration in the T/C group. Results showed that group T demonstrated a lower level of responding than groups preexposed to T and C. Generalisation of conditioned responding seemed to be enhanced when stimuli shared elements of familiarity. This supports findings from Best and Batson (1977) and Honey (1990). The alternative interpretation in terms of sensory preconditioning was not supported. The prediction (Hall, 2001) was that with short ITIs, associations between stimuli were more likely to form, and would be stronger than those formed with long ITIs, resulting in greater generalisation. However, the T/C groups showed similar conditioned responding during each trial of the test, despite groups T/C 140 and T/C 420 increasing responding from trial 1 to trial 2. T/C groups displayed lower rates of responding than group T. This indicates enhanced generalisation in T/C groups.

Hall's (2001) concerns cannot be completely dispelled however. The longest ITI used in the current experiment was 420 s; however, it is possible that learning was at asymptote at this duration. With an even longer ITI, associations may be reduced, and so generalisation may not be enhanced. It was not practical to use a longer ITI with the current method, however, so this idea was not tested.

To test predictions concerning sensory preconditioning further, it may be interesting to test intermixed and blocked preexposed. Alonso and Hall (1999) gave rats either concurrent or blocked preexposure to two flavours, A and B. A was aversively conditioned; and in a test,

consumption of B was recorded. Rats given concurrent preexposure showed greater generalisation of conditioned response (consumption) than a group given blocked preexposure. The concurrent exposure may have allowed frequent A-B pairings, which may then have led to a B – A – CR chain. The present procedure used intermixed trials. If a group given blocked preexposure had lower levels of suppression than the intermixed groups, this would indicate sensory preconditioning, and could provide support for Hall's theory (2001).

4.2 Experiment 10. Generalisation in Subjects with Perirhinal Cortex Lesions

My aim in Experiment 10 was to test the performance of rats with lesions to the perirhinal cortex on the generalisation task (Experiment 9). Perirhinal cortex is important for recognition memory; for example, in object recognition, animals with perirhinal lesions are impaired in discriminating between novel and familiar stimuli (e.g., Cowell et al., 2006; Mumby & Pinel, 1994). Findings from Experiment 9 suggested that generalisation of the CR occurred to a greater extent between stimuli that were matched in terms of familiarity. For this to occur, subjects need to be able to discriminate stimuli in terms of familiarity or novelty. Subjects with perirhinal lesions may not exhibit generalised suppression because they cannot form or recall familiarity representations, a finding that may be instructive in selecting among theories of perirhinal cortex function. A tone generalisation test was given after the test stage in this experiment; rats were presented with tones of 2.0, 2.5, 3.0 and 3.5 kHz. This test was conducted to examine the effect of perirhinal lesions on auditory

discrimination. This was necessary to confirm that results of the generalisation test were not due to a deficit in auditory processing. For example, if group T were unable to distinguish between the tone and the clicker, they may show suppression to both stimuli. Conducting a test between tones of different frequencies was a more sensitive test of auditory discrimination than testing between different types of auditory stimuli, for example, a test using a tone and white noise, because it requires finer auditory discrimination.

The representational-hierarchical model (Cowell et al., 2010a, 2010b) may have difficulty predicting impairment in the performance of subjects with perirhinal lesions in our task. This is because the model is based on visual representations, whereas this task uses auditory stimuli. If the model was expanded to include representations of auditory stimuli, it could still have difficulty because it does not account for familiarity representations. The model states that perirhinal cortex is important for holding representations of complex visual stimuli. However, neurons in perirhinal cortex respond differently to novel and familiar stimuli (e.g., Brown & Aggleton, 2001). This indicates that perirhinal cortex may be important for discriminating novel and familiar stimuli. These psychologically based concepts are not formulised in the representational-hierarchical model.

4.2.1 Method.

Subjects.

The subjects were 32 rats (Harlan, UK) of the same sex and strain as Experiment 9. They were kept in the same conditions as Experiment 9.

Until the experiment began, all rats had free access to food and water. Rats' mean weight was 331 g (range of 300 - 365 g). Once the experiment began, food was restricted to reduce the rats' weight gradually by 10 - 20%.

Surgery.

Prior to the experiment, 16 rats underwent surgery to produce perirhinal cortex lesions (group PeRh) and a further 16 rats had control lesions (group Sham), in which surgery was performed but no neurotoxin applied.

Rats were anaesthetized with isoflurane mixed with oxygen and kept anaesthetized during the procedure with a lower concentration of isoflurane. Rats were placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA) with the incisor bar set to -3.3 mm. Rats' scalps were shaved, and an incision was made along the midline of the scalp. The skulls were exposed and bone was removed from both hemispheres (at approximately 3-7 mm caudally of bregma) using a dental burr. Injections were made with a 2 μL Hamilton syringe (Hamilton, Bonaduz, Switzerland) that was fixed to a moveable arm of the stereotaxic frame. The plunger of the syringe was attached to an electronic microdrive (Model KDS 310; KD Scientific, New Hope, PA), which regulated the volume and rate of infusion of the neurotoxin. Lesions were made with ibotenic acid (Sigma Aldrich, Gillingham, UK), which was dissolved in sterile phosphate-buffered saline (7.4 pH) to produce a 63-mM solution. This was infused at a rate of 0.03 μL/min. Injections were made at -3.0 mm rostral-caudal (RC, medial lateral [ML] ±5.8, dorsal ventral [DV] - 4.0, 0.120 μl), -4.0mm (ML ±6.1, DV- 3.8, 0.100 μl), 5.0mm (ML ±6.5, DV- 4.0, 0.070 μl), 6.0mm (ML ±6.7,

DV- 3.5, 0.050 μl) and 7.0mm (ML ±6.3, DV- 3.1, 0.035 μl) posterior to bregma. The needle was left in place for two minutes after each injection. The same procedure was used for the sham surgery except only the dura was perforated with a 25-gauge Microlance needle (BD, Drogheda, Ireland). At the end of the procedure, the scalp incisions were sutured. Those rats given lesions to perirhinal cortex received an injection of saline and glucose (5 ml, subcutaneous). Rats were placed in individual recovery boxes until they exhibited normal behaviour (approximately 24 hours). The recovery box was held in a darkened room, lit with a red lamp. Following recovery, the rats were returned to their home cages. They were then allowed to recover for at least two weeks before the experimental procedures began.

After the experiment, rats with perirhinal cortex lesions were anaesthetised with an overdose of sodium pentobarbital (200 mg/kg, Euthatal) and were transcardiallly perfused with 0.9% saline and 10% formal saline. The brains were then removed and placed in 10% formal saline solution. Before sectioning, each brain was placed in 20% sucrose until saturated. The brain was sectioned at 40 μm using a cryostat-microtome (Leica Microsystems Ltd, Milton Keynes, UK) and every fifth section was mounted for analysis. Sections were stained with cresyl violet and histologically examined.

Apparatus.

The apparatus used was that described in Experiment 9. As well as the 2.0-kHz tone and the clicker, three other tones were used: 2.5 kHz, 3.0

kHz, and 3.5 kHz. These measured 85 dBA and were presented through the same speaker as the 2.0 kHz tone.

Procedure.

The training, preexposure, conditioning and test stages were identical to those of Experiment 9, with the following exceptions. There were two preexposure groups: T/C and C (Table 2). In each preexposure group, half (n = 8) the subjects had lesions and the other half had sham surgery (n = 8). The details for the preexposure session for Group T/C were identical to that of Group T/C 280 in Experiment 9. Group C was presented with eight clicker trials in a session that lasted 80 minutes and had a mean ITI of 420 s. The conditioning stage was identical to that of Experiment 9, except that the role of the tone and clicker were reversed, so that the tone was paired with the shock. The test was also identical to that of Experiment 9; however, order of trials was C C C C T T T T. This was so the important test stimulus C was given before the tone trials. A second test was performed in which the 2.0 kHz tone was presented along with the higher-frequency tones (2.5, 3.0, 3.5 kHz). There were 16 trials, four of each tone. These were presented in a random order with the constraint that each tone occurred in every successive block of four trials. The ITI was variable with a mean of 280 s.

Data collation and analysis methods.

These were the same as in Experiment 9.

4.2.2 Results and Discussion.

Histology.

Two rats in group PeRh had asymmetrical damage to perirhinal cortex so their data were excluded from the analysis (Table 3). In the remainder of the subjects that had lesions, all damage was bilateral. Lesions began at -3 mm caudal to bregma and extended to -6 mm. Figure 31 depicts cell loss in the animals with the smallest and largest lesions. There was some damage to areas adjacent to the perirhinal cortex: dorsally, including ventral temporal association areas (8 cases), and ventrally, including lateral entorhinal area (4 cases) and amygdala (7 cases). There was one case of damage to the CA2 region of the hippocampus. In most cases, the damage in these extra-perirhinal areas was unilateral.

Table 2.

Surgical and preexposure groups.

Surgery	**Sham lesion**	**Perirhinal lesion**
Preexposure	**(control)**	
Tone + Click (T/C)	*n* = 8	*n* = 8 (7)
Click (C)	*n* = 8	*n* = 8 (7)

Note. T = tone, C = clicker. Numbers in parentheses indicate *n* after exclusions following histological analysis.

For diagrams of lesion reconstructions please see Robinson, Whitt, Horsley, and Jones (2010). Familiarity-based stimulus generalization of conditioned suppression in rats is dependent on the perirhinal cortex. Behavioral Neuroscience, 124, 587–599.

Figure 31. Reconstructions of lesions.

Training.

All rats successfully learned to recover food pellets from the food tray. In the last session of lever training, rats were pressing levers at a mean of 14.89 presses per minute.

Preexposure.

The first session of preexposure was examined as rats showed some recovery of unconditioned suppression, after which responses remained level across sessions.

Pre-CS responses per minute.

Responding during the pre-CS period was similar across clicker and tone trials (Figure 32). In the tone trials, group PeRh T/C had a lower level of responses than group Sham T/C. These descriptions were partially confirmed with an ANOVA with factors of trial, preexposure group (T/C or C) and surgery (PeRh or Sham). For the clicker trials, there was an effect of trial, $F(7, 182) = 2.18$, $p = .038$, $\eta_P^2 = .08$, no effect of surgery, $F(1, 26) = 3.26$, $p = .083$, $\eta_P^2 = .11$, or preexposure group, $F < 1$, $\eta_P^2 = .03$. There was no interaction between surgery and preexposure, $F < 1$, $\eta_P^2 = .01$, no Trial x Preexposure interaction, $F(7, 182) = 1.26$, $p = .275$, $\eta_P^2 = .05$, no Trial x Surgery interaction, $F < 1$, $\eta_P^2 = .04$, and no three-way interaction of trial, preexposure and surgery, $F < 1$, $\eta_P^2 = .02$. For the tone trials an ANOVA with factors of trial and surgery revealed no effect of trial, $F(7, 91) = 1.32$, $p = .249$, $\eta_P^2 = .09$, but an effect of surgery, $F(1, 13) = 6.18$, $p = .027$, $\eta_P^2 = .32$, and a near-significant interaction between these factors, $F(7, 91) = 2.04$, $p = .058$, $\eta_P^2 = .14$.

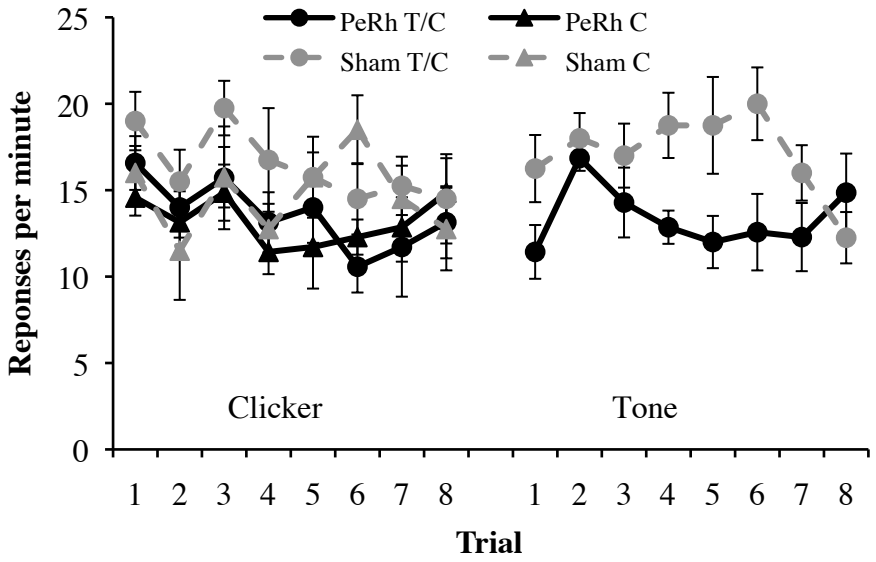


Figure 32. Mean responses per minute prior to the onset of the clicker and tone trials during the first session of preexposure. Error bars represent one standard error of the mean.

CS responses per minute.

During the first two trials of the clicker, all groups had a low level of responses (Figure 33, upper left panel); however, by the third trial, responses had increased and from then stayed at a level rate. An ANOVA with trials and between-group variables of surgery and preexposure group supported this description. There was a significant effect of trial, $F(7, 182) = 10.74$, $p < .001$, $\eta_P^2 = .29$, but no significant effects of either surgery, $F(1, 16) = 3.39$, $p = .077$, $\eta_P^2 = .12$, or preexposure, $F < 1$, $\eta_P^2 = .01$. There was no interaction between these between-subject variables, $F < 1$, $\eta_P^2 = .01$. There was a significant Trial x Preexposure interaction, $F(7, 182) = 3.10$, $p = .004$, $\eta_P^2 = .11$, but no Trial x Surgery interaction, $F < 1$, $\eta_P^2 = .02$, nor a three-way interaction, $F < 1$, $\eta_P^2 = .02$.

The significant Trial x Preexposure interaction was analysed using simple main effects (SME) with pooled error terms. Both the C group and the T/C group increased responding over trials, $F(7, 20) = 4.99$, $p = .002$, $\eta_P^2 = .64$, $F(7, 20) = 2.89$, $p = .029$, $\eta_P^2 = .50$, respectively. Group C had a lower level of responding than group T/C at trials two, $F(1, 26) = 4.97$, $p = .035$, $\eta_P^2 = .16$, and three, $F(1, 16) = 5.45$, $p = .028$, $\eta_P^2 = .17$, but not at any other trial, lowest $p = .197$ (trial 1).

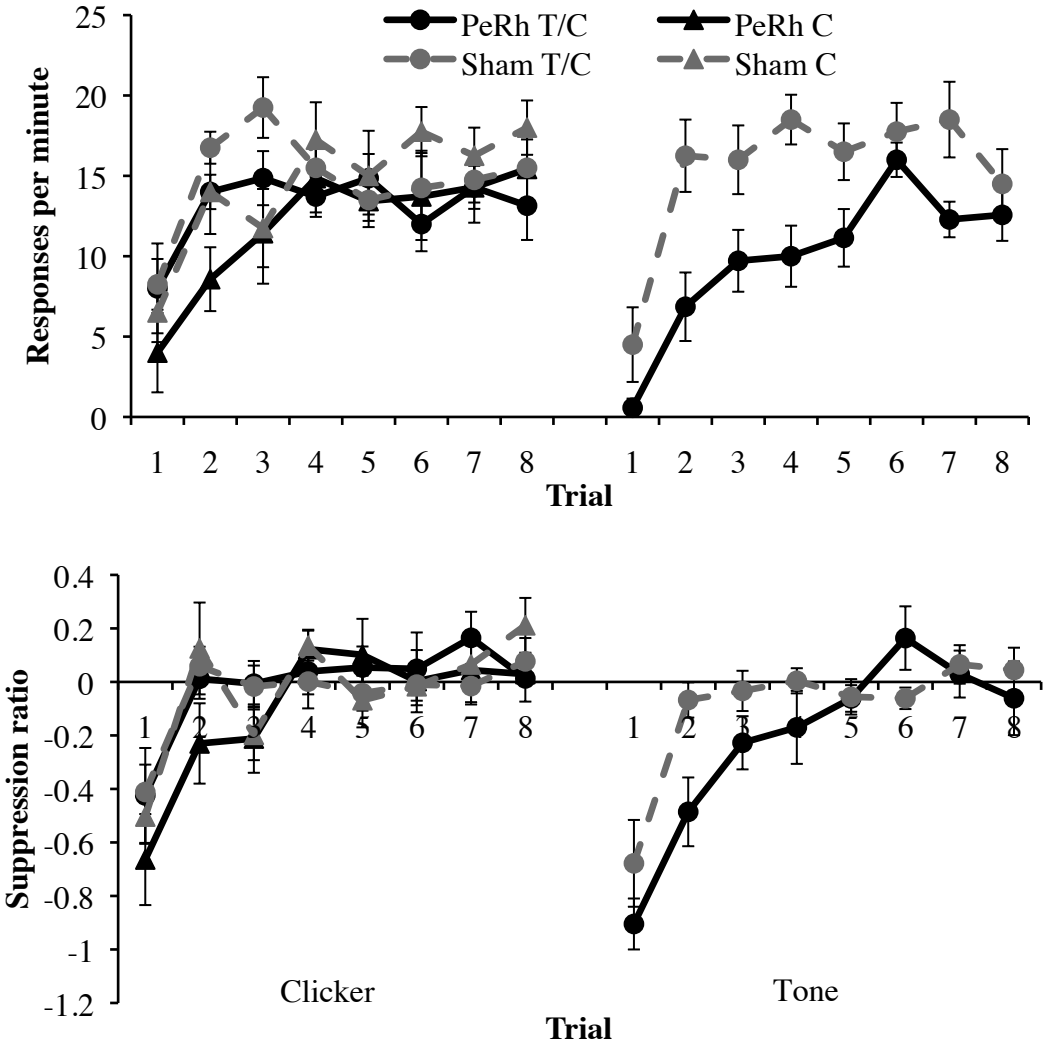


Figure 33. Mean responses per minute (top graph) and suppression ratios (lower graph) of rats during the first preexposure session. Clicker and Tone refer to those trials. Error bars represent the standard error of the mean.

Responding to T (Figure 33, upper right panel) was also analysed with an ANOVA, with variables of trial and surgery. There was a significant effect of trial, $F(7, 91) = 15.26$, $p < .001$, $\eta_P^2 = .54$, a significant effect of surgery, $F(1, 13) = 10.79$, $p = .006$, $\eta_P^2 = .45$, but no interaction between these, $F(7, 91) = 1.56$, $p = .159$, $\eta_P^2 = .11$. Responding during the tone trials increased over the session, and group Sham T/C made more responses than group PeRh T/C.

Ratios.

The pattern of results shown by the ratios was similar to that shown by the response per minute data; there was some suppression in the first trial, but this decreased and stayed stable for the rest of the session (Figure 33, lower left panel). An ANOVA of suppression ratios to C with between-subject variables of surgery and preexposure supported this description. A main effect of trial was found, $F(7, 182) = 15.47$, $p < .001$, $\eta_P^2 = .37$. There was no effect of surgery, $F < 1$, $\eta_P^2 = .01$, or preexposure, $F < 1$, $\eta_P^2 = .03$, and no interaction between these factors, $F(1, 16) = 1.27$, $p = .270$, $\eta_P^2 = .05$. There were no interactions with any other variables; trial did not interact with surgery, $F(7, 182) = 1.27$, $p = .267$, $\eta_P^2 = .05$, or preexposure, $F(7, 182) = 1.18$, $p = .314$, $\eta_P^2 = .04$. There was no three-way interaction between the variables, $F < 1$, $\eta_P^2 = .01$. The intercept was significantly different from zero, $F(1, 26) = 4.35$, $p = .047$, $\eta_P^2 = .05$.

Responding to T (Figure 33, lower right panel) was also analysed, using variables of trial and surgical group. There was a significant effect of trial, $F(7, 91) = 19.62$, $p < .001$, $\eta_P^2 = .60$, no effect of surgery, $F(1, 13) = 2.77$, $p = .120$, $\eta_P^2 = .18$, but there was an interaction between these factors,

$F(7, 91) = 2.25$, $p = .037$, $\eta_P^2 = .15$. The intercept was significant, $F(1, 13) = 19.78$, $p = .001$, $\eta_P^2 = .60$.

The interaction was explored using SMEs. Group Sham T/C showed no differences in ratios over the session, $F(7, 7) = 2.75$, $p = .103$, $\eta_P^2 = .73$, but suppression of group PeRh T/C decreased, $F(7, 7) = 7.41$, $p = .008$, $\eta_P^2 = .88$. Group PeRh showed more suppression than group Sham at trial two, $F(1, 13) = 9.06$, $p = .010$, $\eta_P^2 = .41$, but not at any other trial, lowest $p = .080$ (trial 6).

Overall, these results show that responding during the clicker increased over trials in the session and that the groups did not differ. Responding to the tone also increased over the session, but there was some difference between group Sham T/C and group PeRh T/C. This may be due to group Sham T/C recovering from suppression fairly quickly, but group PeRh T/C being slower to recover. The ratio calculations show that this may be because responding of group Sham T/C was steady throughout the session, but group PeRh T/C showed suppression in early trials, which decreased over the session. These differences are not of concern, as all groups were responding similarly by the end of the session.

Conditioning.

Pre-CS responses per minute.

Responding prior to trials in each conditioning session declined from session one to session two. Group PeRh had lower response levels

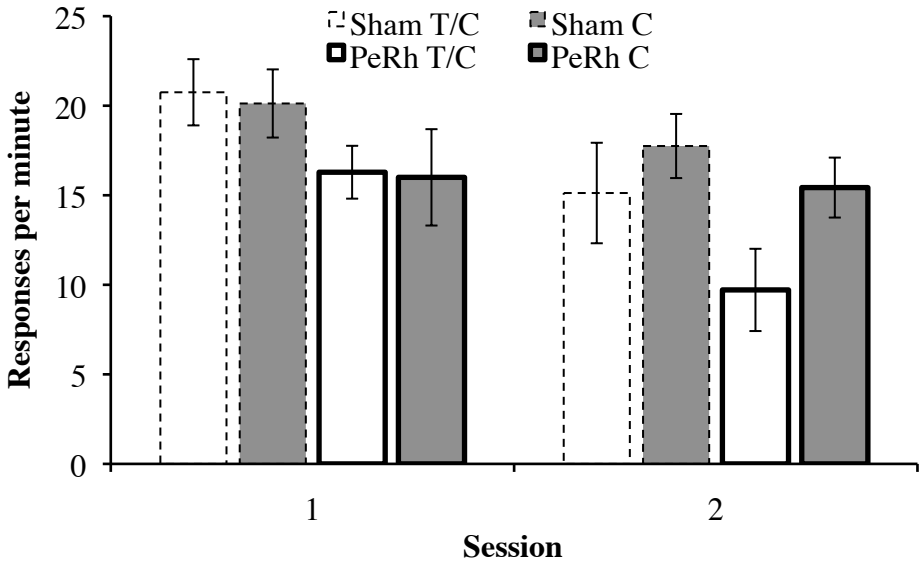


Figure 34. Mean responses per minute prior to the onset of the clicker (averaged over session) for both conditioning sessions. Error bars represent one standard error of the mean.

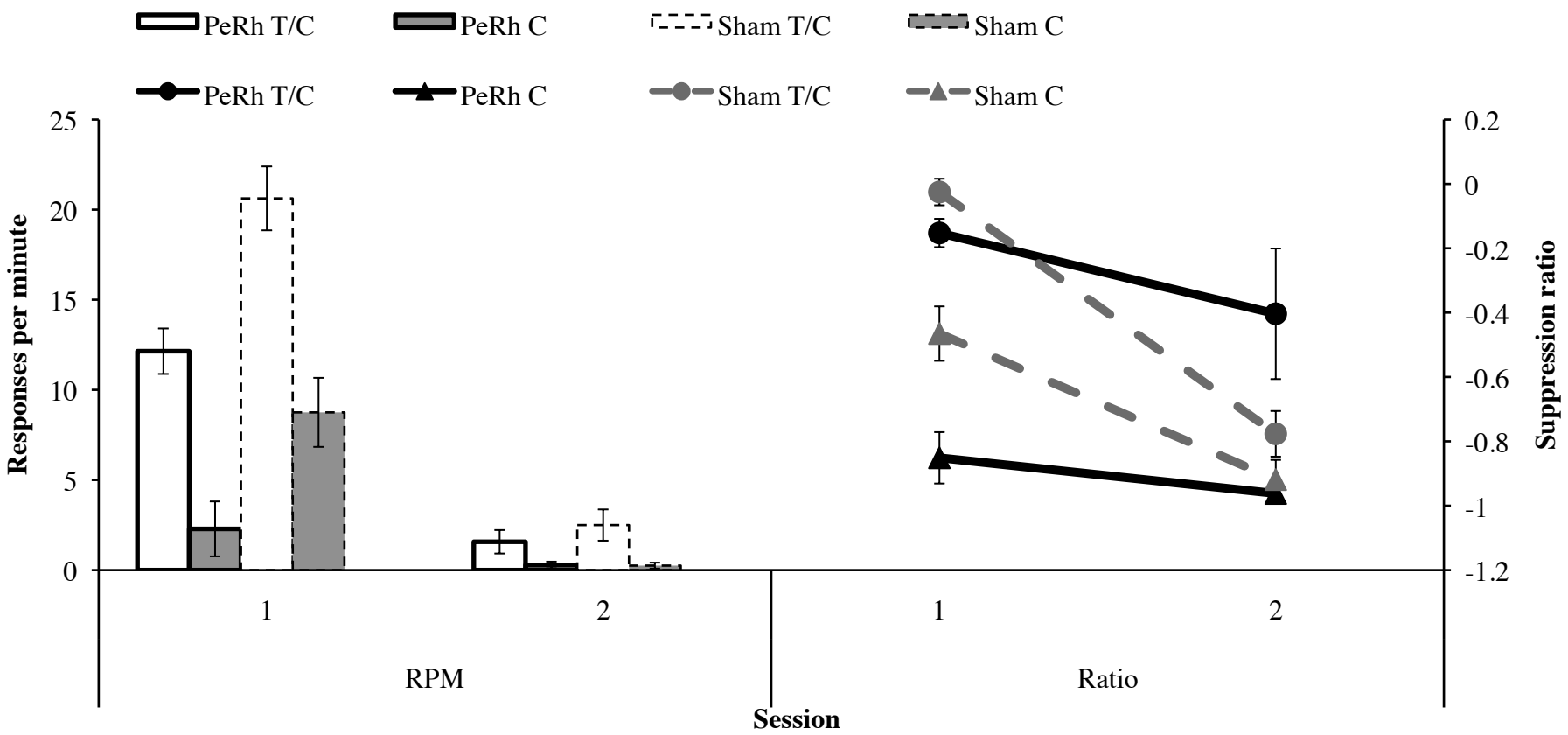


Figure 35. Responses per minute (left side) and suppression ratios (right side) during the two conditioning sessions (2 trials per session). Error bars represent one standard error of the mean.

than group Sham (Figure 34). This description was supported by an ANOVA with session and preexposure (T/C or C) and surgery (PeRh or Sham) as factors. There was an effect of trial, $F(1, 26) = 6.85$, $p = .015$, $\eta_P^2 = .21$, and surgery, $F(1, 26) = 6.90$, $p = .014$, $\eta_P^2 = .21$, and no effect of preexposure, $F(1, 26) = 1.43$, $p = .243$, $\eta_P^2 = .05$. There was no interaction between preexposure and surgery, $F < 1$, $\eta_P^2 = .01$, no Trial x Preexposure interaction, $F(1, 26) = 2.56$, $p = .122$, $\eta_P^2 = .09$, no Trial x Surgery interaction, $F < 1$, $\eta_P^2 < .01$, and no three-way interaction, $F < 1$, $\eta_P^2 < .01$.

CS responses per minute.

Results from the conditioning stage (T+) showed that during the first session of conditioning, responses of the C groups were low; this may have been the result of unconditioned suppression. However, by the second session, response levels of all groups were low (Figure 35).

An ANOVA of session, surgery and preexposure supported this description. There was a significant effect of session, $F(1, 26) = 131.60$, $p < .001$, $\eta_P^2 = .84$, and significant effects of surgery, $F(1, 26) = 18.73$, $p < .001$, $\eta_P^2 = .42$, and preexposure, $F(1, 26) = 47.67$, $p < .001$, $\eta_P^2 = .65$, but there was no interaction of these between-subject factors, $F < 1$, $\eta_P^2 = .03$. There were significant interactions of Session x Surgery, $F(1, 16) = 16.92$, $p < .001$, $\eta_P^2 = .39$, and Session x Preexposure, $F(1, 16) = 28.36$, $p < .001$, $\eta_P^2 = .52$. There was no three-way interaction, $F < 1$, $\eta_P^2 < .01$.

SMEs analyses were conducted on each interaction. In the Session x Surgery interaction it was found that group PeRh had a lower level of responses than group Sham in session 1, $F(1, 26) = 19.87$, $p < .001$, $\eta_P^2 = .43$, but not in the second session, $F < 1$, $\eta_P^2 = .02$. Responding declined

in both group PeRh and group Sham, $F(1, 26) = 25.38$, $p < .001$, $\eta_P^2 = .49$, and, $F(1, 26) = 130.12$, $p < .001$, $\eta_P^2 = .83$, respectively.

In the Session x Preexposure interaction it was found that there were group differences in both sessions; responding of the T/C groups was generally higher than the C groups: for session 1, $F(1, 26) = 42.01$, $p < .001$, $\eta_P^2 = .62$, for session 2, $F(1, 26) = 9.67$, $p = .004$, $\eta_P^2 = .27$. Both groups' responding declined over the two sessions: for the C groups, $F(1, 26) = 18.89$, $p < .001$, $\eta_P^2 = .42$, and for the T/C groups, $F(1, 26) = 141.08$, $p < .001$, $\eta_P^2 = .84$.

Ratios.

The suppression ratios calculated suggested a similar pattern of results to that indicated by the responses per minute data. Suppression increased in all groups by the second session (Figure 35). An ANOVA with variables of surgery and preexposure supported this description. There was an effect of session, $F(1, 26) = 44.84$, $p < .001$, $\eta_P^2 = .63$, and an effect of preexposure, $F(1, 26) = 46.59$, $p < .001$, $\eta_P^2 = .64$, but no effect of surgery, $F < 1$, $\eta_P^2 = .02$. There was an interaction between surgery and preexposure, $F(1, 26) = 6.27$, $p = .019$, $\eta_P^2 = .19$, and a significant Session x Surgery interaction, $F(1, 26) = 12.98$, $p = .001$, $\eta_P^2 = .33$. There was no Session x Preexposure interaction, $F(1, 26) = 3.51$, $p = .072$, $\eta_P^2 = .12$, and no three-way interaction, $F < 1$, $\eta_P^2 = .45$.

SMEs analyses were conducted to investigate the interactions. In the Surgery x Preexposure interaction, group PeRh T/C showed less suppression than group PeRh C, $F(1, 26) = 40.78$, $p <. 001$, $\eta_P^2 = .61$, and group Sham T/C showed less suppression than group Sham C, $F(1, 26) =$

10.01, $p = .004$, $\eta_P^2 = .28$. Also, group Sham C showed less suppression than group PeRh C, $F(1, 26) = 5.07$, $p = .033$, $\eta_P^2 = .16$, but this difference was not apparent in the T/C groups, $F(1, 26) = 1.66$, $p = .209$, $\eta_P^2 = .06$.

In the Session x Surgery interaction, there was a group effect in the first session, $F(1, 26) = 15.13$, $p = .001$, $\eta_P^2 = .37$, PeRh groups showed more suppression than the Sham groups, but this was not apparent in the second session, $F(1, 26) = 2.35$, $p = .137$, $\eta_P^2 = .08$. Both groups increased suppression from session one to session two; for group Sham, $F(1, 26) = 48.26$, $p < .001$, $0_P^2 = .65$, and for group PeRh, $F(1, 26) = 4.89$, $p = .036$, $\eta_P^2 = .16$.

Test.

Pre-CS responses per minute.

Responding of PeRh groups was lower than those of the Sham groups (Figure 36). An ANOVA of trial, preexposure and surgery supported this description. There was an effect of surgery, $F(1, 26) = 11.34$, $p = .002$, $\eta_P^2 = .30$, but no effect of trial, $F < 1$, $\eta_P^2 < .01$, or preexposure, $F < 1$, $\eta_P^2 < .01$. There was no interaction between preexposure and surgery, $F < 1$, $\eta_P^2 = .04$, no Trial x Preexposure interaction, $F < 1$, $\eta_P^2 = .02$, and no Trial x Surgery interaction, $F < 1$, $\eta_P^2 = .01$. There was no three-way interaction, $F(1, 26) = 1.62$, $p = .215$, $\eta_P^2 = .06$.

CS responses per minute.

During the first trial of the test stage, responding by all groups, except group Sham C, was low (Figure 37). Responding then increased slightly on the second trial. An ANOVA of responses made during the first two clicker trials of the test stage, with variables of surgery and

preexposure, partially supported this description. There was a significant effect of trial, $F(1, 26) = 5.60$, $p = .026$, $\eta_P^2 = .18$, a significant effect of surgery, $F(1, 26) = 13.57$, $p = .001$, $\eta_P^2 = .34$, but no effect of preexposure, $F(1, 26) = 3.20$, $p = .085$, $\eta_P^2 = .11$. There was no significant interaction of the between-subject variables, Preexposure x Surgery interaction, $F(1, 26) = 2.79$, $p = .107$, $\eta_P^2 = .10$. There were no interactions of any variable with trial, Trial x Preexposure, $F(1, 26) = 1.55$, $p = .224$, $\eta_P^2 = .06$, Trial x Surgery, $F < 1$, $\eta_P^2 = .02$, and no three-way interaction, $F < 1$, $\eta_P^2 < .01$.

Ratios.

Suppression ratios were all below zero in the first trial, and increased during the second trial (Figure 37). The groups' ratios were similar, apart from that of group Sham C, which showed less suppression than the other groups. An ANOVA partially supported this description. There was a significant effect of trial, $F(1, 26) = 6.93$, $p = .014$, $\eta_P^2 = .21$, a significant effect of surgery, $F(1, 26) = 13.33$, $p = .001$, $\eta_P^2 = .34$, but no effect of preexposure, $F(1, 26) = 2.54$, $p = .123$, $\eta_P^2 = .09$. There was a near-significant interaction of the between-subject variables, $F(1, 26) = 3.88$, $p = .060$, $\eta_P^2 = .13$. There were no interactions with trial, Trial x Preexposure, $F < 1$, $\eta_P^2 = .02$, Trial x Surgery, $F(1, 26) = 1.15$, $p = .294$, $\eta_P^2 = .04$, and no three-way interaction, $F < 1$, $\eta_P^2 < .01$.

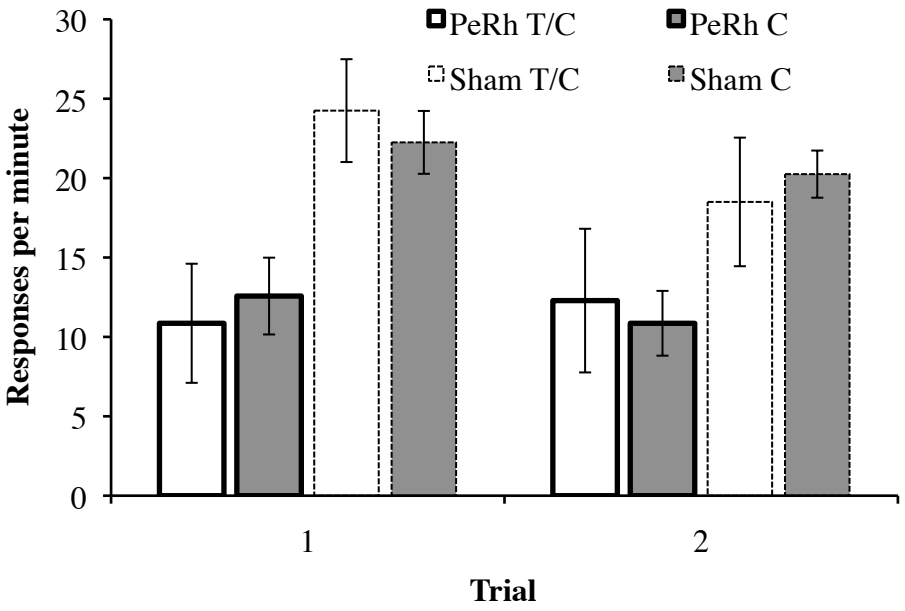


Figure 36. Responses per minute prior to the onset of the clicker in the test session. Error bars represent one standard error of the mean.

SMEs analyses were used to investigate the near-significant interaction of Preexposure x Surgery. These showed that group Sham C showed less suppression than group Sham T/C, $F(1, 26) = 6.81$, $p = .015$, $\eta_P^2 = .21$, but this difference was not seen between group PeRh T/C and group PeRh C, $F < 1$, $\eta_P^2 < .01$. Group PeRh C showed more suppression than group Sham C, $F(1, 26) = 15.80$, $p < .001$, $\eta_P^2 = .38$, but group PeRh T/C and group Sham T/C did not show any difference, $F(1, 26) = 1.41$, $p = .245$, $\eta_P^2 = .05$.

These results were similar to those reported in Experiment 9. When preexposed to the conditioning stimulus and the test stimulus, normal (non-operated) rats in Experiment 9 and sham-operated rats in the present experiment showed suppression to the test stimulus. Subjects that were preexposed to only one stimulus showed less suppression. When the conditioning stimulus and test stimulus were both familiar, generalisation of the CR was more apparent. In the present experiment, although results were not significant, there was some indication that rats with lesions to perirhinal cortex did not show a difference in preexposure treatment. This may indicate that the perirhinal cortex has a role in processing the familiarity of stimuli.

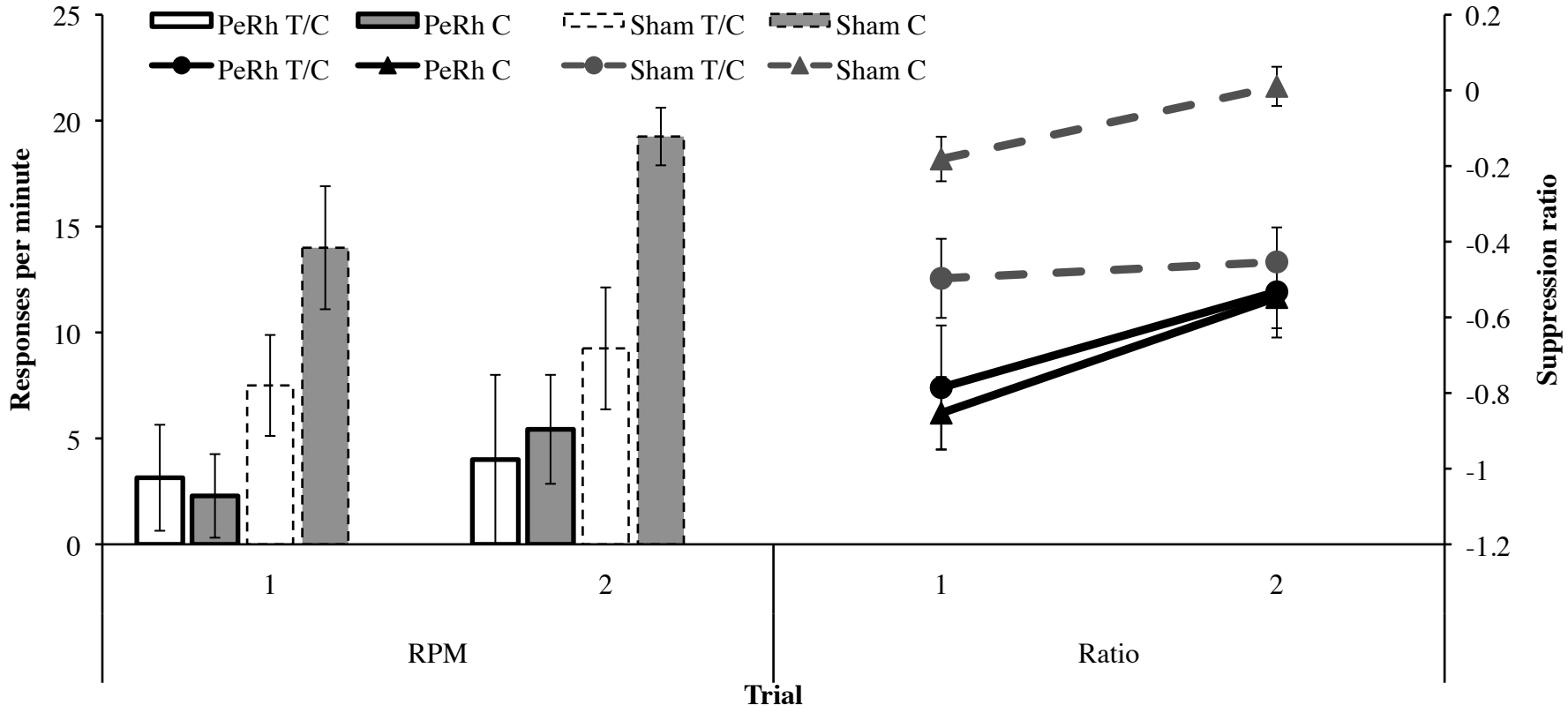


Figure 37. Mean responses per minute (left graph) and suppression ratios (right side) during the first two clicker trials of the test stage. Error bars represent one standard error of the mean.

Tone test.

Pre-CS responses per minute.

Responding during the pre-CS period was similar for each surgical group, but the PeRh groups had a lower level of responding to the Sham groups (Figure 38). An ANOVA with factors of trial, surgery (PeRh or Sham) and preexposure (T/C or C) supported this description. There was an effect of surgery, $F(1, 26) = 11.20$, $p = .002$, $\eta_P^2 = .30$, but no effect of trial, $F(1, 26) = 3.28$, $p = .082$, $\eta_P^2 = .11$, or preexposure, $F < 1$, $\eta_P^2 < .01$. There was no interaction between surgery and preexposure, $F < 1$, $\eta_P^2 < .01$, no Trial x Preexposure interaction, $F < 1$, $\eta_P^2 < 1$, no Trial x Surgery interaction, $F(1, 26) = 2.83$, $p = .105$, $\eta_P^2 = .10$, and no three-way interaction, $F(1, 26) = 2.41$, $p = .122$, $\eta_P^2 = .09$.

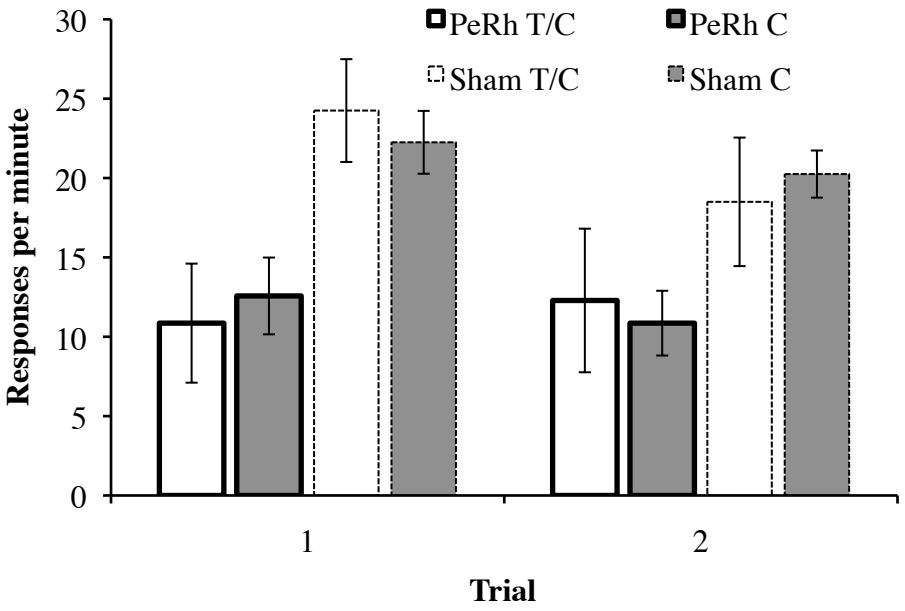


Figure 38. Responses per minute during the pre-CS period of the tone trials. Error bars represent one standard error of the mean.

CS Responses per minute.

Responding to the conditioned stimulus, the tone, was also analysed. Responding during both trials was very low for all groups; groups Sham T/C in trial 1 and PeRh T/C in trial 2 did not make any responses (Figure 39, left panel). An ANOVA of trials, preexposure and surgery confirmed this description. There was no effect of trial, $F < 1$, $\eta_P^2 = .01$, surgery, $F < 1$, $\eta_P^2 < .01$, or preexposure, $F < 1$, $\eta_P^2 < .01$. There was a Preexposure x Surgery interaction, $F(1, 26) = 4.27$, $p = .049$, $\eta_P^2 = .14$. There was no Trial x Surgery interaction, $F(1, 26) = 2.29$, $p = .143$, $\eta_P^2 = .08$, no Trial x Preexposure interaction, $F < 1$, $\eta_P^2 = .01$, and no three-way interaction, $F < 1$, $\eta_P^2 = .01$.

The Preexposure x Surgery interaction was analysed using SMEs. These showed that there was no difference between the Sham groups T/C and C, $F(1, 26) = 2.00$, $p = .170$, $\eta_P^2 = .07$, and no difference between the PeRh groups T/C and C, $F(1, 26) = 2.28$, $p = .143$, $\eta_P^2 = .08$. There was also no difference between groups Sham T/C and group PeRh T/C, $F(1, 26) = 1.73$, $p = .200$, $\eta_P^2 = .06$, or between groups Sham C and group PeRh T/C, $F(1, 26) = 2.59$, $p = .120$, $\eta_P^2 = .09$.

Ratios.

Suppression ratios showed a similar pattern; there was suppression across both trials, the Sham groups were more suppressed than the PeRh groups (Figure 39, right panel). An ANOVA with trials and between-subjects factors of surgery and preexposure supported this description. There was no effect of trial, $F(1, 26) = 4.02$, $p = .055$, $\eta_P^2 = .13$, but an effect of surgery, $F(1, 26) = 4.88$, $p = .036$, $\eta_P^2 = .16$, and no effect of

preexposure, $F(1, 26) = 1.39$, $p = .248$, $\eta_P^2 = .05$. There was no Preexposure x Surgery interaction, $F < 1$, $\eta_P^2 = .02$, no Trial x Preexposure interaction $F(1, 26) = 1.40$, $p = .247$, $\eta_P^2 = .05$, but a Trial x Surgery interaction, $F(1, 26) = 6.23$, $p = .019$, $\eta_P^2 = .19$. There was no significant three-way interaction, $F(1, 26) = 1.74$, $p = .199$, $\eta_P^2 = .06$.

The Trial x Surgery interaction was also explored using SMEs analyses. Sham groups showed more suppression than PeRh groups at trial one, $F(1, 26) = 7.91$, $p = .009$, $\eta_P^2 = .23$. This difference is not apparent in trial two, $F(1, 26) = 1.88$, $p = .182$, $\eta_P^2 = .07$. Suppression in the Sham groups was consistent over trials, $F < 1$, $\eta_P^2 < .01$, but suppression in PeRh groups increased over trials, $F(1, 26) = 9.49$, $p = .005$, $\eta_P^2 = .27$.

These results indicate that PeRh groups showed less suppression to the tone than Sham groups. However, this may reflect the fact that PeRh groups responded less than the Sham groups during the pre-CS.

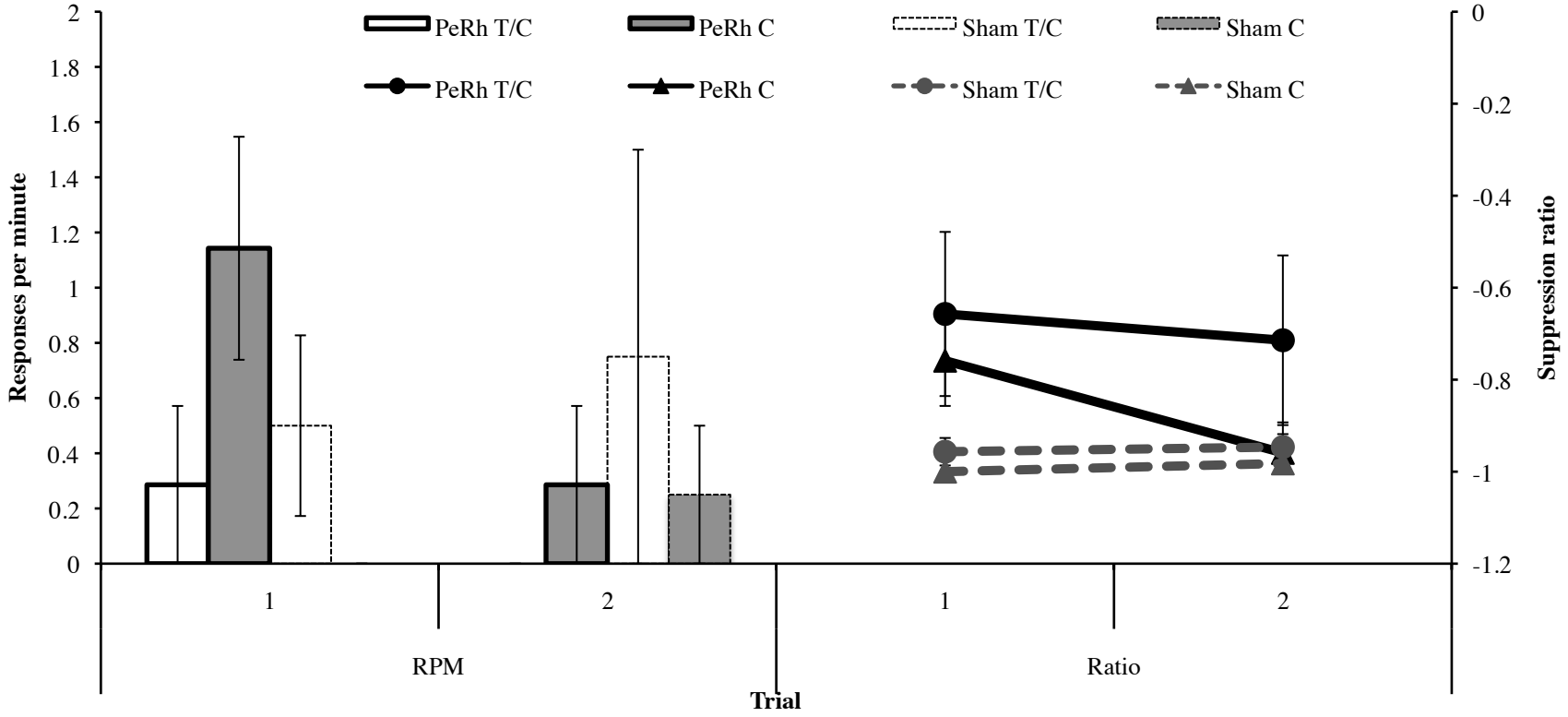


Figure 39. Responses per minute (left graph) and suppression ratios (right graph) during the first two trials of the tone in the test stage. Error bars represent one standard error of the mean. Mean responses for groups Sham T/C in trial 1 and PeRh T/C in trial 2 were equal to zero and not shown.

Generalisation to tones of different frequencies.

On the day following the test with the clicker and the tone, another test was performed to examine rats' responses to tones of higher frequencies. This was to confirm that PeRh groups' responding in the clicker test was not due to a general impairment in auditory discrimination.

Pre-CS responses per minute.

Responding during each pre-CS period for the tone was averaged from the four trials. The PeRh groups showed lower response levels than the Sham groups (Figure 40). An ANOVA with tone, surgery and preexposure confirmed this description. There was no effect of tone, $F(3, 78) = 2.16$, $p = .099$, $\eta_P^2 = .08$, no effect of preexposure, $F < 1$, $\eta_P^2 < .01$, but a significant effect of surgery, $F(1, 26) = 9.05$, $p = .006$, $\eta_P^2 = .26$. There was no interaction between surgery and preexposure, $F < 1$, $\eta_P^2 = .02$, no Tone x Surgery interaction, $F < 1$, $\eta_P^2 = .02$, and no Tone x Preexposure interaction, $F < 1$, $\eta_P^2 = .02$. There was no three-way interaction, $F(3, 78) = 2.16$, $p = .099$, $\eta_P^2 = .08$.

Ratios.

Data were pooled over trials for each frequency, and were presented in a ratio that was calculated by subtracting responses during the 2.0 kHz tone (the conditioning tone) from responses during the presentation of a 2.5 kHz tone, divided by total responding during both these trials. Zero would indicate no differences in responding, and above zero indicates increased responding during the 2.5 kHz tone. Ratios were also calculated for 3.0 kHz, and 3.5 kHz tones.

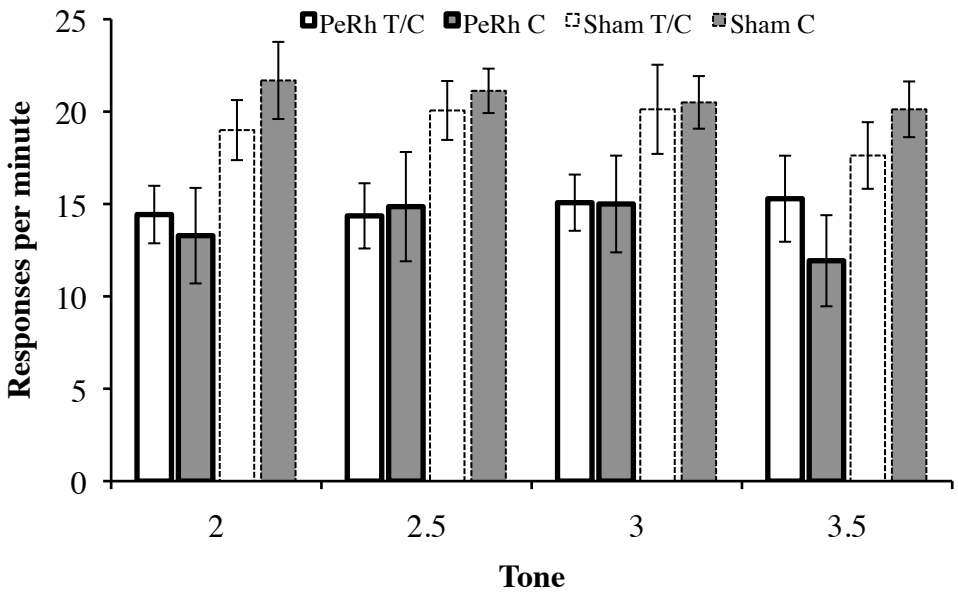


Figure 40. Mean responses for responding during the pre-CS period for the 2.0, 2.5, 3.0, and 3.5 kHz tones. Error bars represent one standard error of the mean.

Results showed that overall, responding was higher for 2.5, 3.0 and 3.5 kHz (Figure 41) than for the 2.0 kHz tone because ratios were above zero. There seems to be less responding to higher frequency tones, possibly due to unconditioned suppression. Those tones more distant in frequency from the 2.0 kHz tone will be less similar than tones close to 2.0 kHz; this may lead to generalisation of habituation of unconditioned suppression from the 2.0 kHz tone to tones that were close in frequency. An ANOVA with tone and surgical group as variables, showed that there was a significant effect of tone, $F(2, 56) = 5.81$, $p = .005$, $\eta_P^2 = .17$, no effect of surgery, $F < 1$, $\eta_P^2 < .01$, and no interaction between these variables, $F(2, 56) = 1.37$, $p = .264$, $\eta_P^2 = .05$. The intercept was significant, $F(1, 28) = 11.30$, $p = .002$, $\eta_P^2 = .29$, indicating that ratios were above zero, responses to 2.5, 3.0, and 3.5 kHz tones were higher than to the 2.0 kHz tone. Within-subjects contrasts revealed a linear trend of tone, $F(1, 28) = 10.40$, $p = .003$, $\eta_P^2 = .27$. Rats were able to discriminate between tones; ratios were highest for the 2.5 kHz tone, even though it was the closest frequency to the 2.0 kHz (conditioning) tone. Because of its proximity in frequency to the 2.0 kHz tone it may seem the most similar, so it might be expected that responding to the 2.5 kHz tone would be similar to the 2.0 kHz tone, however this was not the case. Rats with lesions responded similarly to the control group. This indicates that perirhinal lesions did not affect rats' auditory discrimination in general, suggesting that results from the clicker test reflect deficits in familiarity processing.

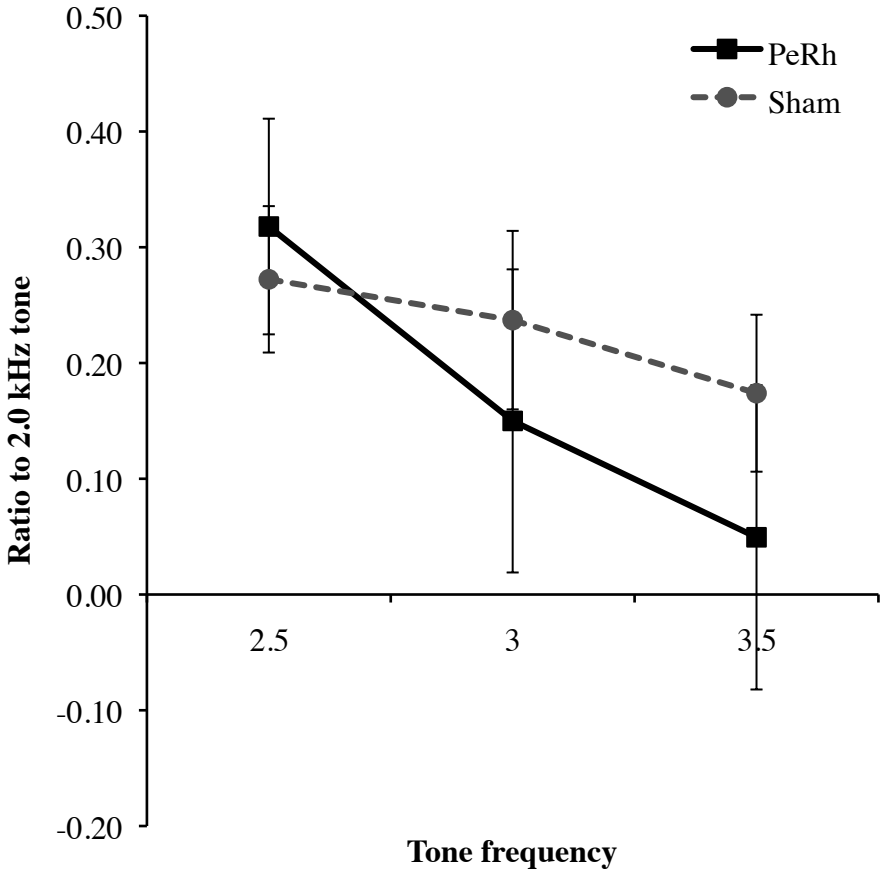


Figure 41. Ratios representing differences in responding to the conditioning tone (2.0 kHz) and tones higher in frequency.

4.2.3 General discussion.

My aim in Experiment 10 was to examine the effect of perirhinal lesions on familiarity generalisation. The results supported those of Experiment 9; rats that were preexposed to two stimuli, T and C, showed enhanced generalisation of suppression between those stimuli after T was made aversive. This may be due to the preexposed stimuli sharing elements of familiarity (e.g., Honey, 1990); generalisation was enhanced between stimuli that were both familiar. Although results were not significant, there was some indication that rats with perirhinal lesions did not show this enhancement. The PeRh groups were more suppressed than the Sham groups during other stages of the experiment; this can be seen in the analysis for pre-CS responding. This may indicate a floor effect. Recognition tasks require discrimination between novelty and familiarity (e.g., Albasser et al., 2009; Mumby et al., 2007; Norman & Eacott, 2005), and so have parallels to the current experiment. This means that the mechanisms used in recognition, and in familiarity generalisation, may rely on performance of the perirhinal cortex. These mechanisms may relate to detection of the novelty or familiarity of the stimulus, which supports Brown and Aggleton's (2001) theory.

The results of the PeRh groups are worth discussing, despite their non-significance. There was an indication that PeRh groups' suppressed responding during the clicker trials similarly. McTighe et al. (2011) found that rats with perirhinal lesions explored novel stimuli for similar durations as familiar stimuli. This may indicate that lesions of perirhinal cortex make novel stimuli seem familiar (McTighe et al., 2011). The suppression shown

by group PeRh T/C may represent generalisation of CR between two familiar stimuli. For group PeRh C, the conditioning stimulus, the tone, may have appeared familiar even though it was novel. This may have occurred just because both stimuli that the animal was exposed to were auditory. The suppression shown by group PeRh C may be a result of generalisation between two stimuli that both seemed familiar.

Research by Albasser et al. (2011) challenged McTighe et al.'s (2011) interpretation. They argued that the current models of perirhinal lesion deficit do not sufficiently explain novelty/familiarity discrimination. According to the representational-hierarchical model (Cowell et al., 2010a, 2010b; McTighe et al., 2010), rats with perirhinal lesions respond to novel objects as though they are familiar so will show decreased exploration of novel objects. Another theory (Brown & Aggleton, 2001) states that perirhinal cortex signals object novelty. Both familiarity and novelty detection are mediated by perirhinal cortex. Perirhinal lesions may decrease exploration of novel objects, but increase exploration of familiar objects. These predictions were not supported by Albasser et al.'s experiments. They conducted object recognition tests with rats that were given perirhinal lesions. Exploration of pairs of familiar objects, and pairs of novel objects was similar between rats with perirhinal lesions and control animals. However, when simultaneously presented with a familiar object and a novel object, rats with perirhinal lesions explored objects similarly. Albasser et al. criticised models of perirhinal lesions, as they do not predict/explain these differences in exploration levels.

These ideas may further explain results from the present experiment. In the test, both PeRh groups demonstrated suppression to the clicker. The T/C group were generalising between two familiar stimuli. Albasser et al. (2011) found PeRh rats responded to familiar objects similarly to controls. This can be seen in the current experiment, T/C groups were conditioned with a familiar stimulus and tested with a familiar stimulus. Responding to familiar stimuli was not impaired: group PeRh T/C showed results similar to the group Sham T/C. Group C were conditioned with a novel stimulus, and tested with a familiar stimulus. Suppression was greater in group PeRh C than group Sham C. This mirrors Albasser et al.'s findings that PeRh rats are impaired in discriminating familiar and novel stimuli. However, as mentioned previously, the PeRh groups showed lower levels of responding in other stages, and during pre-CS periods, and so these results may not reflect deficits in familiarity generalisation.

Results from this experiment indicated that perirhinal cortex might be important in discriminating familiarity. However, it is not clear exactly which stages of the procedure were important. Experiments 11, 12, and 13 investigated which of the stages of the generalisation task were particularly vital to forming familiarity representations.

4.3 Experiment 11. Effects of Scopolamine on Generalisation

Disruption of the cholinergic system has detrimental effects on recognition memory (e.g., Aigner & Mishkin, 1986; Ennaceur & Meliani, 1992; Huston & Aggleton, 1987; Plakke, Ng, & Poremba, 2008; Schon et al., 2005; Winters, Saksida, & Bussey, 2006, 2008). This has been demonstrated through tests such as delayed matching-to-sample, delayed

nonmatching-to-sample, and spontaneous object recognition. For example, Huston and Aggleton (1987) reported that administration of the muscarinic antagonist scopolamine affected performance in a delayed nonmatching-to-sample task. Those rats that were given scopolamine (0.05 mg/kg and above) showed a significant drop in performance in the task, compared to control subjects that were given saline injections. Studies using novel object recognition tasks supported these findings, for example, Woolley, Marsden, Sleight, and Fone (2003) reported that subjects given scopolamine (0.1 mg/kg and above) showed similar exploration of novel and familiar objects.

These reports have obvious parallels to findings that perirhinal lesions affect recognition memory (e.g., Cowell et al., 2006; Mumby & Pinel, 1994). Experiment 10 indicated that recognition and familiarity generalisation might share similar mechanisms, as performance in these tasks may be impaired in subjects with perirhinal lesions. It seems possible that disruption of the cholinergic system would also affect familiarity generalisation. The enhancement of generalisation between familiar stimuli may not be apparent with cholinergic blockade because the subject cannot discriminate between familiar and novel stimuli.

I conducted three experiments to test this prediction. I administered scopolamine systemically before the preexposure stage (Experiment 11 and 13) and the conditioning stage (Experiment 12 and 13) of the procedure to examine which stage was important for familiarity processing (Figure 42). In Experiment 11, subjects were injected with scopolamine before preexposure sessions. This was because object recognition studies found

impairments in recognition when subjects were given scopolamine before the sample stage (Ennaceur & Meliani, 1992; Winters et al., 2006). Scopolamine seems to impair acquisition of stimulus information (Dere, Huston, & De Souza Silva, 2007), so may disrupt acquisition of familiarity of the preexposed stimuli in the present experiment.

The control group in the present experiment were given methylscopolamine (MS). This has identical peripheral effects to scopolamine (S), including an increase in pupil diameter, blurred vision, reduction in salivation, and urinary retention (Julien, Advokat, & Comaty, 2007; Klinkenberg & Blokland, 2010). However, it does not cross the blood-brain barrier, and so has lesser effects on memory (Herz, Teschemacher, Hofstetter, & Kurz, 1965; Woolley et al., 2003).

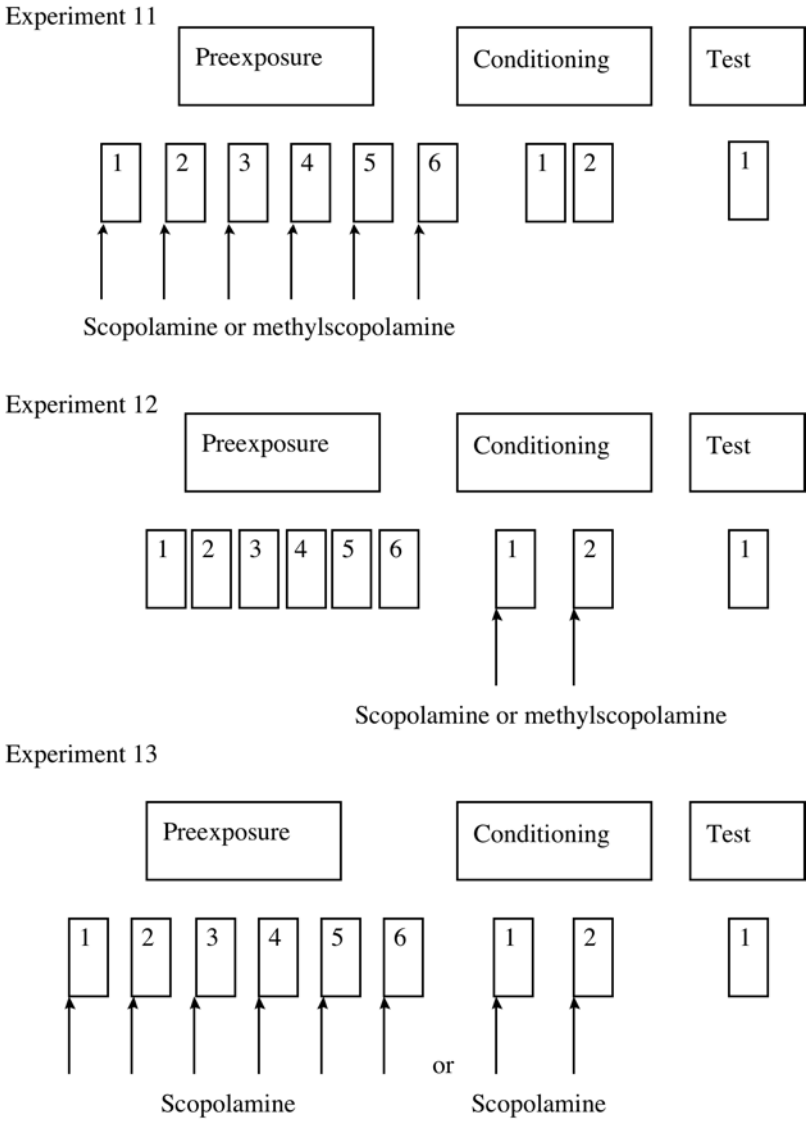


Figure 42. Structure of the generalisation task and administration of scopolamine. Arrows represent injections. In Experiment 11, one group of rats were given scopolamine (S) the other group were given methlyscopolamine (MS) before each preexposure session. In Experiment 12, rats were given S or MS before each conditioning session. In Experiment 13, the control group were not given any injections, a second group were given S before preexposure sessions, and a third group were given S before conditioning sessions.

4.3.1 Method.

Subjects.

The subjects were 32 rats (Charles River, UK) of the same sex and strain as Experiment 9. They were kept in the same conditions as Experiment 9. Food restriction was the same as in Experiment 9. Before food restriction, rats' mean weight was 295 g (range of 255-325 g).

Apparatus.

The apparatus used was the same as in Experiment 9. The lever was only present during lever training sessions and the test sessions, due to welfare issues. Huston and Aggleton (1987) observed gagging as a peripheral effect of scopolamine; in my experiments, food pellets were not used during treatment sessions to avoid this.

Scopolamine and methylscopolamine (Sigma-Aldrich, Gillingham, UK) were each dissolved in 0.9% saline before preexposure sessions. Drugs were administered by intraperitoneal (IP) injection at 1 mg/kg. A 1 ml syringe and 1 in. (25 mm) microlance needle (BD, Oxford, UK) were used.

Procedure.

The procedure and programs used were the same as in Experiment 10. Rats were not given the test with tones of different frequencies. In this experiment, no rats were given surgery, but scopolamine (or methylscopolamine) injections were given before rats were placed in the apparatus for each preexposure session. There were four groups (Table 3).

In the preexposure and conditioning sessions, the VI60 schedule and levers were not operational. Rats were injected 30 minutes before they

were put in the apparatus for the preexposure session. They were injected in the holding room and returned to their home cage for 30 minutes before being taken to the experimental room.

Data collation and analysis methods.

These were the same as in Experiment 9.

Table 3.

Preexposure and drug group assignment.

Drug	**Methylscopolamine**	**Scopolamine (S)**
Prexposure	**(MS)**	
T/C	$n = 8$	$n = 8$
C	$n = 8$	$n = 8$

Note. C = clicker, T = tone.

4.3.2 Results and discussion.

Training.

All rats learnt to retrieve food pellets from the food tray. At the end of lever training, rats responded at a mean rate of 9.93 presses per minute. There are no results to report from the preexposure and conditioning stage because levers were not operational.

Clicker test.

Pre-CS responses per minute.

Responding during the pre-CS period was similar across groups (Figure 43). An ANOVA with trial, drug (MS or S) and preexposure (T/C or C) as factors confirmed this description. There was no effect of trial, $F < 1$, $\eta_P^2 = .03$, no effect of drug, $F < 1$, $\eta_P^2 < .01$, and no effect of preexposure, $F(1, 28) = 1.15$, $p = .294$, $\eta_P^2 = .04$. There was no interaction between drug and preexposure, $F < 1$, $\eta_P^2 < .01$, no Trial x Preexposure interaction, $F(1, 28) = 2.22$, $p = .148$, $\eta_P^2 = .07$, no Trial x Drug interaction, $F(1, 28) = 1.40$, $p = .247$, $\eta_P^2 = .05$, and no three-way interaction, $F(1, 28) = 1.65$, $p = .210$, $\eta_P^2 = .06$.

CS responses per minute.

It was expected that group MS T/C would have low levels of responding (due to generalisation from T), but group MS C would have higher levels of responding because they would not show enhanced generalisation. This pattern of results was not observed in the current experiment (Figure 44, left panel). MS T/C and MS C showed similar levels of responding; whereas group S T/C had a low level of responding compared to group S C.

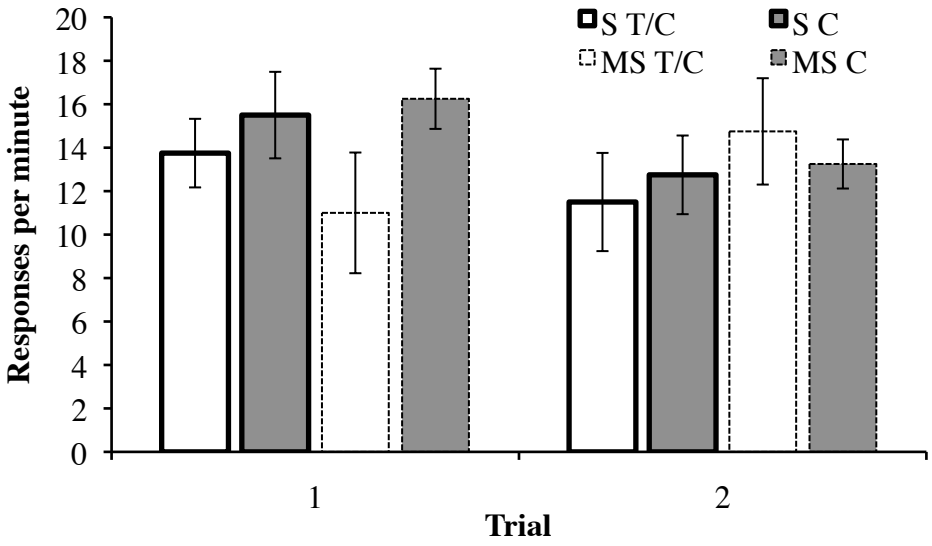


Figure 43. Mean responses during the pre-CS period of the clicker trials. Error bars represent one standard error of the mean.

An ANOVA with trial, and between-subjects factors of drug and preexposure confirmed that results were not in line with the predictions. There was a significant effect of trial, $F(1, 28) = 6.67$, $p = .015$, $\eta_P^2 = .19$, and preexposure, $F(1, 28) = 5.40$, $p = .028$, $\eta_P^2 = .16$, but no effect of drug, $F(1, 28) = 3.27$, $p = .081$, $\eta_P^2 = .11$. There was a significant interaction of Preexposure x Drug, $F(1, 28) = 4.27$, $p = .048$, $\eta_P^2 = .13$, a near significant interaction of Trial x Preexposure, $F(1, 28) = 4.04$, $p = .054$, $\eta_P^2 = .13$, but no interaction of Trial x Drug, $F < 1$, $\eta_P^2 < .01$. There was no three-way interaction, $F < 1$, $\eta_P^2 < .01$.

The interaction of Preexposure x Drug was explored using simple main effects (SME) with different error terms. Group S C responded more than group S T/C, $F(1, 28) = .9.64$, $p = .004$, $\eta_P^2 = .26$, but groups MS C and MS T/C had similar levels of responding, $F < 1$, $\eta_P^2 < .01$. Group S T/C responded less than group MS T/C, $F(1, 28) = 7.50$, $p = .011$, $\eta_P^2 = .21$, but groups S C and MS C had similar responding, $F < 1$, $\eta_P^2 < .01$.

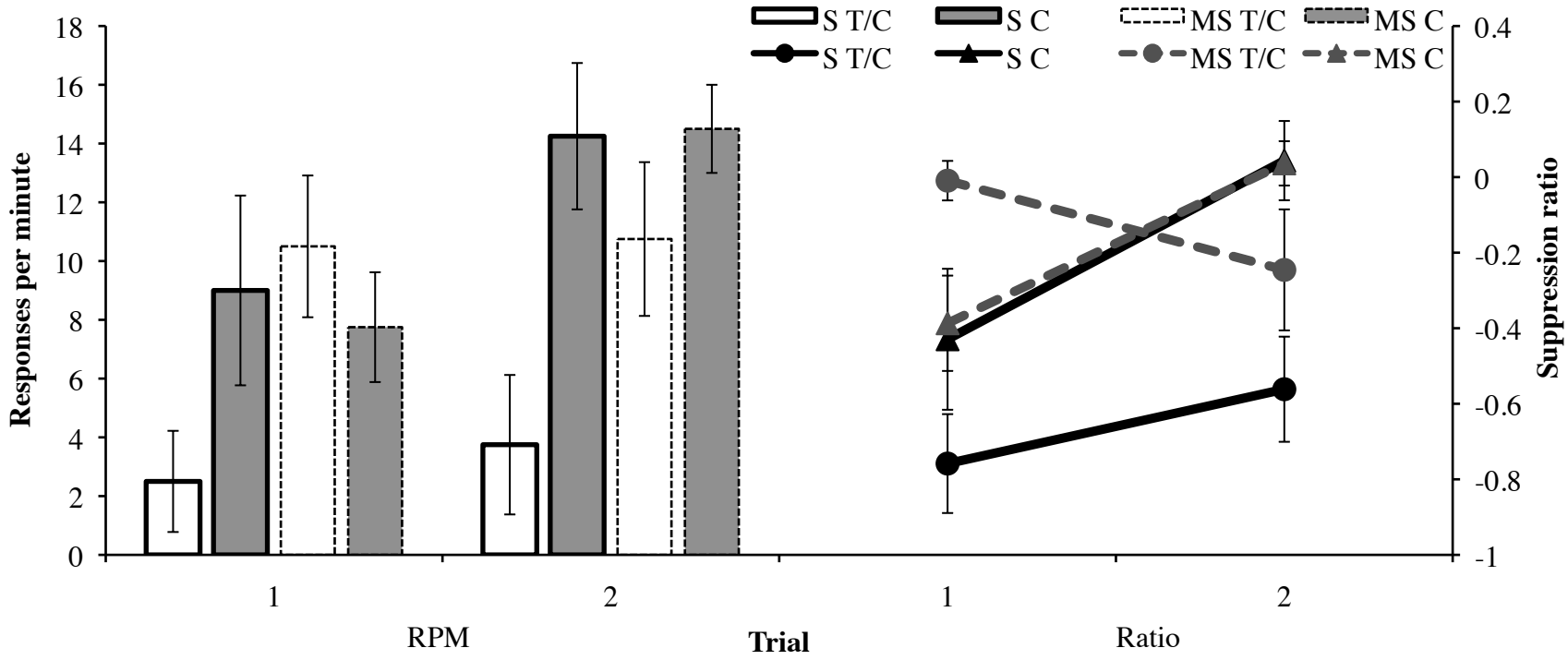


Figure 44. Responses per minute during the first two clicker trials in the test stage (shown by bar graph on the left side) and suppression ratios calculated from responses preceding and during the clicker, (C - Pre C / C + Pre C). S refers to the group that had scopolamine treatment, MS refers to methylscopolamine. T/C refers to the preexposure, tone and clicker, C refers to clicker alone. Error bars represent one standard error of the mean.

Ratios.

The suppression ratios indicate a similar pattern of results to the responses per minute data (Figure 44, right panel). The MS groups showed low levels of suppression (about zero), whereas group S T/C showed some suppression, but group S C did not. An ANOVA with trial, drug, and preexposure confirmed these descriptions. There was a main effect of trial, $F(1, 28) = 7.49$, $p = .011$. $\eta_P^2 = .21$, preexposure, $F(1, 28) = 4.34$, $p = .047$, $\eta_P^2 = .13$, drug, $F(1, 28) = 7.46$, $p = .011$, $\eta_P^2 = .21$, and a significant interaction of Drug x Preexposure, $F(1, 28) = 6.54$, $p = .016$, $\eta_P^2 = .19$. There was also a significant Trial x Preexposure interaction, $F(1, 28) = 8.94$, $p = .006$, $\eta_P^2 = .24$, but no Trial x Drug interaction, $F(1, 28) = 2.38$, $p = .134$, $\eta_P^2 = .78$, and no three-way interaction, $F(1, 28) = 1.50$, $p = .231$, $\eta_P^2 = .05$. The intercept indicated that overall, ratios were significantly different from zero, $F(1, 28) = 32.92$, $p < .001$, $\eta_P^2 = .54$.

The significant Drug x Preexposure interaction was explored with SME analyses. Group S T/C showed more suppression than group S C, $F(1, 28) = 10.76$, $p = .003$, $\eta_P^2 = .28$. MS groups T/C and C showed similar suppression, $F < 1$, $\eta_P^2 < .01$. Group MS T/C showed more suppression than group S T/C, $F(1, 28) = 13.98$, $p = .001$, $\eta_P^2 = .33$, groups MS C and S C showed similar suppression, $F < 1$, $\eta_P^2 < .01$.

SME analysis of the Trial x Preexposure interaction revealed that C groups showed more suppression in the first trial than the second, $F(1, 28) = 16.40$, $p < .001$, $\eta_P^2 = .37$, but the T/C groups had similar ratios on both trials, $F < 1$, $\eta_P^2 < .01$. T/C groups showed more suppression than C groups

on trial two, $F(1, 28) = 13.23$, $p = .001$, $\eta_P^2 = .32$; but not trial one, $F < 1$, $\eta_P^2 < .01$.

Overall, these results did not support the predictions that were made. There was no difference in responding between groups MS T/C and MS C, indicating no enhancement of generalisation in group MS T/C, contrary to findings from Experiments 9 and 10. Because of this it is difficult to account for the differences seen between group S T/C and group S C.

Tone test.

After presentations of the clicker during the test stage, rats were given trials with the conditioned stimulus, the tone, to examine whether groups differed in their response to that stimulus.

Pre-CS responses per minute.

Responding in the pre-CS period was consistent between groups (Figure 45). An ANOVA of trial, preexposure, and drug confirmed this description. There was an effect of trial, $F(1, 28) = 5.10$, $p = .032$, $\eta_P^2 = .15$, no effect of preexposure, $F(1, 28) = 1.61$, $p = .215$, $\eta_P^2 = .05$, and no effect of drug, $F(1, 28) = 2.21$, $p = .149$, $\eta_P^2 = .07$. There was no interaction between preexposure and drug, $F < 1$, $\eta_P^2 < .01$, no Trial x Preexposure interaction, $F(1, 28) = 2.00$, $p = .172$, $\eta_P^2 = .07$, no Trial x Drug interaction, $F < 1$, $\eta_P^2 = .02$, and no three-way interaction, $F < 1$, $\eta_P^2 < .01$.

CS Responses per minute.

During the first trial, responses to the tone were low (Figure 46), but increased on the second trial. An ANOVA with trial, and between-

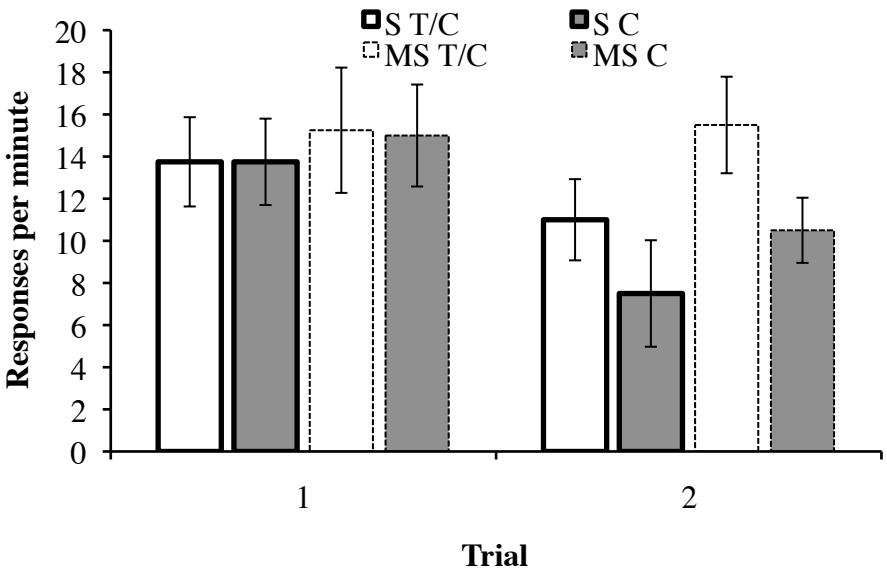


Figure 45. Responses per minute during the pre-CS period of the tone trials. Error bars represent one standard error of the mean.

subject factors of drug and preexposure supported this description. There was a main effect of trial, $F(1, 28) = 12.67$, $p = .001$, $\eta_P^2 = .31$, but no effects of preexposure, $F(1, 28) = 2.04$, $p = .164$, $\eta_P^2 = .07$, or drug, $F < 1$, $\eta_P^2 = .01$. There was no Preexposure x Drug interaction, $F < 1$, $\eta_P^2 < .01$. There were no interactions of the between-subject factors with trial, Trial x Preexposure, $F(1, 28) = 2.56$, $p = .14$, $\eta_P^2 = .08$, Trial x Drug, $F < 1$, $\eta_P^2 = .02$, nor a three-way interaction, $F < 1$, $\eta_P^2 < .01$.

These results indicate that groups had low levels of responding during the first tone trial, but this increased on the second trial, and this pattern was apparent for all groups.

Ratios.

Ratios reflected response results; there was more suppression on the first trial than on the second (Figure 46). An ANOVA with trial, drug and preexposure revealed a main effect of trial, $F(1, 28) = 11.91$, $p = .002$, $\eta_P^2 = .30$, no effect of preexposure, $F < 1$, $\eta_P^2 < .01$, or drug, $F(1, 28) = 2.43$, $p = .13$, $\eta_P^2 = .08$. There was no Preexposure x Drug interaction, $F < 1$, $\eta_P^2 < .01$. There was a significant Trial x Drug interaction, $F(1, 28) = 5.04$, $p = .033$, $\eta_P^2 = .15$. There was no Trial x Preexposure interaction, $F < 1$, $\eta_P^2 < .01$, and no three-way interaction, $F < 1$, $\eta_P^2 < .01$. The intercept indicated that overall, ratios were significantly different from zero, $F(1, 28) = 316.04$, $p < .001$, $\eta_P^2 = .92$.

The significant Trial x Drug interaction was explored with SMEs analyses. Suppression was reduced in trial 2 compared to trial 1 in S groups, $F(1, 28) = 16.23$, $p < .001$, $\eta_P^2 = .37$. MS groups had similar ratios across trials, $F < 1$, $\eta_P^2 = .03$. MS groups were more suppressed than S groups at

trial 2, $F(1, 28) = 4.49$, $p = .043$, $\eta_P^2 = .14$, but not at trial 1, $F(1, 28) = 1.57$, $p = .220$, $\eta_P^2 = .05$.

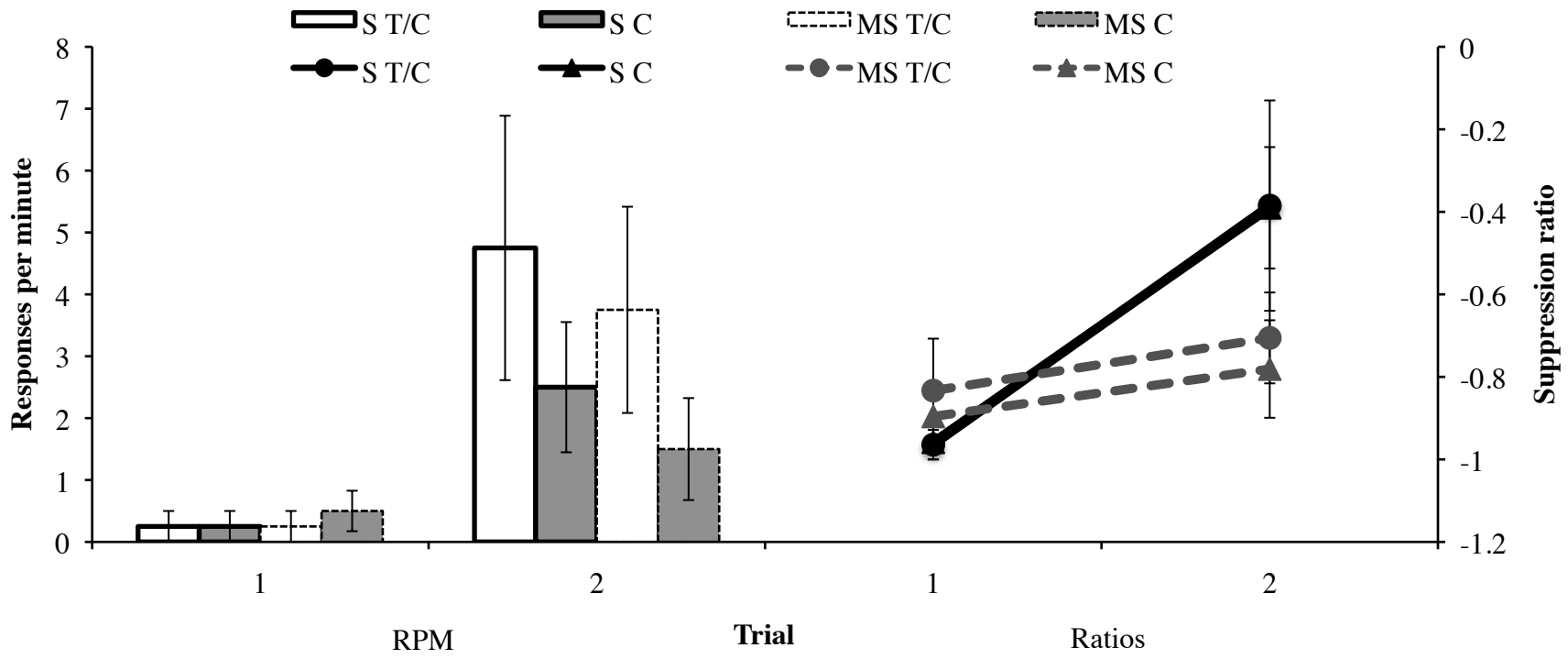


Figure 46. Responses per minute during the first two tone trials in the test stage (shown by bar graph on the left side) and suppression ratios calculated from responses preceding and during the tone, (T - Pre T / T + Pre T). S refers to the group that had scopolamine treatment, MS refers to methylscopolamine. T/C refers to the preexposure, tone and clicker, C refers to clicker alone. Error bars represent one standard error of the mean.

These results show that groups had a high level of suppression during the first tone trial, but only those rats given scopolamine reduced suppression during the second trial. Overall, these results indicate that all groups had conditioned to the tone. The unexpected results seen in the test stage with the clicker trials may not be due to inadequate conditioning to the tone.

4.3.3 General discussion.

The aim of Experiment 11 was to test whether scopolamine administration before the preexposure stage affected familiarity generalisation. It was predicted that generalisation among familiar stimuli may be less apparent in subjects that were given scopolamine, because their ability to discriminate familiarity and novelty may be diminished. These predictions were not supported. The methylscopolamine control groups responded similarly, whether preexposed to T/C or C. This indicates no enhancement of generalisation in the T/C group. The groups given scopolamine differed, depending on whether the preexposure was to T/C or to C. It is difficult to interpret whether the differences in the scopolamine groups were due to generalisation since the results of the control group did not support the familiarity generalisation hypothesis.

All groups showed low levels of responding during the test trials of the conditioned stimulus, the tone. This indicates that the conditioning was successful. The lack of distinction between the methylscopolamine control groups was not due to a weak CR to the tone.

I administered scopolamine before the preexposure sessions because reports from recognition studies showed that scopolamine

impaired performance when it was administered before the sample stage (Aigner, Walker, & Mishkin, 1991; Warburton et al., 2003; Winters et al., 2006). However, the generalisation procedure uses three stages, so it is possible that the conditioning stage was important, rather than the preexposure stage. In the conditioning stage, the CR may become associated with the familiarity elements of the stimuli, and it is this that enhances the generalisation.

4.4 Experiment 12. Scopolamine in Conditioning

Experiment 12 was a replication of Experiment 11; but, instead of administrating scopolamine before preexposure, it was administered before conditioning. Administration of scopolamine may prevent recognition of the stimulus, so that the conditioning stimulus may appear novel. The familiarity elements of the stimulus would not enter into an association with the shock, and so could not mediate generalisation. On test, there may be no difference in responding of group T/C compared to group C.

There may be concern in regards to whether subjects will condition after scopolamine administration, as this would affect generalisation in the test stage. Anagnostaras, Maren, Sage, Goodrich and Fanselow (1999) reported that tone conditioning was disrupted by high doses of scopolamine (100 mg/kg); and, although smaller doses (1 mg/kg) produced some deficit during conditioning trials, overall there was significant learning. Based on these findings, the scopolamine dose used in the present experiment (1 mg/kg) should be low enough to allow conditioning, but also attenuate recognition memory.

4.4.1 Method.

Subjects.

The subjects were two groups of 16 rats (N = 32, Charles River, UK) of the same sex and strain as Experiment 9. They were kept in the same conditions as Experiment 9. Food restriction was the same as in Experiment 9. Before food restriction, rats' mean weight was 219 g (range of 185-250 g).

Apparatus.

The apparatus used was the same as in Experiment 9. Again, the lever was only present during lever training sessions and the test sessions, due to welfare issues. Drugs, concentrations and injection procedures used were the same as those used in Experiment 11.

Procedure.

The experiment was run in two replications. The procedure and programs used were the same as in Experiment 10. As in Experiment 11, rats were given scopolamine or methylscopolamine injections, but this time they were given 30 minutes before rats were placed in the apparatus for the conditioning sessions (Figure 33).

Data collation and analysis methods.

These were the same as in Experiment 9.

4.4.2 Results and discussion.

Only results from the test stage are presented, as there were no levers present during preexposure or conditioning (see above).

Training.

Rats successfully learnt to retrieve food pellets from the food tray. Lever training was also successful; by the last lever training session, rats were responding at a mean of 11.84 presses per minute.

Clicker test.

Pre-CS responses per minute.

Responding during the pre-CS period was similar between groups (Figure 47). An ANOVA with trial, preexposure (T/C or C) and drug (MS or S) supported this description. There was no effect of trial, $F(1, 28) = 2.65$, $p = .115$, $\eta_P^2 = .09$, no effect of preexposure, $F(1, 28) = 2.56$, $p = .121$, $\eta_P^2 = .08$, no effect of drug, $F < 1$, $\eta_P^2 < .01$. There was no interaction between preexposure and drug, $F < 1$, $\eta_P^2 = .02$. There was no Trial x Preexposure interaction, $F < 1$, $\eta_P^2 < .01$, no Trial x Drug interaction, $F < 1$, $\eta_P^2 < .01$, and no three-way interaction, $F(1, 28) = 1.09$, $p = .306$, $\eta_P^2 = .04$.

CS responses per minute.

Results showed that group MS T/C showed lower levels of responding than group MS C (Figure 48), whereas the S groups did not show this difference. However, an ANOVA of trials, preexposure and drug did not support these descriptions. There was no effect of trial, $F < 1$, $\eta_P^2 < .01$, or preexposure, $F(1, 28) = 2.94$, $p = .098$, $\eta_P^2 = .10$, or drug, $F < 1$, $\eta_P^2 < .01$. There was no Preexposure x Drug interaction, $F(1, 28) = 2.94$, $p = .098$, $\eta_P^2 = .10$, nor any interactions with trial, Trial x Preexposure, $F < 1$, $\eta_P^2 < .01$, Trial x Drug, $F(1, 28) = 2.66$, $p = .114$, $\eta_P^2 = .09$, there was no three-way interaction, $F < 1$, $\eta_P^2 < .01$.

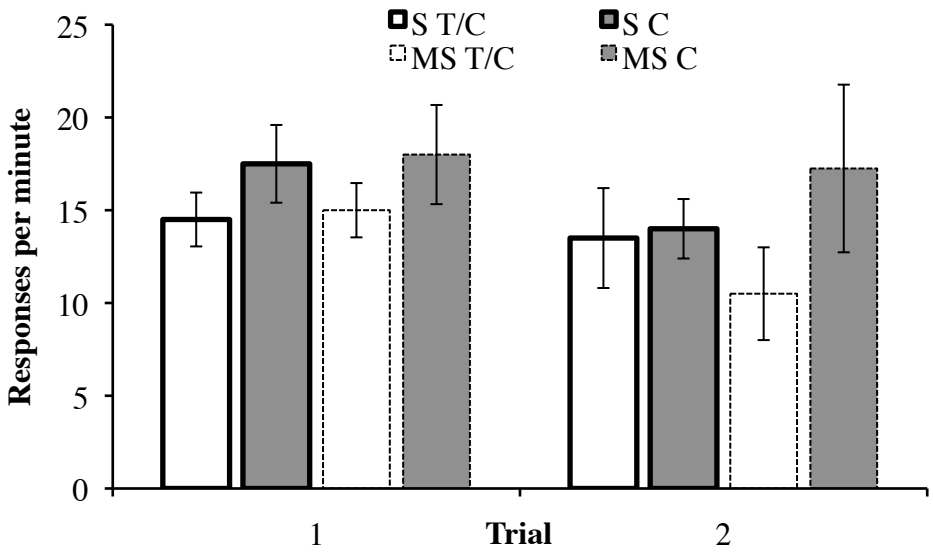


Figure 47. Mean responses during pre-CS period of the clicker trials. Error bars represent one standard error of the mean.

Ratios.

The ratio scores seem to reflect the same pattern of results. The ratios of group MS T/C were lower than of group MS C; however, this seemed to be reversed in the scopolamine groups. An ANOVA with trial, drug and preexposure did not reveal any differences. There was no effect of trial, $F(1, 28) = 1.86$, $p = .184$, $\eta_P^2 = .06$, preexposure, $F < 1$, $\eta_P^2 < .01$, or drug, $F < 1$, $\eta_P^2 < .01$. There was no interaction of Preexposure x Drug, $F(1, 28) = 1.69$, $p = .205$, $\eta_P^2 = .06$. There were no interactions of trial with either preexposure or drug, Fs < 1, highest $\eta_P^2 = .02$. There was no three-way interaction, $F < 1$, $\eta_P^2 = .01$. The intercept indicated that overall, ratios were not significantly different from zero, $F < 1$, $\eta_P^2 < .01$.

Overall, these results do not provide support for the pattern of results seen in the graphs. There were no differences in responding over trials, and no differences involving preexposure groups or drug groups. Unexpectedly, administering scopolamine before conditioning sessions did not seem to affect responding in the test.

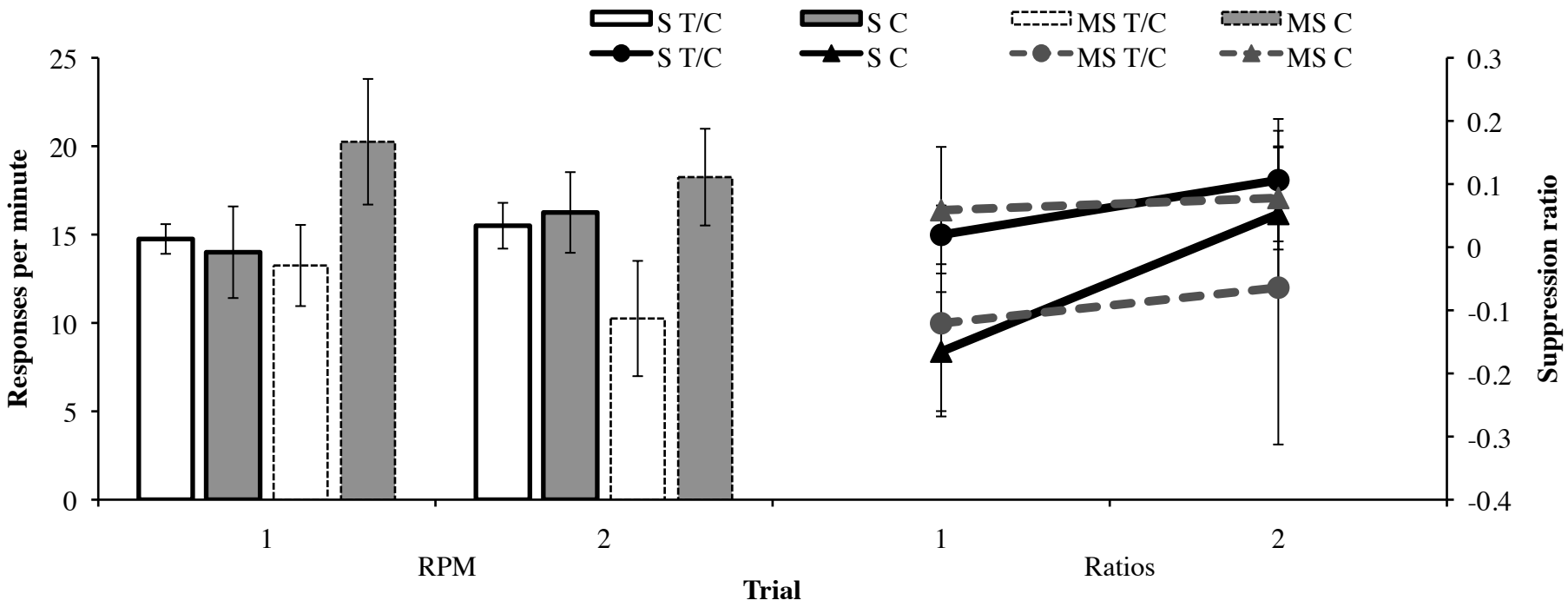


Figure 48. Responses per minute during the first two clicker trials in the test stage (shown by bar graph on the left side) and suppression ratios calculated from responses preceeding and during the clicker, (C - Pre C / C + Pre C). S refers to the group that had scopolamine treatment, MS refers to methylscopolamine. T/C refers to the preexposure, tone and clicker, C refers to clicker alone. Error bars represent one standard error of the mean.

Tone test.

After the clicker trials of the test stage, rats were tested with the tone, and responses were analysed. This was particularly important to check group differences in conditioning.

Pre-CS responses per minute.

Responding during the pre-CS period was consistent between groups and across trials (Figure 49). This was confirmed by an ANOVA with trial, preexposure (T/C or C) and drug (MS or S) as factors. There was no effect of trial, $F(1, 28) = 1.69$, $p = .204$, $\eta_P^2 = .06$, of preexposure, $F(1, 28) = 2.76$, $p = .108$, $\eta_P^2 = .09$, or drug, $F < 1$, $\eta_P^2 < .01$. There was no interaction between preexposure and drug, $F(1, 28) = 1.10$, $p = .303$, $\eta_P^2 = .04$, no Trial x Preexposure interaction, $F < 1$, $\eta_P^2 = .03$, no Trial x Drug interaction, $F < 1$, $\eta_P^2 < .01$, and no three-way interaction, $F(1, 28) = 1.38$, $p = .250$, $\eta_P^2 = .05$.

Responses per minute.

Responses were low on the first tone trial for group MS C, group MS T/C, and group S C. In the second trial, responses increased in all groups (Figure 50). An ANOVA with trial, drug and preexposure partially supported this description. There was a significant effect of trial, $F(1, 28) = 48.44$, $p < .001$, $\eta_P^2 = .63$, and drug, $F(1, 28) = 8.38$, $p = .007$, $\eta_P^2 = .23$. There was no effect of preexposure, $F < 1$, $\eta_P^2 = .02$, and no Preexposure x Drug interaction, $F < 1$, $\eta_P^2 < .01$. There was no interaction of Trial x Preexposure, $F(1, 28) = 2.01$, $p = .17$, $\eta_P^2 = .07$, no Trial x Drug interaction,

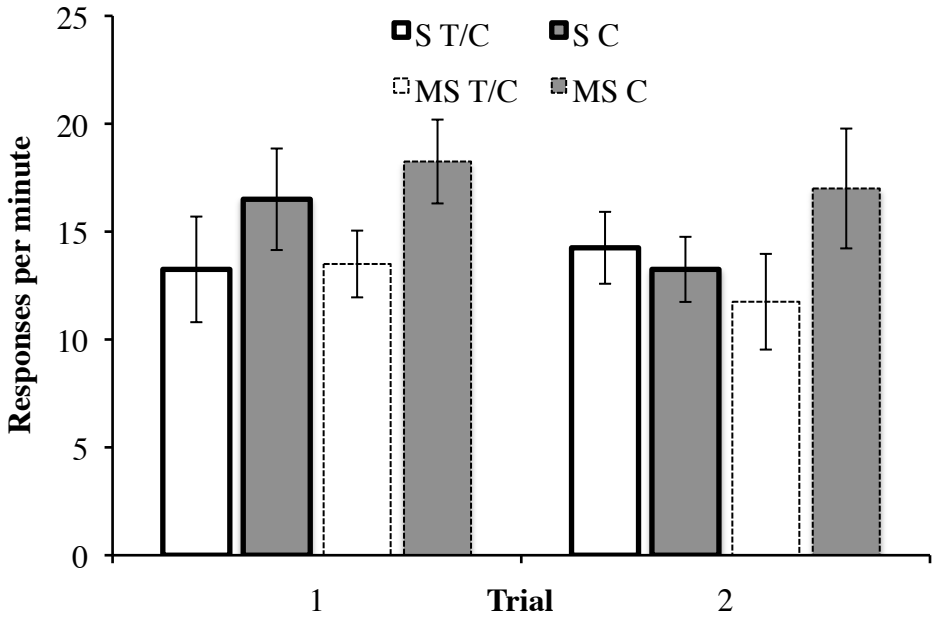


Figure 49. Responses per minute during the pre-CS period of the tone trials. Error bars represent one standard error of the mean.

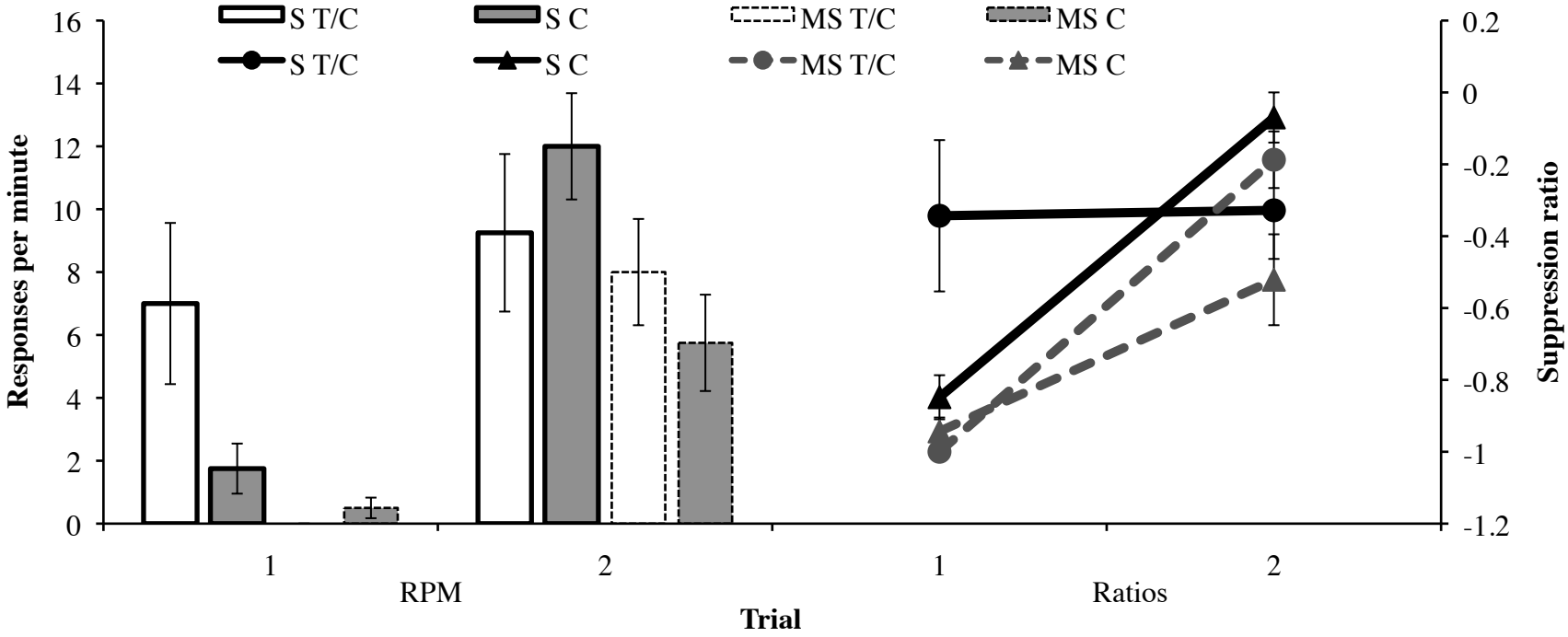


Figure 50. Responses per minute during the first two tone trials in the test stage (shown by bar graph on the left side) and suppression ratios calculated from responses preceding and during the tone, (T - Pre T / T + Pre T). S refers to the group that had scopolamine treatment, MS refers to methylscopolamine. T/C refers to the preexposure, tone and clicker, C refers to clicker alone. Mean responses for group MS T/C were equal to zero, and so not shown. Error bars represent one standard error of the mean.

$F < 1$, $\eta_P^2 < .01$, but a significant three-way interaction, $F(1, 28) = 8.44$, $p = .007$, $\eta_P^2 = .23$.

Simple interaction effects were used to examine the three-way interaction. These showed that all groups except group S T/C increased responding from the first to the second trial, all groups, Fs$(1, 28) > 8.06$, ps $< .008$, $\eta_P^2 > .22$, group S T/C, $F(1, 28) = 1.48$, $p = .234$, $\eta_P^2 = .05$.

Ratios.

Suppression ratios indicated a similar pattern of results; there was more suppression during the first tone trial than the second, except in group S T/C, where suppression was minimal over both trials (Figure 50). An ANOVA of trials, using preexposure and drug as between-subjects factors supported this description. There was a significant effect of trial, $F(1, 28) = 71.59$, $p <. 001$, $\eta_P^2 = .72$, a significant effect of drug, $F(1, 28) = 8.52$, $p = .007$, $\eta_P^2 = .23$, and no effect of preexposure, $F(1, 28) = 2.07$, $p = .161$, $\eta_P^2 = .07$. There was no interaction of Preexposure x Drug, $F < 1$, $\eta_P^2 < .01$. There was no interaction of trial with preexposure, $F(1, 28) = 2.42$, $p = .131$, $\eta_P^2 = .08$, or drug, $F(1, 28) = 3.39$, $p = .076$, $\eta_P^2 = .11$. There was a three-way interaction between all the variables, $F(1, 28) = 23.17$, $p < .001$, $\eta_P^2 = .45$. The intercept indicated that overall, ratios were significantly different from zero, $F(1, 28) = 135.76$, $p < .001$, $\eta_P^2 = .83$.

Simple interaction effects were used to examine the three-way interaction. These revealed that all groups increased responding from the first to the second trial, Fs$(1, 28) > 12.40$, ps $< .001$, $\eta_P^2 > .30$, except group S T/C, $F < 1$, $\eta_P^2 < .01$.

Overall, these results indicated that conditioning was effective for most groups; however, the scopolamine group that was preexposed to T/C only showed minimal suppression. Without an established CR to the tone, generalisation between stimuli would be limited.

4.4.3 General Discussion.

The aim of Experiment 12 was to examine the effects of scopolamine administration, before conditioning, on familiarity generalisation. It was predicted that group S T/C would respond similarly to group S C: familiarity generalisation would be diminished in the test due to disruption of recognition of the conditioned stimulus, the tone. Results did not support these predictions; the effect of preexposure treatment on responding did not differ depending on drug treatment. The MS groups' results tended toward the predicted familiarity generalisation, but differences were not revealed in statistical analysis.

Results regarding the effect of scopolamine on familiarity generalisation may be unclear because group S T/C did not show suppression to the tone. This may mean that the scopolamine treatment adversely affected conditioning; if there was no CR established to the tone, then generalisation would be limited. This was unexpected since other studies report that this dose of scopolamine did not affect conditioning (Anagnostaras et al., 1999). However, group S C did show some suppression in the first trial of the tone test, indicating that scopolamine might not have completely disrupted conditioning. Latent inhibition may also contribute to the lack of suppression in group S T/C; preexposure to T

would have retarded conditioning to T, this may have had an additive effect with the actions of scopolamine potentially disrupting conditioning.

4.5 Experiment 13. Scopolamine in Preexposure or Conditioning

Experiment 13 was conducted to replicate Experiments 11 and 12, to test whether the effects seen in those experiments were reliable. Experiment 13 compared performance of a control group, that had neither scopolamine nor methylscopolamine, with a group that had scopolamine in preexposure, and a group that had scopolamine in conditioning. I did not use methylscopolamine in the control group to avoid subjecting them to the peripheral effects. It is likely that results from Experiments 11 and 12 were due to extraneous factors. In this replication, I expected that performance of the control groups and the effects of scopolamine would be clearer, and that scopolamine would affect familiarity generalisation. However, if effects were found, it would be difficult to determine whether these were a result of central or peripheral actions.

4.5.1 Method.

Subjects.

The subjects were 48 rats (Charles River, UK) of the same sex and strain as Experiment 9. They were kept in the same conditions as Experiment 9. Food restriction was the same as in Experiment 9. Before food restriction, rats' mean weight was 233 g (range of 215 - 250 g).

Apparatus.

The apparatus used was the same as in Experiment 9. Only scopolamine was used in this experiment, concentrations and injection procedures used were the same as in Experiment 11.

Procedure.

The procedure and programs used were the same as in Experiment 10. In this experiment there were six groups (Table 4). The control group received no injections; the preexposure and conditioning groups received injections of scopolamine before preexposure or conditioning respectively.

Due to the number of groups, in the preexposure sessions, only four groups were run per day. This meant that sometimes groups had consecutive days of preexposure, and other times had a maximum of two days in between preexposures sessions.

Another difference was in the test session. The clicker and tone test were conducted on consecutive days, the clicker test first, followed by the tone test. The clicker test consisted of four trials of 30 s duration. The session lasted 23.5 minutes and the mean ITI was 285 s. The tone test was identical to the tone generalisation test in Experiment 10, but only responding to the 2.0 kHz tone was analysed.

Data collation and analysis methods.

These were the same as in Experiment 9.

Table 4

Drug treatments and preexposure groups.

Drug treatment Preexposure	**Control** No drug	**Scopolamine before preexposure**	**Scopolamine before conditioning**
T/C	*n* = 8	*n* = 8	*n* = 8
C	*n* = 8	*n* = 8	*n* = 8

Note. T = tone, C = clicker.

4.5.2 Results and discussion.

Training.

Rats successfully learnt to retrieve food pellets from the food tray. Lever training was successful. In the last training session, rats were pressing the levers at a mean of 9.75 presses per minute.

Clicker test.

Pre-CS responses per minute.

Responding during the pre-CS period was consistent across trials and between groups (Figure 51). An ANOVA with trial, preexposure (T/C or C), and drug (control, preexposure or conditioning) confirmed this description. There was no effect of trial, $F < 1$, $\eta_P^2 = .02$, no effect of preexposure, $F < 1$, $\eta_P^2 < .01$, and no effect of drug, $F(2, 42) = 1.48$, $p = .240$, $\eta_P^2 = .07$. There was no interaction between preexposure and drug, $F < 1$, $\eta_P^2 = .01$, there was no Trial x Preexposure interaction, $F < 1$, $\eta_P^2 < .01$, no Trial x Drug interaction, $F < 1$, $\eta_P^2 < .01$, and no three-way interaction, $F(2, 42) = 1.10$, $p = .342$, $\eta_P^2 = .05$.

CS responses per minute.

Responding was similar across groups (Figure 52). An ANOVA with a within-subject factor of trials, and drug and preexposure as between-subject variables, supported this description. There was a near-significant effect of trial, $F(1, 42) = 3.72$, $p = .061$, $\eta_P^2 = .08$, no effect of preexposure, $F(1, 42) = 1.25$, $p = .270$, $\eta_P^2 = .03$, or drug, $F < 1$, $\eta_P^2 = .01$. There was no Preexposure x Drug interaction, $F < 1$, $\eta_P^2 = .02$. There was a significant Trial x Preexposure interaction, $F(1, 42) = 4.09$, $p = .050$, $\eta_P^2 = .09$, but no

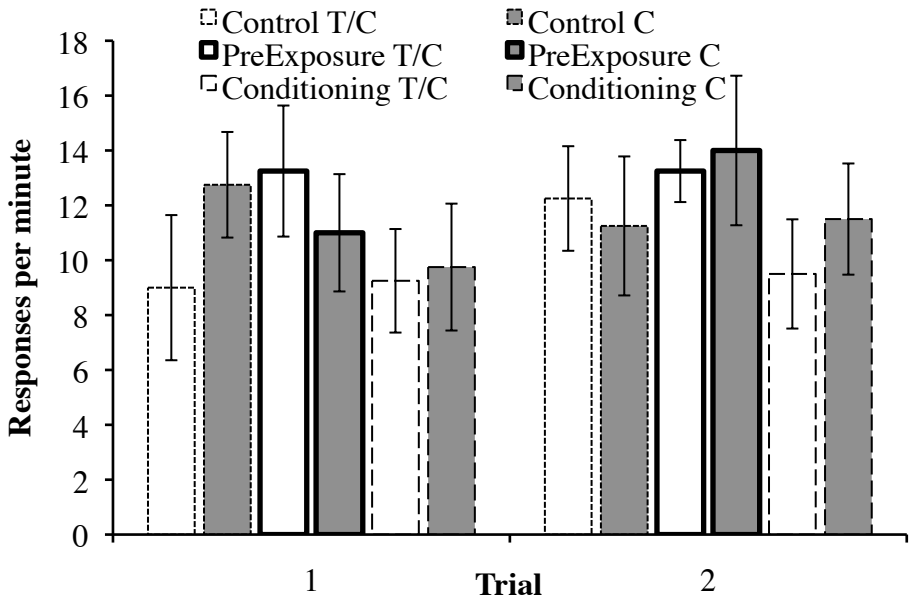


Figure 51. Responses per minute during the pre-CS period of clicker trials. Error bars represent one standard error of the mean.

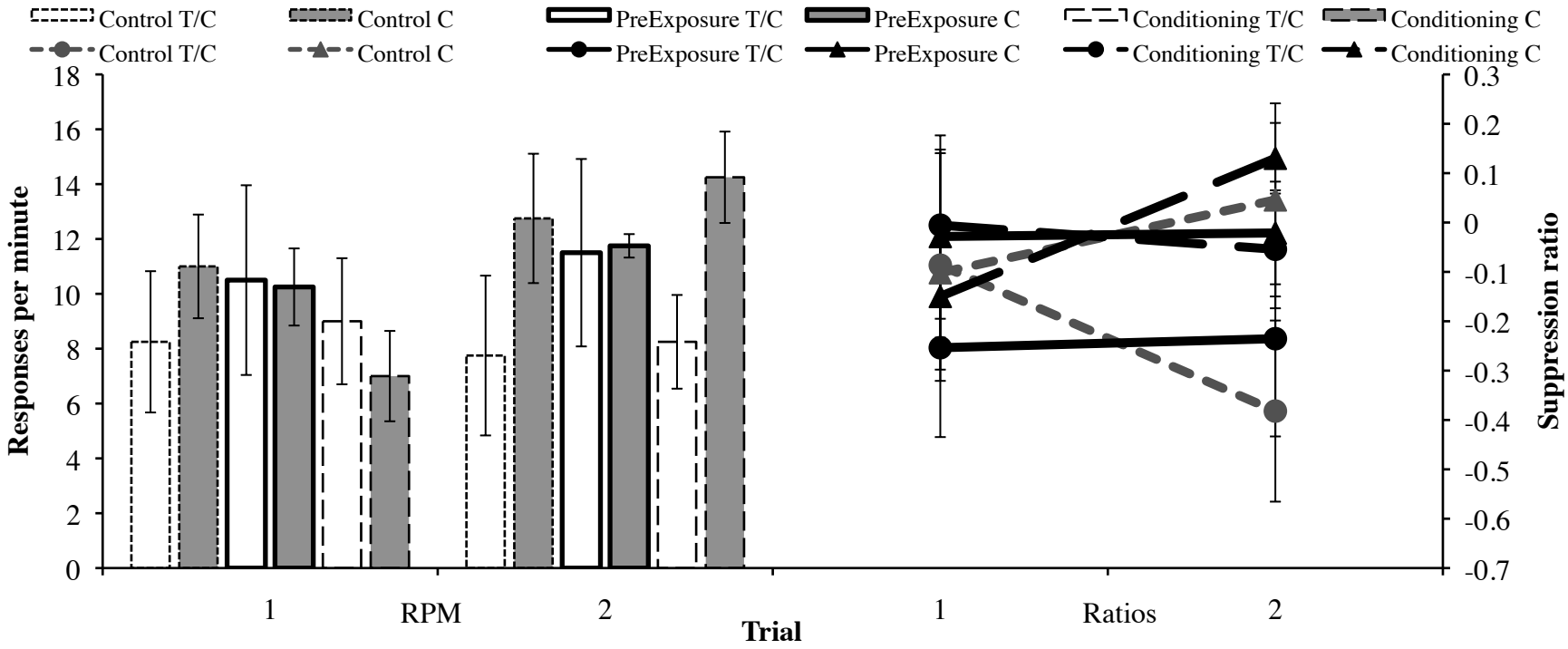


Figure 52. Responses per minute during the first two clicker trials in the test stage (shown by bar graph on the left side) and suppression ratios calculated from responses preceding and during the clicker, (C - Pre C / C + Pre C). Preexposure and conditioning refers to the groups that were given scopolamine during those stages. T/C refers to the preexposure, tone and clicker, C refers to clicker alone. Error bars represent one standard error of the mean.

Trial x Drug interaction, $F < 1$, $\eta_P^2 = .04$, and no three-way interaction, $F(2, 42) = 1.63$, $p = .207$, $\eta_P^2 = .07$.

The Trial x Preexposure interaction was examined using SMEs analyses. In C groups, responses were higher in the second trial than the first trial, $F(1, 42) = 7.80$, $p = .008$, $\eta_P^2 = .16$. Responses of the T/C groups did not differ over trials, $F < 1$, $\eta_P^2 < .01$. T/C and C groups did not differ at trial 1, $F < 1$, $\eta_P^2 < .01$, or trial 2, $F(1, 42) = 3.93$, $p = .054$, $\eta_P^2 = .09$

Ratios.

Suppression ratios indicated that groups responded similarly across trials (Figure 52). An ANOVA with a within-subjects factor of trial and between-subjects factors of drug and preexposure supported this description. There was no effect of trial, $F < 1$, $\eta_P^2 < .01$, preexposure, $F(1, 42) = 1.82$, $p = .184$, $\eta_P^2 = .04$, or drug, $F < 1$, $\eta_P^2 = .02$. There was no Preexposure x Drug interaction, $F < 1$, $\eta_P^2 = .02$. There was no Trial x Preexposure interaction, $F(1, 42) = 2.76$, $p = .104$, $\eta_P^2 = .06$, no Trial x Drug, $F < 1$, $\eta_P^2 = .02$, and no three-way interaction, $F < 1$, $\eta_P^2 = .04$. The intercept indicated that overall, ratios were not significantly different from zero, $F(1, 42) = 2.99$, $p = .091$, $\eta_P^2 = .07$.

Overall, these results do not confirm the expected pattern. While the control group may show a slight indication of higher responding by the C group than the T/C group, the difference was not large enough to be seen in the statistics.

Tone test.

The day after the test of clicker trials, rats were exposed to trials with the conditioned stimulus, the tone.

Pre-CS responses.

Responding during the pre-CS period was similar between groups (Figure 53). An ANOVA with trial, preexposure (T/C or C) and drug (control, preexposure or conditioning) partially supported this description. There was no effect of trial, $F(1, 42) = 1.52$, $p = .224$, $\eta_P^2 = .04$, no effect of preexposure, $F < 1$, $\eta_P^2 < .01$, and no effect of drug, $F < 1$, $\eta_P^2 < .01$. There was no interaction between preexposure and drug, $F < 1$, $\eta_P^2 = .04$. There was a Trial x Preexposure interaction, $F(1, 42) = 6.27$, $p = .016$, $\eta_P^2 = .13$, and a Trial x Drug interaction, $F(2, 42) = 3.67$, $p = .034$, $\eta_P^2 = .15$. There was no three-way interaction, $F < 1$, $\eta_P^2 = .04$.

The Trial x Preexposure interaction was examined using SME analysis. These revealed that the C groups decreased responding from trial one to trial two, $F(1, 42) = 6.98$, $p = .012$, $\eta_P^2 = .14$, the T/C groups' responding did not differ over trials, $F < 1$, $\eta_P^2 = .02$. There was a difference between group T/C and group C at trial two, $F(1, 42) = 4.56$, $p = .039$, $\eta_P^2 = .10$, but not at trial one, $F(1, 42) = 1.25$, $p = .269$, $\eta_P^2 = .03$.

SME analysis on the Trial x Drug interaction revealed that the control group decreased responding in trial two from trial one, $F(1, 42) = 7.39$, $p = .010$, $\eta_P^2 = .15$, there was no difference in responding between trials 1 and 2 in the preexposure group, $F < 1$, $\eta_P^2 < .01$, or in the conditioning group, $F(1, 42) = 1.21$, $p = .278$, $\eta_P^2 = .03$. There was no

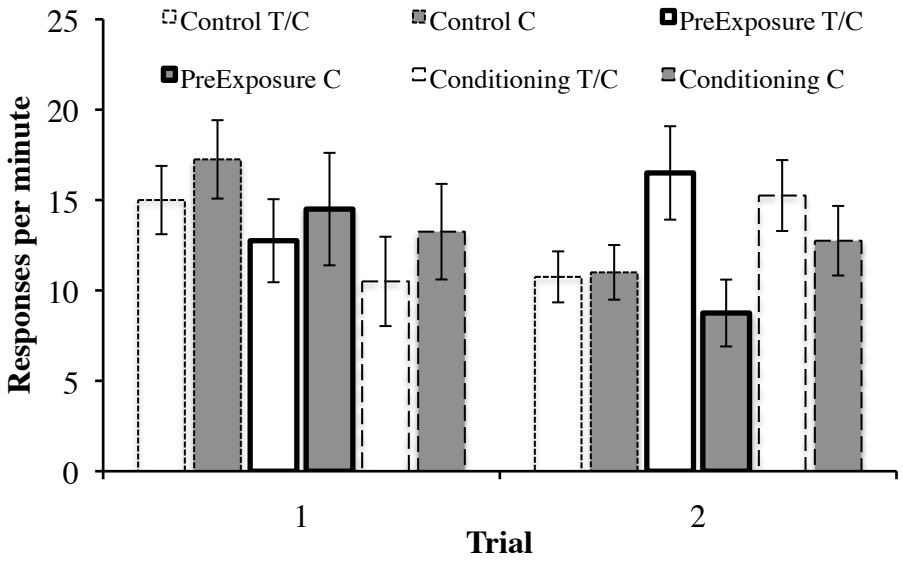


Figure 53. Responses per minute during the pre-CS period of the tone trials. Error bars represent one standard error of the mean.

difference between groups at trial one, $F(2, 42) = 1.51$, $p = .233$, $\eta_P^2 = .07$, or at trial two, $F(2, 42) = 1.34$, $p = .272$, $\eta_P^2 = .06$.

CS responses per minute.

Responding was fairly low during the first tone trial in all groups but group conditioning T/C (Figure 54). During the second trial, responding generally increased. An ANOVA of responses with drug and preexposure showed that there was a main effect of trial, $F(1, 42) = 8.98$, $p = .005$, $\eta_P^2 = .18$, and of drug, $F(2, 42) = 11.90$, $p < .001$, $\eta_P^2 = .36$. There was no effect of preexposure, $F(1, 42) = 1.97$, $p = .168$, $\eta_P^2 = .05$, no Preexposure x Drug interaction, $F < 1$, $\eta_P^2 < .01$. There was a Trial x Drug interaction, $F(2, 42) = 6.25$, $p = .028$, $\eta_P^2 = .16$, but no Trial x Preexposure interaction, $F(1, 42) = 2.18$, $p = .147$, $\eta_P^2 = .05$. There was a significant three-way interaction, $F(2, 42) = 6.25$, $p = .004$, $\eta_P^2 = .23$.

The three-way interaction was examined using simple interaction effects. These revealed that both prexposure groups and group conditioning C increased, but group conditioning T/C decreased, responding from the first trial to the second trial, Fs$(1, 42) > 5.66$, $p < .022$, $\eta_P^2 > .11$. The control groups' responding was similar across trials, Fs$(1, 42) < 3.09$, $p > .086$, $\eta_P^2 < .07$. Conditioning group T/C had a higher level of responding than group conditioning C in trial 1, $F(1, 42) = 8.93$, $p = .005$, $\eta_P^2 = .18$. Responding did not differ between preexposure groups for any other drug group at any trial, lowest $p = .312$. There was a difference in responding in the drug groups that were preexposed to C at trial 2, $F(2, 42) = 5.51$, $p = .008$; group conditioning C responded more than group control C, $p = .002$, $\eta_P^2 = .21$, no other group comparisons were significant, lowest p

= .066. There were no drug group differences of those rats that were preexposed to C in trial 1, $F(2, 42) = 2.17$, $p = .127$, $\eta_P^2 = .09$. There was a drug group difference in responding in trial 1 in those rats preexposed to T/C, $F(2, 42) = 14.11$, $p < .001$, $\eta_P^2 = .40$; group conditioning T/C responded more than group control T/C, $p < .001$, and group preexposure T/C, $p < .001$, which did not differ, $p = .879$. There were no drug group differences of responding in those animals preexposed to T/C in trial 2, $F < 1$, $\eta_P^2 = .05$.

Ratios.

Suppression ratios indicated that suppression decreased from trial one to trial two and that groups responded similarly across trials, except the conditioning groups (Figure 54). An ANOVA of trials, with drug and preexposure as between-subject variables, partially supported this description. There was a significant effect of trial, $F(1, 42) = 19.79$, $p <$. 001, $\eta_P^2 = .32$, of preexposure, $F(1, 42) = 4.50$, $p = .040$, $\eta_P^2 = .10$, drug, $F(2, 42) = 19.13$, $p < .001$, $\eta_P^2 = .48$. There was no Preexposure x Drug interaction, $F < 1$, $\eta_P^2 = .03$. There was a significant Trial x Drug interaction, $F(2, 42) = 6.53$, $p = .003$, $\eta_P^2 = .24$, but no Trial x Preexposure interaction, $F(1, 42) = 1.70$, $p = .199$, $\eta_P^2 = .04$. There was a significant three-way interaction, $F(2, 42) = 5.66$, $p = .007$, $\eta_P^2 = .21$. The intercept indicated that ratios across groups were significantly different from zero, $F(1, 42) = 173.22$, $p < .001$, $\eta_P^2 = .81$.

The three-way interaction was examined using simple interaction effects analyses. These revealed that group conditioning T/C increased, but that groups conditioning T/C and C, preexposure T/C and C, and control

T/C) decreased, suppression from trial one to trial two, all Fs(1, 42) > 4.87, ps < .033, η_P^2 > .10. Suppression ratios of group control C were similar across trials, F(1, 42) = 2.05, p = .159, η_P^2 = .05. Conditioning group T/C showed less suppression than group conditioning C in trial 1, F(1, 42) = 17.88, p < .001, η_P^2 = .30. Responding did not differ between preexposure type for any other drug group at any trial, lowest p = .119. There was a difference in suppression in the drug groups that were preexposed to C at trial 1, F(2, 42) = 6.14, p = .004, η_P^2 = .23; group conditioning C was less suppressed than group control C, p = .002, and group preexposure C, p = .005, which did not differ, p = .783. There was also a drug group difference of those rats that were preexposed to C in trial 2, F(2, 42) = 4.53, p = .027, η_P^2 = .18; group conditioning C was less suppressed than group control C, p = .004. There were no other group differences in trial 2, lowest p = .106. There was a drug group difference in responding in trial 1 in those rats preexposed to T/C, F(2, 42) = 31.89, p < .001, η_P^2 = .60; group conditioning T/C was less suppressed than group control T/C, p < .001, and group preexposure T/C, p < .001, which did not differ, p = .773. There were no drug group differences of responding in those animals preexposed to T/C in trial 2, F < 1, η_P^2 = .03.

Overall, these results indicate that conditioning was effective for most groups; however, there may be some concerns with regards to the higher level of responding in conditioning T/C group.

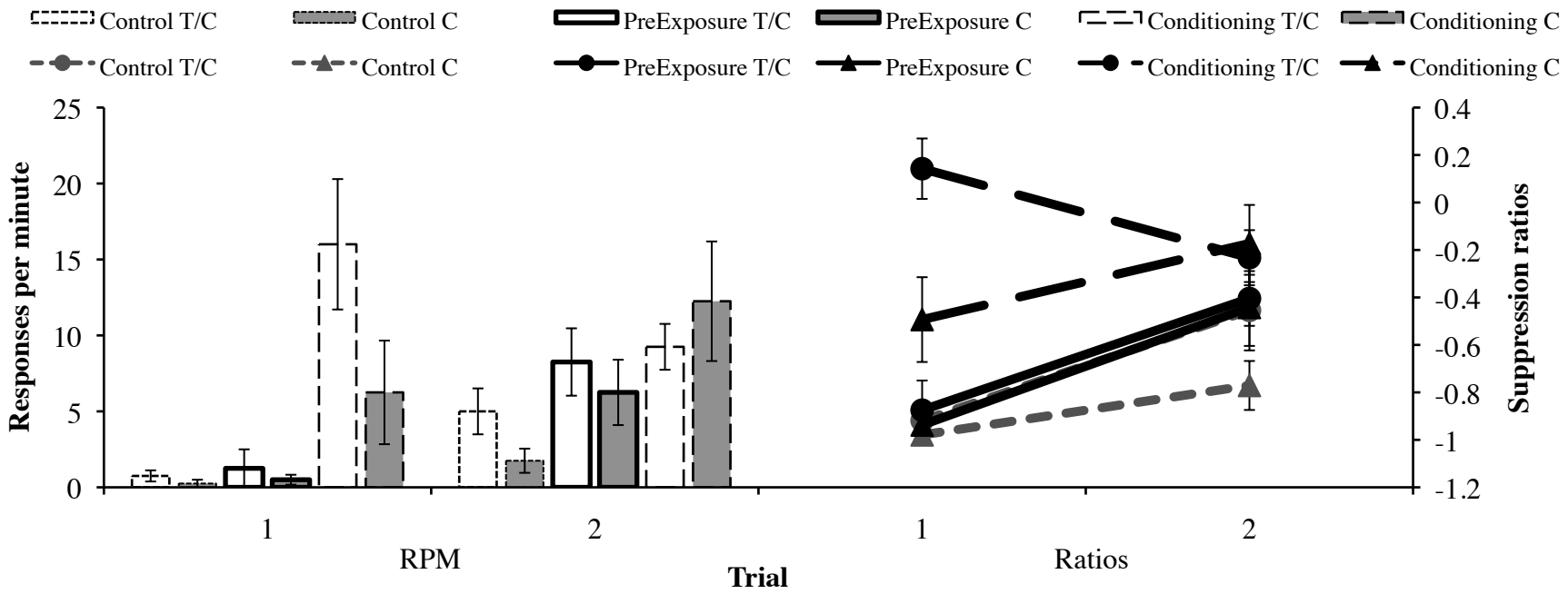


Figure 54. Responses per minute and standard error of the mean, during the first two tone trials in the test stage (shown by bar graph on the left side) and suppression ratios calculated from responses preceding and during the tone, (T - Pre T / T + Pre T). Preexposure and conditioning refers to the groups that were given scopolamine during those stages. T/C refers to the preexposure, tone and clicker, C refers to clicker alone.

4.5.3 General discussion.

The aim of the present experiment was to replicate Experiments 11 and 12, to investigate the effects of scopolamine on familiarity generalisation. Scopolamine was administered before the preexposure stage and before the conditioning stage. The control groups T/C and C tended towards differences in responding; but as this was not supported by the statistical analysis other comparisons can only allow tentative conclusions. Groups T/C and C, which were administered scopolamine before conditioning, also showed similar responses in the test stage. This might be due to scopolamine diminishing familiarity generalisation. However, analyses showed that scopolamine administration might have disrupted conditioning in the conditioning groups. Results from the preexposure T/C and C groups were also unclear. Group preexposure T/C seemed to show more suppression than group preexposure C, though this was not revealed in the analysis. This disparity was reported in Experiment 11. However, it is difficult to interpret these findings without a clear result in the control groups.

These results may be so variable because the rats were in different drug stages across stages of the experiment. There may be effects of state dependent learning. Performance in a test may be impaired if the animal is in a different drug state to that it was in when the response was established (Overton, 1984). In the present experiment, this may be seen in the conditioning groups; animals were in a drug state when the shock was presented, but were drug free in the test stage. In Experiments 11, 12, and 13, all groups were tested without any drug administration, whereas, in

previous stages subjects had been drugged. However, this is difficult to resolve without using a different procedure. Rats could not be given drugs during the test stage, as there might be very little responding due to the peripheral effects of the drug. Another way of measuring results could be to video record freezing behaviour. Rats would not need any lever training if this method were used.

In Experiments 11, 12, and 13, preexposure lasted for six sessions, so there is a chance that the rats developed some tolerance to the effects of the scopolamine. In a study using monkeys, Aigner et al. (1987) limited scopolamine doses to a maximum of two injections per week, and reported that five out of seven subjects showed evidence of developing tolerance to the effects of scopolamine. Doses in my experiments were much closer together. Even in Experiment 13, which was the experiment that had least consecutive treatment sessions, rats in the preexposure groups received six injections within nine days. However, spacing the preexposure to give a maximum of two injections per week would mean that the preexposure stage would last three weeks. This may mean that dishabituation of the UR may occur.

In a recent review of the validity of scopolamine as a model for cognitive impairment, Klinkenberg and Blokland (2010) pointed out that scopolamine can have peripheral effects on attention and discrimination. Researchers need to ensure impairments in cognitive processes are due to the central actions of scopolamine and not these peripheral effects. Using methylscopolamine may control for the peripheral effects; however, methylyscoplamine can affect performance in discrimination tasks (e.g.,

Herremans, Hijzen, Olivier, & Slangen, 1995). Klinkenberg and Blokland suggest using antagonists that select for just one of the five subtypes of muscarinic receptors, M1 receptors. This may reduce peripheral effects to give a more effective way to induce cognitive deficits. Future research with the generalisation procedure could employ this selective antagonist, and this may reduce the peripheral effects of scopolamine.

4.6 Chapter discussion

In this chapter, I examined enhancement of generalisation among familiar stimuli and tested whether it was affected by perirhinal lesions and scopolamine administration. These manipulations affect discrimination in recognition memory tasks, so it was likely they might have a similar affect on familiarity generalisation. The experiments presented in this chapter showed some support for this (Experiments 9, 10), but others were inconclusive (Experiments 11, 12, 13).

Experiment 9 supported the main predictions that generalisation is enhanced between two stimuli that are both familiar or both novel (see Honey, 1990). Experiment 9 also allayed concerns of sensory preconditioning (Hall, 2001); results showed that the length of the ITI between trials did not make a difference to the enhancement of generalisation in the T/C groups.

Results from Experiment 10 suggested that lesions of the perirhinal cortex might have disrupted familiarity generalisation, although results were not significant. This finding parallels research that reported that perirhinal lesions affected object recognition (e.g., Albasser et al., 2009;

Norman & Eacott, 2005). This may suggest there is a common process in these tasks, probably located in perirhinal cortex.

There were no significant findings from Experiments 11, 12, and 13. Results from the control groups did not replicate those seen in Experiment 9. It was, therefore, difficult to interpret results of the experimental groups. The differences in stages may have affected performance; the preexposure and conditioning stage were conducted off baseline, therefore the test was quite different as rats were presented with levers and food. The systemic administration of scopolamine may have too many peripheral effects. Infusion of scopolamine into perirhinal cortex (Warburton et al., 2003) may reduce these peripheral effects and make central effects (e.g., amnesia) more apparent.

The mixed results of control groups across generalisation experiments may be of concern. There was no indication of enhanced familiarity generalisation in Experiment 11, in which control groups received methylscopolamine before preexposure. This may be due to methylscopolamine having vigorous peripheral effects on subjects. In the test, the animals' drug state may have been so different from the preexposure stage that there was no transfer of learning (see Overton, 1984). Klinkenberg and Blokland (2010) urged caution when using methylscopolamine as a control for peripheral effects as it can affect rats' performance in discrimination tasks (Herremans et al., 1995; van Haaren & van Hest, 1989). This detrimental effect may be due to state dependency (Ennaceur & Meliani, 1992). Discrimination of novelty/familiarity by rats

in the present experiments may have been attenuated by these actions of methylscopolamine.

The elemental theory for stimulus generalisation, proposed by McLaren and Mackintosh (2002), seems to be the most fitting for familiarity generalisation. This theory would need to be modified to accept that familiarity may be represented by stimulus elements. Generalisation may occur between stimuli that share familiarity elements. A CR established to one familiar stimulus may be elicited by another familiar stimulus (see, Best & Batson, 1977; Honey 1990). McLaren and Mackintosh, however, predicted a decrease in generalisation when stimuli were familiar because latent inhibition may reduce the associability of the familiar stimulus. Latent inhibition could not explain results from Experiment 9 and 10, and so give support for enhanced generalisation among familiar stimuli as reported by Honey (1990).

The representation-hierarchical model (Cowell et al., 2010a, 2010b) would have difficulty explaining results seen in this chapter. It is a model based on visual representations, and as the present experiments used auditory stimuli it may not be able to account for the results reported here, even if it were extended to include auditory stimuli. Results, although not significant, may suggest that perirhinal cortex might be important for representing abstract features of stimuli such as familiarity/novelty. This is supported by findings that showed more neuronal activation in perirhinal cortex for novel stimuli than for familiar stimuli (Wan et al., 1999). The representational-hierarchical model does not account for these

psychological processes, and so is not a fitting model for the current findings.

The aim of the experiments presented in this chapter was to investigate enhancement of generalisation among stimuli that were familiar. An elemental model of generalisation seemed most fitting to explain results; the results suggested that familiarity might be represented among stimulus elements. The representational-hierarchical model was insufficient to explain the results, as it does not account for the psychological concept of familiarity.

Chapter 5. General Discussion

My aim in this thesis was to examine the possibility that associative learning processes may contribute to recognition. If this were the case, I aimed to specify those processes. I presented experiments that gave some support for object recognition involving associative processes. A familiarity generalisation procedure was also used to demonstrate further effects of associative processes in recognition memory; this procedure had not previously been used to test recognition memory.

5.1 Summary of findings

5.1.1 Priming in object recognition.

In Chapter 2, I presented experiments that were designed to test discrimination of objects when subjects' memory of them had been primed. This aimed to demonstrate that pairing stimuli might result in associations between stimuli that would influence later discrimination. Experiment 1 demonstrated that my apparatus and general procedure were effective for allowing successful discrimination between novel and familiar stimuli. The results showed that rats were able to discriminate between novel and familiar objects, and contexts. This supports findings in the extant literature (e.g. Berlyne, 1950; Dember, 1956; Ennaceur & Delacour, 1988). This discrimination between novel and familiar stimuli may be due to the difference in activation of stimulus elements (e.g., Wagner, 1981); the familiar stimulus will be in A2 and the novel stimulus in A1. The presentation of novel stimuli activates those elements into A1, resulting in more exploratory behaviour toward those stimuli. Familiar stimuli are primed by previous presentation (self-generated), or by an association with

another stimulus, for example the context (retrieval-generated), so their elements will be in A2 on test. These differences in activation result in the discriminations seen in object recognition experiments.

Extending this application, Experiment 2 included familiarisation stages to confirm that self-generated priming mechanisms were effective in producing discrimination of stimuli, even when stimuli had been preexposed. Rats still showed discrimination between a stimulus that was recently presented (primed to A2) and a stimulus that was presented not so recently. These results are also attributable to a retrieval-generated priming explanation; presentation of P in stage 2 may have led to stronger associations made with the context for P. In the test stage, this stronger association primed more of P's elements to A2, whereas there may have been more of Q's elements in A1. This discrimination was found using objects and a combination of objects and contexts. This indicated that the parameters set out in this experiment were appropriate for the following experiments.

In Experiment 3 and 4, I examined possible associative activation of stimulus representations, and tested discrimination of a primed stimulus versus a non-primed stimulus. Results showed that rats spent more time with the stimulus that was not primed. These results indicate the possibility that associative processes were active in this type of task. The use of an object task is an important development in the literature. My findings support those of Honey and Good (2000), who found similar results in an orienting response experiment, and Sanderson and Bannerman (2011), who reported similar findings with mice in a spatial task. These findings suggest

that subjects were sensitive to the co-occurrence of two stimuli, which may have become associated, and that these associations affected later behaviour. This seems to be a reliable finding across different experimental paradigms.

5.1.2 The spacing effect in object recognition.

In Chapter 3, I presented results from a set of experiments that examined rats' discrimination of novel and familiar visual stimuli, and tested if this discrimination was affected by the spacing of the stimulus exposures. Experiment 5 was designed as a visual version of an object recognition experiment. I found that rats still exhibited discrimination between familiar and novel stimuli. This indicated that rats were able to process visual stimuli in order to make later discriminations. These results supported those of Forwood et al. (2007), who also used two-dimensional visual stimuli in a recognition task. The findings from Experiment 5 enabled me to conduct the spacing experiments with confidence that rats could discriminate the stimuli.

Experiments 6 and 7 tested effects of presenting stimuli with a short interval (massed) or with a long interval (spaced). The difference between Experiments 6 and 7 was the stimuli used. Experiment 6 used insert stimuli; while these were designed to be visual stimuli, it was possible that rats could sample them in the interval between presentations using the texture. Experiment 7, therefore, was designed to resolve this issue by screening the stimuli in transparent vases. In the intervals, both stimuli would feel the same, and only visual cues could be used to discriminate between them. Results indicated that discrimination between familiar and

novel stimuli was generally longer lasting if the stimuli were spaced. These findings supported other research that reported the beneficial effect of spaced training (e.g., Davis, 1970; Sanderson & Bannerman, 2011; Sunsay & Bouton, 2008). My results also support those of Anderson et al. (2008), who reported that the spacing effect was apparent in an object recognition experiment. My experiments resolved methodological issues that Anderson et al.'s procedure faced (e.g., handling, single trial or multiple trials).

The SOP model (Wagner, 1976, 1981) predicts that spacing stimulus presentations will result in better learning, and thus better memory. In massed exposure, there is not enough time for elements to decay and reactivate before the next stimulus presentation. The elements relating to that stimulus remain in A2, and cannot be reactivated into A1, resulting in less behaviour. With spaced exposure, there is more time for the stimulus's elements to decay to inactive, allowing better reactivation to A1. This process may only be operational at short delays. This may also reflect a retrieval-generated process; in spaced exposure, there is more time for the object to be associated with the context. This leads to better priming in the test. This cannot occur with massed exposures because the next stimulus presentation restricts activation of elements to A1 and so limits associations forming between the context and the object.

The results from Experiments 6 and 7 could have been due to self-generated or retrieval-generated processes. With a ten-minute delay between the sample and test stage, it was difficult to discriminate which of these was operational. Experiment 8 was designed to test the effect of shortening or lengthening the delay between the sample and the test stage.

Results indicated that at short delays, rats showed good discrimination in both massed and spaced conditions. At a longer delay, this discrimination was much reduced. There was a trend toward better discrimination when stimulus exposures were spaced, as opposed to massed. Short delays seem to overcome the spacing effect, which may indicate that self-generated processes were active, and may be based on the last stimulus presentation. These results support those of Sanderson and Bannerman (2011) who reported greater short-term habituation with a short interval (1 minute) between training and the test.

I predicted that at long delays, retrieval-generated priming would be optimal. Over the delay, activation of stimulus elements would have decayed to inactive, meaning there would be no discrimination in the test with self-generated priming. For the familiar stimulus to be activated to A2 and the novel stimulus to be activated to A1, a prior association between the context and the object would be needed. After a delay, the context would activate the familiar stimulus's elements to A2. This association would be best formed through spaced presentations of stimuli (Wagner, 1976). Results tended toward this effect, though were not significant.

5.1.3 Familiarity generalisation.

ITIs and the action of lesions to perirhinal cortex.

Chapters 3 and 4 demonstrated that associative processes might be involved in recognition memory. The aim of Chapter 5 was similar, but incorporated a different task. The task examined rats' generalisation of conditioned suppression. All rats were preexposed to the test stimulus. Preexposure to the conditioned stimulus differed: rats were either

preexposed or not preexposed to the conditioned stimulus. Experiment 9 showed that rats' suppression was greater when the conditioned stimulus was preexposed. This may be due to the familiarity of the stimuli. For one group, the conditioned and test stimulus were both familiar. For the other group, the conditioned stimulus was novel and the test stimulus was familiar. Rats'generalisation of conditioned response was enhanced when the conditioned stimulus and the test stimulus were both familiar. These results support those of Honey (1990) who showed that generalisation is more apparent between stimuli that are matched in terms of familiarity or novelty. A further aim of this experiment was to test the inter-trial interval in groups preexposed to both the conditioned and the test stimuli. This was tested because a short ITI may lead to stronger associations between the preexposed stimuli (T and C) compared to a longer ITI. In the test stage, generalisation between the conditioned (C) and test (T) stimulus may be due to a T - C - US association (see, Hall, 2001). The duration of the inter-trial interval did not affect the enhancement of generalisation suggesting that within-event learning was not apparent.

Generalisation can be explained using elemental associative theory (McLaren & Mackintosh, 2002). When two stimuli have common elements, generalisation between them may be enhanced. This theory may apply to familiarity generalisation, if it is accepted that familiarity may be represented by elements. My results suggest that with many preexposure trials, animals seem to become familiar with the stimuli. Bennett et al.'s experiment (1994) had few preexposure trials, meaning that familiarity may not have become apparent. The results from my experiments provide

support for familiarity generalisation as reported by Best and Batson (1977) and Honey (1990).

This procedure was used to test performance of rats with lesions to the perirhinal cortex (Experiment 10). Results were not significant but there was a suggestion that unlike sham animals, the lesion groups showed no differences in suppression between rats that were preexposed to both the conditioned stimulus and the test stimulus or to the test stimulus alone. In other words, there was no enhancement of generalisation between familiar stimuli. The lesion seemed to affect the familiarity or novelty elements of the stimulus, meaning that elements may not have encoded, or been recalled, sufficiently. This result parallels numerous studies that reported deficits in recognition memory of subjects with perirhinal lesions (e.g., Aggleton et al., 2010; Albasser et al., 2009; Mumby et al., 2007).

Actions of an anticholinergic drug (scopolamine).

In Experiments 11, 12, and 13, I examined the effects of scopolamine administration on generalisation. In Experiment 11, scopolamine was administered before preexposure sessions; in Experiment 12, scopolamine was administered before conditioning sessions. Results from these experiments were inconclusive, so Experiment 13 was run to replicate drug treatments of Experiment 11, 12, along with a control group.

Results from all these experiments were difficult to interpret, as the performance of the control groups was not clear, and did not replicate results seen in Experiments 9 and 10. There was some indication of the generalisation enhancement between the conditioned and test stimulus in the control group in Experiment 13, but this did not reach significant levels.

Administering scopolamine before conditioning sessions seemed to affect conditioning, even at the small doses used. The scopolamine may have affected stimulus processing by acting on the basal forebrain cholinergic system, the scopolamine decreases the processing that the tone receives (Young, Bohenek, & Fanselow, 1995) and so the tone-shock association would be weak. There were conflicting results concerning administration of scopolamine before preexposure. In Experiment 11, performance of rats given scopolamine was comparable to non-drugged rats in Experiment 9; however, scopolamine seemed to affect suppression in Experiment 13.

These results were unexpected, since previous reports suggested that scopolamine affected object recognition when administered before the sample stage (e.g., Dere et al., 2007; Winters et al., 2006). I administered scopolamine before appropriate stages of the generalisation experiment, preexposure and conditioning stages. It was not possible to administer scopolamine before the test, as there may have been little responding due to decreased appetite (Huston & Aggleton, 1987). The test could have been conducted on a lever baseline but without reinforcement; however, rats may have stopped pressing levers once there was no reward. This also relates to issues of state dependency (see, Overton, 1984); the test was always conducted drug-free and with levers, so it could be that behaviour was affected because the learning state and the test state were not identical. One possible way to resolve these issues would be to record freezing behaviour instead of an instrumental response. It may then possible to administer methylscopolamine in the test to imitate the peripheral actions of scopolamine but not affect memory retrieval.

5.2 Implications and future research

5.2.1 Object recognition.

Results from Chapter 2 suggest that exploratory behaviour toward objects may involve differences in stimulus activation, particularly when one stimulus is in A1 and another is in A2. These activation states can result from self-generated priming, as demonstrated in Experiment 1, and from retrieval-generated priming, as demonstrated by Experiments 3 and 4. These findings expand on those of Sanderson and Bannerman (2011), and provide support for the generalisability of these findings across experimental paradigms.

Discrimination between expected and unexpected stimuli has been reported elsewhere (e.g., Dix & Aggleton, 1999). For example, in the test stage of Dix and Aggleton's experiment (Figure 2), a context was exposed with both preexposed objects. The results showed that the rats explored the unexpected object more than the expected object. This, however, may not be due to retrieval-generated priming. In the test stage, the incongruent stimulus may have been perceived differently because it was presented in a new context (e.g., Lovibond, Preston, & Mackintosh, 1984). This difference in object perception may have led to the object seeming, at least partially, novel, and so have increased exploratory behaviour. In my experiments, the priming stimulus, X, was presented before the test. This prevented any generalisation decrement.

Only associative models make predictions concerning priming of stimuli (e.g., Wagner, 1976, 1981). Retrieval-generated priming accounts for the results. Presentation of X in stage 2 activates its associate object's

elements to A2. This A2 activation inhibits exploration in the test. The representational-hierarchical model (Cowell et al., 2010a, 2010b) could not explain these effects. The model assumes exploration of novel stimuli. Only stimuli that have been previously encountered are represented in the brain. If new stimuli are different from these representations, there is exploration of them. In my experiment, in the test stimuli are equated in terms of familiarity. According to this account, as the test stimuli have both been presented once in stage one they will both be represented in the brain. Thus at test, according to this theory, exploration of stimuli would be similar. This model may need to incorporate an explanation concerning consequences of pairing stimuli to accommodate these results.

Neural models (e.g., Brown & Aggleton, 2001) may also have difficulty with these results because of the presentation of stimuli in stage 1 of Experiment 3 and 4. This presentation may have made the rats familiar with those stimuli. This may mean that in the test stage, there would have been no familiarity/novelty signals. My results suggest that current models may need alteration or expansion.

Although results suggest involvement of retrieval-generated processes, self-generated priming may still be present. All stages were conducted in one session, and the duration between stages was 10 minutes. This may not have allowed for complete decay of elements. A longer delay (e.g., 24 hours) between stage one and stage two may encourage decay of elements from stage one, thus reducing the influence of self-generated priming. Priming in stage two would then be due mainly to retrieval-generated processes, and so activation of stimulus elements in the test may

be different, i.e., the primed stimulus would have mainly A2 activation, the unprimed stimulus elements would be activated to A1.

These experiments may be conducted using human participants, to enhance generalisability of priming processes. The task could be easily altered to a task in which participants view pictures of objects. Humans show a bias towards novel stimuli, as rats do. Fagan (1970) reported that infants paid more attention to novel stimuli in a visual task. Eye tracking may be used as an indirect way to measure attentional processes. This research may highlight self-generated and retrieval-generated processes in human memory.

Results from Chapter 3 suggested spaced presentation of stimuli enhanced discrimination between familiar and novel stimuli. These results were generally in line with predictions from SOP. Although results from Experiment 8 did not completely replicate other findings (e.g., Sanderson & Bannerman 2011), this was more likely to be to do with my procedure rather than being a contradiction to those experiments or theory. For example, my prediction that discrimination may be enhanced after spaced exposures (rather than massed) was not strongly supported. Future research with a longer ITI may help to confirm that prediction. For example, Sanderson and Bannerman (2011) used a 24-hour interval between stimulus presentations. This is considerably longer than the 4-minute interval I used here, and it is likely that a longer interval would enhance the spacing effect. The longer interval would give greater opportunity for the context to become associated with the object (Wagner, 1976).

Barela (1999) and Sunsay and Bouton (2008) both discussed the possibility of two mechanisms, one for short inter-trial intervals (ITIs), and one for longer ITIs. This idea also seems to apply to the delays between sample and test. Self-generated priming is most effective at short delays, whatever the ITI, whereas retrieval-generated processes are important at longer delays, once the activation of stimulus elements can no longer have an effect.

The representational-hierarchical model (Cowell et al., 2010a, 2010b) could not predict effects concerning spacing of stimuli; however, it does predict delay effects. With a long delay between sample and test, there would be many interfering visual items; these would reduce the subjects' memory of the item they were presented with in the sample stage. In the test, the stimuli may now have features in common with the familiar stimulus and the interfering stimuli, so recognition would be impaired. This was partially supported by my results; although there were no significant differences in discrimination between short and long delay groups, with a short delay rats' discrimination of the novel object was significant, but was not with a long delay. The proposal that interfering stimuli during a delay may affect stimulus recognition could not be applied to the ITI, however. During the ITI, lights were extinguished to eliminate visual examination of stimuli, meaning that rats could not view extraneous stimuli that may have interfered with the representation of the sample object. This again suggests that the RH model needs some alteration to explain non-lesion recognition effects.

Results supported those of Chapter 3, suggesting that there does seem to be a suggestion of associative processes in these memory tests. Both these chapters use object recognition. This is an important addition to the literature as this method is widely used in animal research to test memory.

5.2.2 Familiarity generalisation.

I regard my results from Chapter 4 (Experiments 9 and 10) as demonstrating enhanced generalisation between stimuli that shared familiarity elements. McLaren and Mackintosh (2000, 2002) suggested that stimuli may share common elements, and that generalisation may be enhanced between stimuli that share common elements. My results suggest that this theory should be expanded to include psychological concepts, such as familiarity. The findings also have implications for Hall's (2001) predictions, that generalisation may be due to sensory preconditioning. More specifically, in the preexposure stage, T and C may become associated. Shorter ITIs may have enhanced this association. However, results from Experiment 9 did not support these predictions, results showed that generalisation was similar between groups. However, Hall's concerns may still apply because, although it is unlikely, the ITIs that were used may have still allowed associations between T and C resulting in the enhanced generalisation that was seen in the T/C groups. To test this further, ITIs longer than an average of 420 s could be used. This may reduce generalisation if Hall's theory was correct.

The generalisation procedure provided a novel way to examine familiarity processing. There was an indication that rats with perirhinal

lesions may have been impaired in this task. This impairment may not be due to the representation of the stimulus since the representational-hierarchical account (Cowell et al., 2010a, 2010b) would have difficulty predicting results seen in Experiment 10. Firstly, Cowell et al.'s account is based on visual processing rather than auditory stimuli; and secondly, familiarity generalisation requires the subject to have some concept of stimuli being familiar or novel (Honey, 1990). This psychological construct is not included in the representational-hierarchical model, and so it is inadequate to explain my results.

Results from Experiment 10 indicate the role of auditory stimuli in familiarity/novelty discrimination. Previous studies with auditory stimuli and subjects with perirhinal lesions (e.g., Kowalska et al., 2001; Wan et al., 2001) did not report any performance deficits in subjects with perirhinal lesions. Kowalska et al. (2001) reported that subjects with perirhinal lesions performed similarly to controls in an auditory delayed match-to-sample task. In Wan et al.'s study, perirhinal cortex neurons did not differentially activate to novel and familiar sounds. They suggest that the sounds used were processed at the levels of the individual features, rather than as a complex configuration. In the present experiment, I used a tone and a clicker, stimuli that do not seem complex. Following Wan et al.'s suggestion, there might be no involvement of perirhinal cortex in my experiment. My results dispute this. One possible explanation for this discrepancy is that the auditory stimuli in Wan et al.'s experiment were very short (3 s) compared to 30 s, and multiple trials, in my experiment. In

Wan et al.'s study, there may not have been enough time for stimuli to become familiar.

Experiment 10 provided some suggestion that perirhinal lesions affected familiarity generalisation. This procedure could be used to test other brain regions that are involved in recognition memory. For example, Squire, Wixted, and Clark (2007) concluded, from a review of the literature, that familiarity signals were apparent in hippocampus as well as perirhinal cortex. Using the familiarity generalisation procedure may provide further evidence for this. If the hippocampus is implicated in familiarity processing, lesions in that region may have similar effects to perirhinal lesions, i.e., no enhancement of generalisation between familiar stimuli.

The results from the familiarity generalisation task were generally reliable; it was only in the drug experiments that the expected pattern of results altered. Findings from the scopolamine experiments may differ from those of the perirhinal lesion experiment because the drug effects were temporary, and may have altered over the course of the session (i.e., effects may have worn off whilst the animal was in the apparatus, or they developed drug tolerance over sessions). The drug experiments were conducted off baseline until the test session, meaning that there were problems of state dependency. Lesion effects were more permanent and present throughout the experiment so do not suffer these limitations. However, with lesions it was difficult to know which stage was important for the detriment in performance.

Considering the problems in the scopolamine studies, improvements to that method are required. The scopolamine dosage used may have been too high. This seems highly likely in view of the findings that scopolamine administration may have affected conditioning (Experiment 12). In future the dose could be reduced, this may help to minimise peripheral effects. Another way to do this is to use M1 receptor antagonists in place of scopolamine (Klinkenberg & Blokland, 2010). M1 receptors are mainly found in the brain (Caulfield, 1993), so antagonists may only affect cognitive processes.

Another area of investigation manipulating the cholinergic system may explore enhancing memory. Physostigmine is a cholinesterase inhibitor, meaning that choline levels in the postsynaptic neuron remain high. Its administration is reported to enhance memory (Aigner & Mishkin, 1986; Davis et al., 1978). This may lead to better discrimination between familiar and novel items, and so perhaps enhance generalisation further.

5.3 Conclusion

In this thesis, I suggested that recognition memory (discrimination between familiar and novel stimuli) might involve associative processes. Associative processes (e.g., Wagner, 1976, 1981) are likely to drive priming and spacing effects in object recognition. Familiarity processing was also apparent in generalisation. Generalisation was enhanced between familiar stimuli an effect that can be explained by an associative elemental theory (McLaren & Mackintosh, 2002), as long as familiarity could be represented by elements. These findings lead me to conclude that the psychological processes of detecting familiarity/novelty reflect associative

processes. Future research is needed to further support and extend these findings.

References

Aggleton, J. P., Albasser, M. M., Aggleton, D. J., Poirier, G. L., & Pearce, J. M. (2010). Lesions of the rat perirhinal cortex spare the acquisition of a complex configural visual discrimination yet impair object recognition. *Behavioral Neuroscience, 124*, 55-68.

Aigner, T. G., Mishkin, M. (1986). The effects of physostigmine and scopolamine on recognition memory in monkeys. *Behavioral and Neural Biology, 45*, 81-87.

Aigner, T. G., Mitchell, S. J., Aggleton, J. P., DeLong, M. R., Struble, R. G., Price, D. L, … Mishkin, M. (1987). Effects of scopolamine and physostigmine on recognition memory in monkeys with ibotenic-acid lesions of the nucleus basalis of Meynert. *Psychopharmacology, 92*, 292-300.

Aigner, T. G., Walker, D. L., & Mishkin, M. (1991). Comparison of the effects of scopolamine administered before and after acquisition in a test of visual recognition memory in monkeys. *Behavioral and Neural Biology, 55*, 61-67.

Albasser, M. M., Amin, E., Iordanova, M. D., Brown, M. W., Pearce, J. M., & Aggleton, J. P. (2011). Perirhinal lesions uncover subsidiary systems in the rat for the detection of novel and familiar objects. *European Journal of Neuroscience, 34*, 331-342.

Albasser, M. M., Davies, M., Futter J. E., & Aggleton, J. P. (2009). Magnitude of the object recognition deficit associated with perirhinal cortex damage in rats: Effects of varying the lesion extent and the duration of the sample period. *Behavioural Neuroscience, 123*, 115-124.

Alonso, G., & Hall, G. (1999). Stimulus comparison and stimulus association processes in the perceptual learning effect. *Behavioural Processes, 48*, 11-23.

Anagnostaras, S. G., Maren, S., Sage, J. R., Goodrich, S. G., & Fanselow, M. S. (1999). Scopolamine and Pavlovian fear conditioning in rats: Dose-effect analysis. *Neuropsychopharmacology, 21*, 731-744.

Anderson, M. J., Jablonski, S. A., & Klimas, D. B. (2008). Spaced initial stimulus familiarization enhances novelty preference in Long-Evans rats. *Behavioural Processes, 78,* 481-486.

Atkinson, R. C., & Shiffrin, R. M. (1968). Human memory: A proposed system and its control processes. In K. W. Spence & J. T Spence (Eds.), *The Psychology of Learning and Motivation* (Vol. 2, pp. 90-195). New York; Academic Press.

Barela, P. B. (1999). Theoretical mechanisms underlying the trial-spacing effect in Pavlovian fear conditioning. *Journal of Experimental Psychology: Animal Behavior Processes, 25*, 177-193.

Barker, G. R. I., Bird, F., Alexander, V., Warburton, E. C. (2007). Recognition memory for objects in place, and temporal order: A disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *The Journal of Neuroscience, 27*, 2948-2957.

Bartko, S. J., Winters, B. D., Cowell, R. A., Saksida, L. M., & Bussey, T. J. (2007). Perirhinal cortex resolves feature ambiguity in configural

object recognition and perceptual oddity tasks. *Learning & Memory, 14*, 821-832.

Baxter, M. G., & Murray, E. A. (2001). Impairments in visual discrimination learning and recognition memory produced by neurotoxic lesions of rhinal cortex in rhesus monkeys. *European Journal of Neuroscience, 13, 1228-1238*.

Bennett, C. H., Wills, S. J., Wells, J. O., & Mackintosh, N. J. (1994). Reduced generalization following preexposure: Latent inhibition of common elements or a difference in familiarity? *Journal of Experimental Psychology: Animal Behavior Processes, 20*, 232-239.

Berlyne, D. E. (1950). Novelty and curiosity as determinants of exploratory behaviour. *The British Journal of Psychology, 41*, 68-80.

Best, M. R., & Batson, J. D. (1977). Enhancing the expression of flavor neophobia: Some effects of the ingestion-illness contingency. *Journal of Experimental Psychology: Animal Behavior Processes, 3,* 132-143.

Blough, D. S. (1975). Steady state data and a quantitative model of operant generalization and discrimination. *Journal of Experimental Psychology: Animal Behavior Processes, 1*, 3-21.

Brandon, S. E., Vogel, E. H., & Wagner, A. R. (2003). Stimulus representation in SOP: I Theoretical rationalization and some implications. *Behavioural Processes, 62*, 5-25.

Brown, M. W., & Aggleton, J P. (2001). Recognition memory: What are the roles of the perirhinal cortex and hippocampus? *Nature Reviews: Neuroscience, 2*, 51-61.

Buckley, M. J., Gaffan, D. & Murray, E. A. (1997). Functional double dissociation between two inferior temporal cortical areas: Perirhinal cortex versus middle temporal gyrus. *Journal of Neurophysiology, 77*, 587-598.

Buckley, M. J., & Gaffan, D. (1997). Impairment of visual object-discrimination learning after perirhinal cortex ablation. *Behavioral Neuroscience, 111*, 467-475.

Buckley, M. J., & Gaffan, D. (1998). Perirhinal cortex ablation impairs visual object identification. *The Journal of Neuroscience, 18*, 2268-2275.

Burn, C. C. (2008). What is it like to be a rat? Rat sensory perception and its implications for experimental design and rat welfare. *Applied Animal Behaviour Science, 112,* 1–32.

Burwell, R. D. (2001). Borders and cytoartitecture of the perirhinal and postrhinal cortices in the rat. *The Journal of Comparative Neurology, 437*, 17-41.

Burwell, R. D., & Amaral, D. G. (1998). Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *The Journal of Comparative Neurology, 398*, 179-205.

Bussey, T. J., & Saksida, L. M. (2005). Object memory and perception in the medial temporal lobe: An alternative approach. *Current Opinion in Neurobiology, 15*, 730-737.

Bussey, T. J., & Saksida, L. M. (2007). Memory, perception, and the ventral visual-perirhinal-hippocampal stream: Thinking outside of the boxes. *Hippocampus, 17*, 898-908.

Bussey, T. J., Saksida, L. M., & Murray, E. A. (2002). Perirhinal cortex resolves feature ambiguity in complex visual discriminations. *European Journal of Neuroscience, 15,* 365-374.

Campolattaro, M. M., & Freeman, J. H. (2006a). Perirhinal cortex lesions impair simultaneous but not serial feature-positive discrimination learning. *Behavioural Neuroscience, 120*, 970-975.

Campolattaro, M. M., & Freeman, J. H. (2006b). Perirhinal cortex lesions impair feature-negative discrimination. *Neurobiology of Learning and Memory, 86,* 205-213.

Caulfield, M. P. (1993). Muscarinic receptors – characterization, coupling and function. *Pharmacology & Therapeutics, 58*, 319-379.

Cepeda, N. J., Pashler, H., Vul, E., Wixted, J. T., & Rohrer, D. (2006). Distributed practice in verbal recall tasks: A review and quantitative synthesis. *Psychological Bulletin, 132,* 354-380.

Clark, R. E., & Martin, S. J. (2005). Interrogating rodents regarding their object and spatial memory. *Current Opinion in Neurobiology, 15*, 593-598.

Clark, R. E., Reinagel, P., Broadbent, N. J., Flister, E. D., Squire, L. R. (2011). Intact performance on feature-ambiguous discriminations in rats with lesions of the perirhinal cortex. *Neuron, 70*, 132-140.

Cowell, R. A., Bussey, T. J., & Saksida, L. M. (2006). Why does brain damage impair memory? A connectionist model of object recognition memory in perirhinal cortex. *The Journal of Neuroscience, 26*, 12186-12197.

Cowell, R. A., Bussey, T. J., & Saksida, L. M. (2010a). Functional dissociations within the ventral object processing pathway: Cognitive modules or a hierarchical continuum? *Journal of Cognitive Neuroscience, 22*, 2460-2479.

Cowell, R. A., Bussey, T. J., & Saksida, L. M. (2010b). Components of recognition memory: Dissociable cognitive processes or just differences in representational complexity? *Hippocampus, 20*, 1245-1262.

Davies, M., Machin, P. E., Sanderson, D. J., Pearce, J. M., Aggleton, J. P. (2007). Neurotoxic lesions of the rat perirhinal and postrhinal cortices and their impact on biconditional visual discrimination tasks. *Behavioural Brain Research, 176*, 274-283.

Davis, K. L., Mohs, R. C., Tinklenberg, J. R., Pfefferbaum, A., Hollister, L. E., & Kopell, B. S. (1978). Physostigmine: Improvement of long-term memory processes in normal humans. *Science, 201*, 272-274.

Davis, M. (1970). Effects of interstimulus interval length and variability on startle-response habituation in the rat. *Journal of Comparative and Physiological Psychology, 72*, 177-192.

Dember, W. N. (1956). Response by the rat to environmental change. *Journal of Comparative and Physiological Psychology, 49,* 93-95.

Dere, E., Huston, J., P., De Souza Silva, M. A. (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neuroscience and Biobehavioral Reviews, 31,* 673-704.

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: Evidence of object-location and object-context recognition. *Behavioural Brain Research, 99*, 191-200.

Domjan, M. (1980). Effects of the intertrial interval on taste-aversion learning in rats. *Physiology & Behavior, 25*, 117-125.

Dwyer, D. M. (2003). Learning about cues in their absence: Evidence from flavour preferences and aversions. *The Quarterly Journal of Experimental Psychology, 56B,* 56-67.

Eacott, M. J., & Norman, G. (2004). Integrated memory for object, place, and context in rats: A possible model of episodic-like memory? *The Journal of Neuroscience, 24*, 1948-1953.

Ebbinghaus, H. (1964). *Memory: A contribution to experimental psychology.* New York: Dover.

Ennaceur, A. (2010). One-trial object recognition in rats and mice: Methodological and theoretical issues. *Behavioural Brain Research, 215*, 244-254.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research, 31*, 47-59.

Ennaceur, A., & Meliani, K. (1992). Effects of physostigmine and scopolamine on rats' performances in object-recognition and radial-maze tests. *Psychopharmacology, 109*, 321-330.

Ennaceur, A., Neave, N., & Aggleton, J. P. (1997). Spontaneous object recognition and object location memory in rats: The effects of lesions in the cingulated cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research, 113*, 509-519.

Fagan, J. F. (1970). Memory in the infant. *Journal of Experimental Child Psychology, 9,* 217-226.

Fanselow, M. S., DeCola, J. P., Young, S. L. (1993). Mechanisms responsible for reduced contextual conditioning with massed unsignaled unconditional stimuli. *Journal of Experimental Psychology: Animal Behavior Processes, 19,* 121-137.

Fanselow, M., S., & Tighe, T. J. (1988). Contextual conditioning with massed versus distributed unconditional stimuli in the absence of explicit conditional stimuli. *Journal of Experimental Psychology: Animal Behavior Processes, 14*, 187-199.

Forwood, S. E., Bartko, S. J., Saksida, L. M., & Bussey, T. J. (2007). Rats spontaneously discriminate purely visual, two-dimensional stimuli in tests of recognition memory and perceptual oddity. *Behavioral Neuroscience, 121*, 1032-1042.

Gaffan, D., & Murray, E. A. (1992). Monkeys (Macaca fascicularis) with rhinal cortex ablations succeed in object discrimination learning despite 24-hr intertrial intervals and fail at matching to sample despite double sample presentations. *Behavior Neuroscience, 106*, 30-38.

Gaffan, E. A., Eacott, M. J., & Simpson, E. L. (2000). Perirhinal cortex ablation in rats selectively impairs object identification in a simultaneous visual comparison task. *Behavioral Neuroscience, 114*, 18-31.

Gaskin, S., Tardif, M., Piterkin, P., Kayello, L., & Mumby, D. G. (2010). Object familiarization and novel-object preference in rats. *Behavioural Processes, 83*, 61-71.

Guić-Robles, E., Valdivieso, C, & Guajardo, G. (1989). Rats can learn a roughness discrimination using only their vibrissal system. *Behavioural Brain Research, 31*, 285-289.

Hall, G. (2001). Perceptual learning: Association and differentiation. In R. R. Mowrer & S. B. Klein (Eds.), *Handbook of contemporary learning theories* (pp. 367-407). Mahwah, NJ: Lawrence Erlbaum.

Haselgrove, M., Robinson, J., Nelson, A., & Pearce, J. M. (2008). Analysis of an ambiguous-feature discrimination. *The Quarterly Journal of Experimental Psychology, 61,* 1710-1725.

Herremans, A. H. J., Hijzen, T. H., Olivier, B., & Slangen, J. L. (1995). Cholinergic drug effects on a delayed conditional discrimination task in the rat. *Behavioral Neuroscience, 109,* 426-435.

Herz, A., Teschemacher, H., Hofstetter, A., & Kurz, H. (1965). The importance of lipid-solubility for the central action of cholinolytic drugs. *International Journal of Neuropharmacology, 4,* 207-218.

Hintzman, D. L. (1974). Theoretical implication of the spacing effect. In R. L. Solso (Ed.), *Theories in cognitive psychology: The Loyola symposium* (pp. 77-99). Potomac: Lawrence Erlbaum.

Hintzman, D. L., Summers, J. J., & Block, R. A. (1975). What causes the spacing effect? Some effects of repetition, duration, and spacing on memory for pictures. *Memory & Cognition, 3*, 287-294.

Honey, R. C. (1990). Stimulus generalization as a function of stimulus novelty and familiarity in rats. *Journal of Experimental Psychology: Animal Behavior Processes, 16*, 178-184.

Honey, R. C., & Good, M. (2000). Associative modulation of the orienting response: Distinct effects revealed by hippocampal lesions. *Journal of Experimental Psychology, 26*, 3-14.

Honey, R. C., Good, M., & Manser, K. L. (1998). Negative priming in associative learning: Evidence from a serial-habituation procedure. *Journal of Experimental Psychology: Animal Behavior Processes, 24,* 229-237.

Honig, W. K., & Urcuioli, P. J. (1981). The legacy of Guttman and Kalish (1956): 25 years of research on stimulus generalization. (1981). *Journal of the experimental analysis of behavior, 36*, 405-445.

Hughes, R. N. (2007). Rats' responsiveness to tactile changes encountered in the dark, and the role of the mystacial vibrissae. *Behavioural Brain Research, 179,* 273-280.

Huston, A. E., & Aggleton, J. P. (1987). The effects of cholinergic drugs upon recognition memory in rats. *The Quarterly Journal of Experimental Psychology, 39B,* 297-314.

Iordanova, M. D., Good, M. A., & Honey, R. C. (2008). Configural learning without reinforcement: Integrated memories for correlated of what, where, and when. *The Quarterly Journal of Experimental Psychology, 61*, 1785-1792.

Jacobs, G. H., Fenwick, J. A., & Williams, G. A. (2001). Cone-based vision of rats for ultraviolet and visible lights. *The Journal of Experimental Biology, 204*, 2439-2446.

Julien, R. M., Advokat, C. D., & Comaty, J. E. (2007). *A primer of drug action: A comprehensive guide to the actions, uses, and side effects of psychoactive drugs* (11th ed.). New York, NY: Worth.

Kaye, H., & Pearce, J. M. (1984). The strength of the orienting response during Pavlovian conditioning. *Journal of Experimental Psychology: Animal Behavior Processes, 10,* 90-109.

Klinkenberg, I., & Blokland, A. (2010). The validity of scopolamine as a pharmacological model for cognitive impairment: A review of animal behavioural studies. *Neuroscience and Biobehavioral Reviews, 34*, 1307-1350.

Kowalska, D. M., Kuśmierek, P., Kosmal, A., & Mishkin, M. (2001). Neither perirhinal/entorhinal nor hippocampal lesions impair short-term auditory recognition in dogs. *Neuroscience, 104*, 965-978.

Levy, D. A., Shrager, Y., & Squire, L. R. (2005). Intact visual discrimination of complex and feature-ambiguous stimuli in the absence of perirhinal cortex. *Learning & Memory, 12*, 61-66.

Lovibond, P. F., Preston, G. C., & Mackintosh, N. J. (1984). Context specificity of conditioning, extinction, and latent inhibition. *Journal of Experimental Psychology: Animal Behavior Processes, 10*, 360-375.

Lubow, R. E., & Moore, A. U. (1959). Latent inhibition: The effect of nonreinforced pre-exposure to the conditional stimulus. *Journal of Comparative and Physiological Psychology, 52,* 415-419.

Mandler, G. (1980). Recognizing: The judgement of previous occurrence. *Psychological review, 87*, 252-271.

McLaren, I. P. L., & Mackintosh, N. J. (2000). An elemental model of associative learning: I. Latent inhibition and perceptual learning. *Animal Learning & Behavior, 28,* 211-246.

McLaren, I. P. L., & Mackintosh, N. J. (2002). Associative learning and elemental representation: II. Generalization and discrimination. *Animal Learning & Behavior, 30*, 177-200.

McTighe, S. M., Cowell, R. A., Winters, B. D., Bussey, T. J., & Saksida, L. M. (2010). Paradoxical false memory for objects after brain damage. *Science, 330*, 1408-1410.

Menzel, R., Manz, G., Menzel, R., & Greggers, U. (2001). Massed and spaced learning in honeybees: The role of CS, US, the intertribal interval, and the test interval. *Learning and Memory, 8*, 198-208.

Meunier, M., Bachevalier, J., Mishkin, M., & Murray, E. A. (1993). Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *The Journal of Neuroscience, 13*, 5418-5432.

Mitchell, J. B., & Laiacona, J. (1998). The medial frontal cortex and temporal memory: Tests using spontaneous exploratory behaviour in the rat. *Behavioural Brain Research*, *97*, 107-113.

Mumby, D. G., Glenn, M. J., Nesbitt, C., Kyriazis, D. A. (2002). Dissociation in retrograde memory for object discriminations and object recognition in rats with perirhinal cortex damage. Behavioural Brain Research, 132, 215-226.

Mumby, D. G., & Pinel, J. P. J. (1994). Rhinal cortex lesions and object recognition in rats. *Behavioral Neuroscience, 108*, 11-18.

Mumby, D. G., Piterkin, P., Lecluse, V., & Lehmann, H. (2007). Perirhinal cortex damage and anterograde object-recognition in rats after long retention intervals. *Behavioural Brain Research, 185*, 82-87.

Norman, G., & Eacott, M. J. (2005). Dissociable effects of lesions to the perirhinal cortex and the postrhinal cortex on memory for context and objects in rats. *Behavioral Neuroscience, 119*, 557-566.

Otto, T., & Eichenbaum, H. (1992). Complementary roles of the orbital prefrontal cortex and the perirhinal-entorhinal cortices in an odor-guided delayed-nonmatching-to-sample task. *Behavioral Neuroscience, 106*, 762-775.

Overton, D. A. (1984). State dependent learning and drug discriminations. In Iversen, L. L., Iversen, S. D., & Snyder, S. H (Eds.), *Handbook of Psychopharmacology* (Vol 18, pp. 59-127). New York: Plenum Press.

Paxinos, G., & Watson, C. (2005). *The rat brain in stereotaxic coordinates* (5th ed.). San Diego, CA: Academic Press.

Plakke, B., Ng, CW., Poremba, A. (2008). Scopolamine impairs auditory delayed matching-to-sample performance in monkeys. *Neuroscience Letters, 438*, 126-130.

Reed, P., & Adams, L. (1996). Influence of salient stimuli on rats' performance in an eight-arm radial maze. *Learning and Motivation, 27*, 294-306.

Rescorla, R. A., & Wagner, A. R. (1972). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In A. H. Black & W. F. Prokasy (Eds.), *Classical Conditioning II* (pp. 64-99). New York: Appleton-Century-Crofts.

Rutten, K., Reneerkens, O. A. H., Hamers, H., Sik, A., McGregor, I. S., Prickaerts, J., & Blokland, A. (2008). Automated scoring of novel object recognition in rats. *Journal of Neuroscience Methods, 171*, 72-77.

Saksida, L. M. (2009). Remembering outside the box. *Science, 325*, 40-41.

Sanderson, D. J., & Bannerman, D. M. (2011). Competitive short-term and long-term memory processes in spatial habituation. *Journal of Experimental Psychology: Animal Behaviour Processes,* 37, 189-199.

Sanderson, D. J., McHugh, S. B., Good, M. A., Sprengel, R., Seeburg, P. H., Rawlins, J. N. P., Bannerman, D. M. (2010). Spatial working memory deficits in GluA1 AMPA receptor subunit knockout mice reflect impaired short-term habituation: Evidence for Wagner's dual-process memory model. *Neuropsychologia, 48*, 2303-2315.

Schon, K., Atri, A., Hasselmo, M. E., Tricarico, M. D., LoPresri, M. L., & Stern, C. E. (2005). Scopolamine reduces persistent activity related to long-term encoding in the parahippocampal gyrus during delayed matching in humans. *The Journal of Neuroscience, 25*, 9112-9123.

Silvers, J. M., Harrod, S. B., Mactutus, C. F., & Booze, R. M. (2007). Automation of the novel object recognition task for use in adolescent rats. *Journal of Neuroscience Methods, 166,* 99-103.

Squire, L. R., Wixted, J. T., & Clark, R. E. (2007). Recognition memory and the medial temporal lobe: A new perspective. Nature Reviews Neuroscience, 8, 872-883.

Sunsay, C., & Bouton, M. E. (2008). Analysis of a trial-spacing effect with relatively long intertrial intervals. *Learning & Behavior, 36*, 104-115.

Sunsay, C., Stetson, L., & Bouton, M. E. (2004). Memory priming and trial spacing effects in Pavlovian learning. *Learning and Behavior, 32*, 220-229.

Szél, A., & Röhlich, P. (1992). Two types of rat retina detected by anti-visual pigment antibodies. *Experimental Eye Research, 55*, 47-52.

Tomsic, D., Berón de Astrada, M., Sztarker, J., & Maldonado, H. (2009). Behavioral and neuronal attributes of short- and long-term habituation in the crab Chasmagnathus. *Neurobiology of Learning and Memory, 92*, 176-182.

VanElzakker, M., Fevurly, R. D., Breindel, T., & Spencer, R. L. (2008). Environmental novelty is associated with a selective increase in Fos expression in the output elements of the hippocampal formation and the perirhinal cortex. *Learning & Memory, 15*, 899-908.

van Haaren, F., & van Hest, A. (1989). The effects of scopolamine and methylscopolamine on visual and auditory discriminations in male and female Wistar rats. *Pharmacology Biochemistry & Behavior, 32*, 707-710.

Wagner, A. R. (1976). Priming in STM: An information processing mechanism for self-generated or retrieval-generated depression in performance. In T. J. Tighe & R. N. Leaton (Eds.), *Habituation: Perspectives from child development, animal behavior, and neurophysiology* (pp. 95-128). Hillsdale, NJ: Erlbaum.

Wagner, A. R. (1978). Expectancies and the priming of STM. In S. H. Hulse, H. Fowler, & W. K. Honig (Eds.), *Cognitive Processes in Animal Behavior*. Hillsdale, NJ: Erlbaum.

Wagner, A. R. (1981). SOP: A model of automatic memory processing in animal behavior. In N.E Spears & R.R. Miller (Eds.), *Information processing in animals: Memory mechanisms* (pp. 5-47). Hillsdale, NJ: Erlbaum.

Wan, H., Aggleton, J. P., & Brown, M. W. (1999). Different contributions of the hippocampus and perirhinal cortex to recognition memory. *The Journal of Neuroscience, 19*, 1142-1148.

Wan, H., Warburton, E. C., Kuśmierek, P., Aggleton, J. P., Kowalska, D. M., & Brown, M. W. (2001). Fos imaging reveals differential neuronal activation of areas of rat temporal cortex by novel and familiar sounds. *European Journal of Neuroscience, 14*, 118-124.

Warburton, E. C., Koder, T., Cho, K, Massey, P. V., Duguid, G., Barker, G. R. I., . . . Brown, M. W. (2003). Cholinergic neurotransmission is essential for perirhinal cortical plasticity and recognition memory. *Neuron, 38*, 987-996.

Winters, B. D., & Reid, J. M. (2010). A distributed cortical representation underlies crossmodal object recognition in rats. *The Journal of Neuroscience, 30*, 6253-6261.

Winters, B. D., Saksida, L. M., & Bussey, T. J. (2006). Paradoxical facilitation of object recognition memory after infusion of scopolamine into perirhinal cortex: Implications for cholinergic system function. *The Journal of Neuroscience, 26*, 9520-9529.

Winters, B. D., Saksida, L. M., & Bussey, T. J. (2008). Object recognition memory: Neurobiological mechanisms of encoding, consolidation and retrieval. *Neuroscience and Biobehavioral Reviews, 32*, 1055-1070.

Woolley, M. L., Marseden, C. A., Sleight, A. J., & Fone, K. C. F. (2003). Reversal of a cholinergic-induced deficit in a rodent model of

recognition memory by the selective 5-HT_6 receptor antagonist, Ro 04-6790. *Psychopharmacology, 170*, 358-367.

Young, S. L., Bohenek, D. L., Fanselow, M. S. (1995). Scopolamine impairs acquisition and facilitates consolidation of fear conditioning: Differential effects of tone vs context conditioning. *Neurobiology of learning and memory, 63,* 174-180.

Zhu, X. O., Brown, M. W., McCabe, B. J., & Aggleton, J. P. (1995). Effects of the novelty or familiarity of visual stimuli on the expression of the immediate early gene c-fos in rat brain. *Neuroscience, 69*, 821-829.

Zola-Morgan, S., Squire, L. R., Amaral, D. G., & Suzuki, W. A. (1989). Lesions of perirhinal and parahippocampal cortex that spare the amygdala and hippocampal formation produce severe memory impairment. *The Journal of Neuroscience, 9*, 4355-4370.

Appendix 1: Experiment 14. Ambiguous-Feature Discrimination

Perirhinal cortex is reported to have a role in solving ambiguous visual discriminations (Bartko et al., 2007; Bussey et al., 2002). The representational-hierarchical account (Cowell et al., 2010a, 2010b, Saksida, 2009) suggests that this is because the perirhinal cortex is needed to hold complex representations of stimuli. However, there are reports that find no deficit in rats (Aggleton, Albasser, Aggleton, Poirier, & Pearce, 2010; Clark, Reinagel, Broadbent, Flister, & Squire, 2011; Davies, Machin, Sanderson, Pearce, & Aggleton, 2007) or in humans (Levy, Shrager, & Squire, 2005) with perirhinal lesions in solving ambiguous discriminations. The same subjects were impaired in object recognition (Aggleton et al., 2010; Clark et al., 2011). This suggests that perirhinal cortex is important for discriminating familiarity and novelty, rather than processing perceptual features of stimuli.

Discriminations that use a combination of visual and non-visual stimuli are difficult to learn for subjects with lesions to the perirhinal cortex (Campolattaro & Freeman, 2006a, 2006b). Campolattaro and Freeman reported that in a feature-negative (A+/AX-), or a feature-positive discrimination (A-/AX+), rats with perirhinal lesions were slow to learn the discrimination, but by the end of training, they had acquired the discrimination to the level of the control group. These discriminations are not full configural discriminations, however, as the subject only needs to learn the role of the feature CS in order to change their responding. A full configural design such as A+/B-/AX-/BX+ (Haselgrove, Robinson, Nelson, & Pearce, 2008) would highlight whether perirhinal cortex lesions affect

the acquisition of an ambiguous discrimination. The subject could not rely on the feature (X) to predict the presence or absence of the US; but has to have a full representation of the stimulus for successful discrimination.

The aim of the present study was to refine results seen by Campolattaro and Freeman (2006a, 2006b). An ambiguous-feature discrimination task (A+/B-/AX-/BX+) was used to ensure that subjects learned the complex representation of the entire stimulus to solve the discrimination. According to visual accounts (e.g., Cowell et al., 2010a, 2010b), subjects with perirhinal lesions would not be able to solve the discrimination; however, other reports suggest that there would be no deficit in the perirhinal subjects (Aggleton et al., 2010; Claark et al., 2011). This task used auditory stimuli in combination with visual stimuli, so it would be valuable to see if there were any differences between this task and a purely visual one.

Method

Subjects.

The subjects were 24 male hooded-Lister rats (*Rattus norvegicus*), supplied by Harlan (UK). All rats, except one that was housed individually, were housed in pairs. Rats were kept in a room with a 12-hour light cycle with an 0700 onset. The temperature in the holding room and the experimental room was 20°C ± 2°C with a humidity of 50%.

Until the experiment began, all rats had free access to food and water. After the experiment began, food was restricted to reduce the rats' weight gradually by 10-20%. Water was still freely available throughout the experiment. Rats' weights were recorded at the beginning of the

experiment and monitored throughout. Rats were fed once a day after each experimental session.

All subjects had been used in previous behavioural experiments and so were familiar with some of the stimuli that were used.

Surgery.

Prior to the experiment, 16 rats underwent surgery to produce perirhinal cortex lesions (group PeRh) and a further eight rats had control lesions (group Sham), in which surgery was performed but no neurotoxin applied.

Rats were anaesthetized with isoflurane mixed with oxygen and kept anaesthetized during the procedure with a lower concentration of isoflurane. Rats were placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). The skulls were exposed and bone was removed using a dental burr. Injections were made with a 2 ng the procedure with a lower concentration of isoflurane. Rats were placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA).syringe was attached to an electronic microdrive (Model KDS 310; KD Scientific, New Hope, PA), which regulated the volume and rate of infusion of the neurotoxin. Lesions were made with ibotenic acid (Sigma Aldrich, Gillingham, UK), which was dissolved in sterile phosphate-buffered saline (7.4 pH) to produce a 63-mM solution. This was infused at a rate of 0.03 μL/min. Injections were made at -3.0 mm rostral-caudal (RC, medial lateral [ML] ±5.8, dorsal ventral [DV] - 4.0, 0.120 μl), -4.0mm (ML ±6.1, DV- 3.8, 0.100 μl), 5.0mm (ML ±6.5, DV- 4.0, 0.070 μl), 6.0mm (ML ±6.7, DV- 3.5, 0.050 μl) and 7.0mm (ML ±6.3, DV- 3.1, 0.035 μl) posterior to bregma. The needle was left in place

two minutes after each injection. The same procedure was used for the sham surgery except only their duras were perforated with a 25-gauge Microlance needle (BD, Drogheda, Ireland). Following surgery, rats given lesions were injected with saline and glucose (5 ml, subcutaneous) and placed in individual recovery boxes overnight. The following day they were returned to their home cages. They were then were allowed to recover for at least two weeks, before testing.

Rats with perirhinal cortex lesions were anaethestized with an overdose of sodium pentobarbital (200 mg/kg) and were transcardiallly perfused with 0.3% saline and 10% formal saline. The brains were then removed and placed in 10% formal saline solution. Before sectioning each brain was placed in 20% sucrose until saturated. The brain was sectioned at 40 μm using a cryostat-microtome (Leica Microsystems Ltd, Milton Keynes, UK) and every fifth section was mounted for analysis. They were then stained with cresyl violet and examined.

Apparatus.

The apparatus consisted of eight identical operant boxes (Campden Instruments Ltd., Loughborough, UK); each enclosed within a larger chamber, which was light and sound attenuated. These chambers were equipped with an exhaust fan for ventilation and giving a background noise of 70 dB. The boxes measured 24.5 x 23.0 x 21.0 cm. Three of the walls and the ceiling were aluminium and the fourth was a transparent plastic door. The floor consisted of stainless steel rods. The plastic door was hinged at the floor to allow access to the box, and was secured using a catch on the top of the ceiling. Each box contained a concave food tray

(magazine) that was set into the right side wall. A food dispenser delivered pellets (45 mg, Noyes, Lancaster, NH) into this tray. A sprung transparent plastic flap (6 cm high, 5 cm wide) covered this tray and could be pushed inwards to collect pellets. The flaps could be opened between 10-15 mm into the tray. This pushing actuated a microswitch and was recorded as a response. Each box also contained three lights, one of which was the ceiling light and was not used in this experiment. The other two lights were fixed on the right side wall and were equal distance from the midline of the wall (12.5 cm apart; 15.0 cm above the floor). These wall lights produced 130 lux. A heavy-duty relay was fitted to the top of every box and operated at 10 Hz to produce a pulse of clicks. These ranged from 80-83 dB across boxes. The ceiling of each box contained a loudspeaker to produce a 2 kHz tone. These ranged from 89-96 dB across boxes.

A computer with Windows XP system was used to run MED-PCIV (MED Associates Inc.). This controlled presentation of stimuli, food dispensation and recording of responses.

Procedure.

One rat that was given lesions died before testing began. All rats were given magazine training. Food was dispensed every minute for 20 minutes, to train the rats to retrieve food from the tray by pushing the flap. All rats received two sessions of training.

Following magazine training, rats began the ambiguous-feature training. This consisted of trials of A+ B- AX- BX+ where A and B were either a tone or a clicker, and X was a light. Group 1 (PeRh, $n = 8$) received A+ B- AX- BX+ and group 2 (sham, $n = 4$) received A+ B- AX-

BX+ and groups 3 (PeRh, *n* = 7) and 4 (sham, *n* = 4) received A- B+ AX+ BX-.

Each CS was presented for 10 seconds. In the compounds AX and BX, CSs were presented together, meaning the onset and termination of the wall lights would coincide with the onset and termination of the tone or the clicker. Thirty-two of these 10-second trials were given each session. Trials were block randomised. Inter-trial intervals (ITI) had a mean of 70 seconds. The full sequence of trial sequences and ITIs occurred in three blocks of 32 trials (96 in total). ITIs were counted from the termination of one stimulus to the onset of another. Conditioned responses (CRs) were measured as the number of entries into the magazine during the presentation of each CS.

Results

Histology.

Damage in six subjects in group PeRh was negligible or unilateral so data of those subjects were deleted from analyses. Results are based on data from the remaining 17 rats, group PeRh $n = 9$, and sham group $n = 8$. Cell loss in the remaining 9 rats with lesions was as intended. Lesions began at -3 mm from bregma and extended to -7 mm. There was some damage to dorsally adjacent areas, including ventral temporal association areas (5 cases). There were two cases of damage to the ventral auditory area. The damage in these extra-perirhinal areas was unilateral.

Training.

All rats successfully learnt how to retrieve food pellets from the magazine tray.

Ambiguous-feature discrimination.

The first four trials were deleted from analysis due to a programming error. Results are from the remaining 12 trials, in three blocks, each block containing four trials. Results are presented in corrected responses; this was calculated by subtracting the responding during pre-CS from responding during the CS. This was used to give a clearer indication of responses during the CS.

The first analysis focused on 12 trials that were grouped into three blocks of four trials (Figure 56). There seemed to be lower responses to the non-reinforced stimuli, and higher responses to the positively reinforced

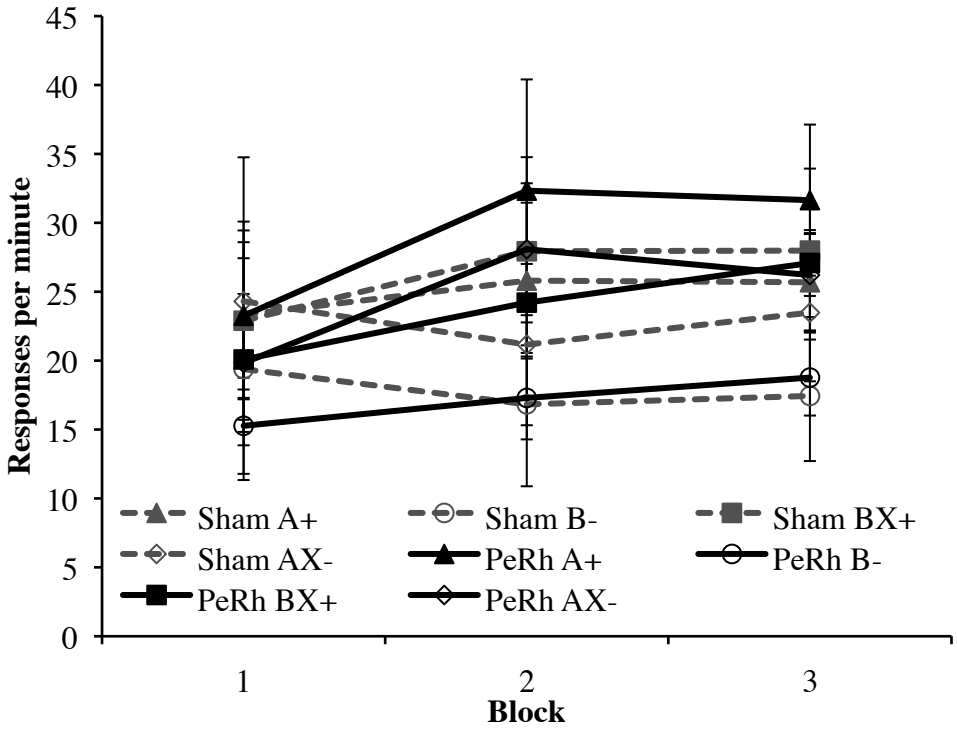


Figure 56. Mean corrected responses per minute (and standard error of the mean) for each block (four trials per block) of all four stimuli for the control (sham) group and the perirhinal lesion (PeRh) group.

stimuli, both the sham group and the PeRh group showed similar responding. An ANOVA on the three blocks, and four types of stimulus was conducted with surgery as a between-subjects factor. There was only a significant effect of stimulus, $F(3, 45) = 5.99$, $p = .002$, $\eta_P^2 = .285$. There was no effect of block, $F(2, 30) = 1.28$, $p = .294$, $\eta_P^2 = .078$, or surgery, $F < 1$, $\eta_P^2 = .001$. There were no interactions of surgery with either stimulus, $F < 1$, $\eta_P^2 = .047$, or block, $F < 1$, $\eta_P^2 = .046$, and no interaction between Block x Stimulus, $F(6, 90) = 1.34$, $p = .247$, $\eta_P^2 = .082$, and no three-way interaction, $F < 1$, $\eta_P^2 = .062$. These results indicate that responses were different between stimuli, but that no other factors, including surgery affected responses to stimuli.

An analysis of all the trials does not show a very clear picture of the data. For the rats to solve the discrimination it was expected that levels of responding to the reinforced stimuli (A+, BX+) would be higher than that of the non-reinforced stimuli (B-, AX-). Responses to positively reinforced stimuli were compared to non-reinforced stimuli.

There were more responses to the positively reinforced stimuli than the negatively reinforced stimuli (Figure 57). An ANOVA with group as the between-subjects factor, and block and reinforcement as within-subject factors supported this description. There was a significant effect of reinforcement, $F(1, 15) = 35.90$, $p < .001$, $\eta_P^2 = .705$, but no effect of surgery, $F < 1$, $\eta_P^2 = .001$, or block, $F(2, 30) = 1.39$, $p = .266$, $\eta_P^2 = .085$.

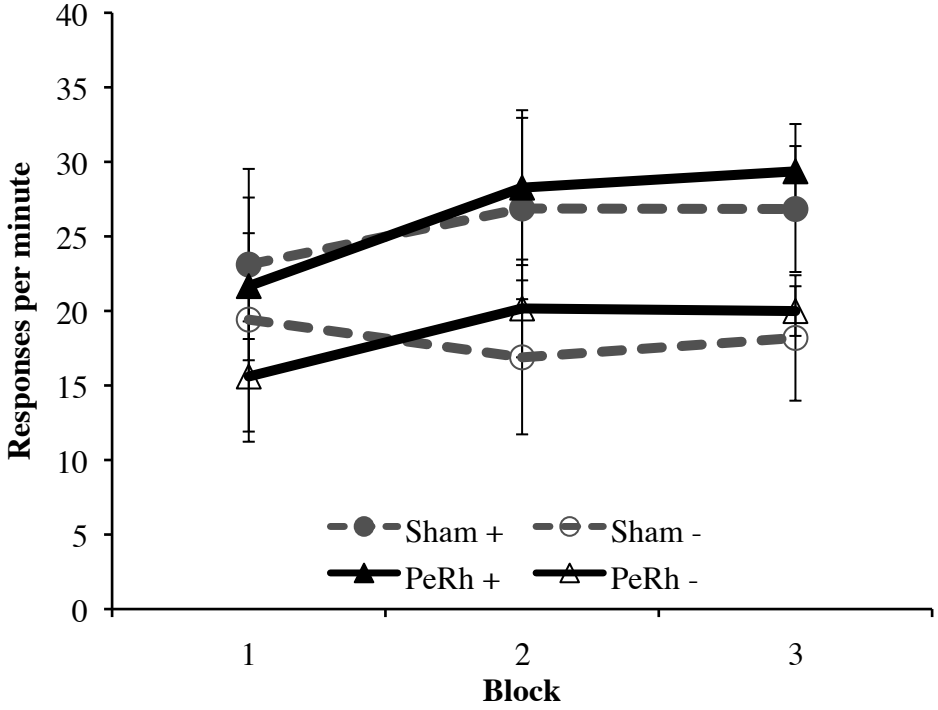


Figure 57. Responses per minute (and standard error of the mean) during three blocks (four trials per block) to positively reinforced (+) and non-reinforced (-) stimuli.

There was a significant Block x Reinforcement interaction, $F(2, 30) = 3.78$, $p = .034$, $\eta_P^2 = .201$. There was no Reinforcement x Surgery interaction, $F < 1$, $\eta_P^2 = .002$, no Block x Surgery interaction, $F < 1$, $\eta_P^2 = .045$, and no three-way interaction, $F < 1$, $\eta_P^2 = .048$.

Simple main effects analyses were conducted to explore the Block x Reinforcement interaction. These found a significant increase in responding across blocks of the reinforced stimulus, $F(2, 14) = 4.42$, $p = .032$, $\eta_P^2 = .387$, but no change in the non-reinforced stimuli, $F < 1$, $\eta_P^2 = .022$.

Results from Haselgrove et al. (2007) showed that there was a difference between learning of a single stimulus and learning of a double-element stimulus. It is possible in the present experiment that rats might learn single elements faster or better than double element stimuli. Rats with perirhinal lesions may be impaired in holding complete representations of complex stimuli (Bussey et al., 2002). It could be possible that rats were only able to learn the discrimination of the single elements, and not the double elements. Ratios were calculated in order to compare differences clearly. Ratios were calculated by subtracting responses to the non-reinforced stimulus (N) from responses to the reinforced stimulus (R), divided by the total responses to N and R, ([R - N]/[R + N]). Zero indicates no difference in responding to stimuli; above zero indicates more responding during the reinforced stimulus. Ratios of responding showed similar levels between groups on single elements, and a lower ratio for the perirhinal group when responding to double elements (Figure 58). An

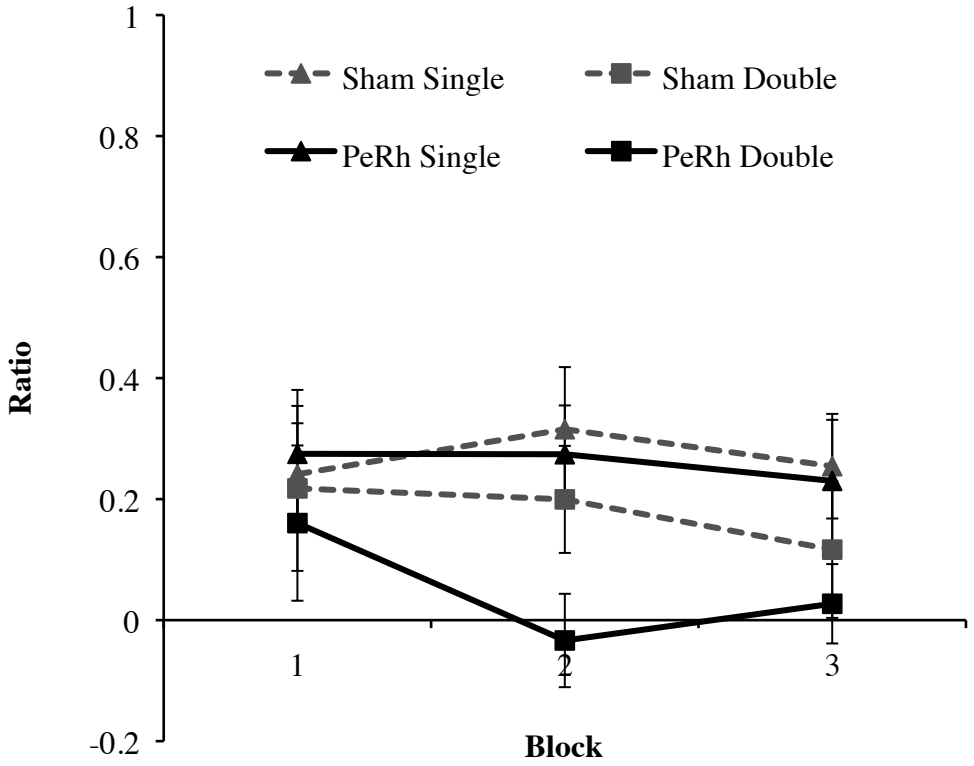


Figure 58. Ratios (and standard error of the mean) of reinforced/nonreinforced stimuli of single element or double element stimuli in both groups. Ratios were calculated by subtracting responses to N (non-reinforced stimulus) from responses to R (reinforced stimulus), divided by the total responses to N and R, ([R - N]/[R + N]). Zero indicates no difference in responding to stimuli; above zero indicates more responding during the reinforced stimulus.

ANOVA of ratios for three blocks, for each group (sham or PeRh) and each set of stimuli (single element or double element) did not show this difference. This revealed no effects of stimulus, $F(1, 15) = 3.38$, $p = .086$, $\eta_P^2 = .184$, or surgery, $F < 1$, $\eta_P^2 = .039$, or block, $F < 1$, $\eta_P^2 = .044$. There was a significant Block x Stimulus interaction, $F(2, 30) = 3.53$, $p = .042$, $\eta_P^2 = .191$. There were no other interactions with Surgery x Stimulus, $F < 1$, $\eta_P^2 = .032$, or Block x Surgery, $F < 1$, $\eta_P^2 = .041$, and no three-way interaction, $F < 1$, $\eta_P^2 = .047$.

The significant Block x Stimulus interaction was explored using SMEs analyses. These showed that there was a significant difference between single element stimuli and double element stimuli in block 2, $F(1, 15) = 6.29$, $p = .024$, $\eta_P^2 = .295$, ratios were higher for single elements than double elements. Although the perirhinal group showed low ratios for the double elements, the statistics do not reveal any differences.

The ambiguous-feature discrimination is essentially made of a feature-positive and a feature-negative discrimination, it could be that rats were solving one of these but not the other; though, results from Campolattaro and Freeman (2006a, 2006b) suggested that rats were able to solve both discriminations; however, presenting them in the same session may have affected performance.

Figure 59 shows mean responding to only the feature-positive aspects of the discrimination (B-/BX+) for the sham group and the PeRh group. Responding to B- remained low over blocks of trials, and responding to BX+ was higher than responding to B- in the second and third block. An ANOVA of responses with surgical group as the between-

subjects factor, and reinforcement (B- or BX+) and block as within-subject factors partially supported this description. There was a significant effect of reinforcement, $F(1, 15) = 33.24$, $p < .001$, $\eta_P^2 = .689$, the rats were able to discriminate between B- and BX+. There was no group effect, $F < 1$, $\eta_P^2 = .005$, no effect of block, $F(2, 30) = 1.40$, $p = .261$, $\eta_P^2 = .086$. There were no interactions of surgery with either reinforcement, $F < 1$, $\eta_P^2 = .028$, or block, $F < 1$, $\eta_P^2 = .026$. There was a near significant Block x Reinforcement interaction, $F(2, 30) = 3.19$, $p = .055$, $\eta_P^2 = .175$, but no three-way interaction, $F < 1$, $\eta_P^2 = .046$.

Figure 60 shows mean responding to only the feature-negative stimuli of the discrimination (A+/AX-) for the control group and the lesion group. Responses were generally higher by the lesion group, but neither group showed any discrimination between the positively reinforced stimulus and the non-reinforced stimulus. An ANOVA on the feature-negative data revealed no significant effects, there was no discrimination between A+ and AX-, $F(1, 15) = 1.62$, $p = .63$, $\eta_P^2 = .097$, no group effect, $F< 1$, $\eta_P^2 = .012$, and no effect of block, $F(2, 30) = 1.15$, $p = .332$, $\eta_P^2 = .071$. There were no interactions with surgery, of block $F(2, 30) = 1.10$, $p = .347$, $\eta_P^2 = .068$, or reinforcement, $F < 1$, $\eta_P^2 = .016$. There was no Block x Reinforcement interaction, $F < 1$, $\eta_P^2 = .040$, and no three-way interaction, $F < 1$, $\eta_P^2 = .022$. These results suggest that although both groups of rats were able to solve the feature-positive discrimination, they did not solve the feature-negative discrimination.

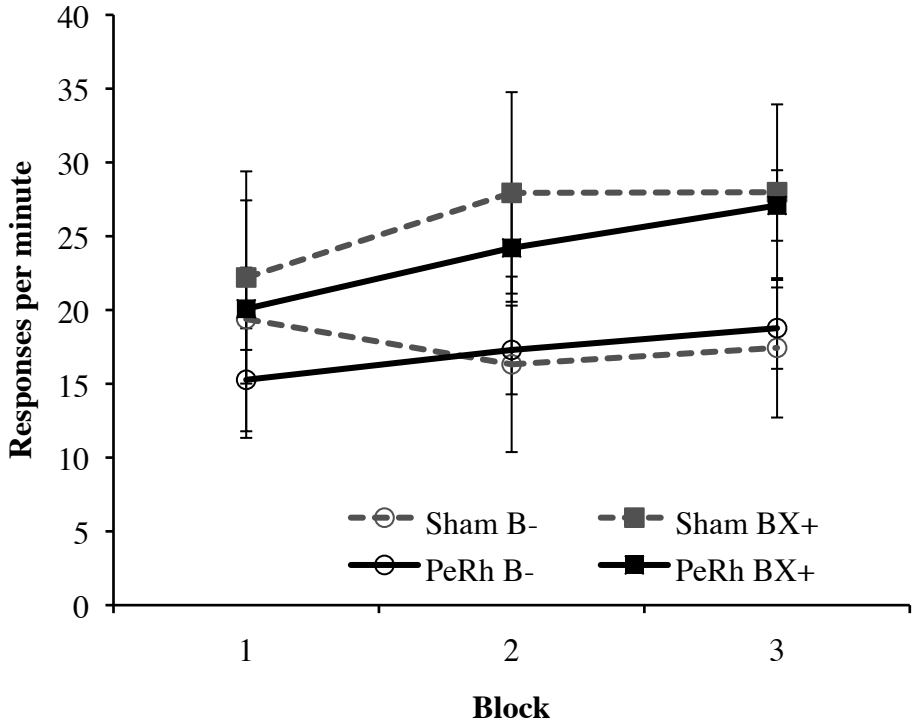


Figure 59. Responses per minute and standard error of the mean to B- and BX+ (feature-positive discrimination).

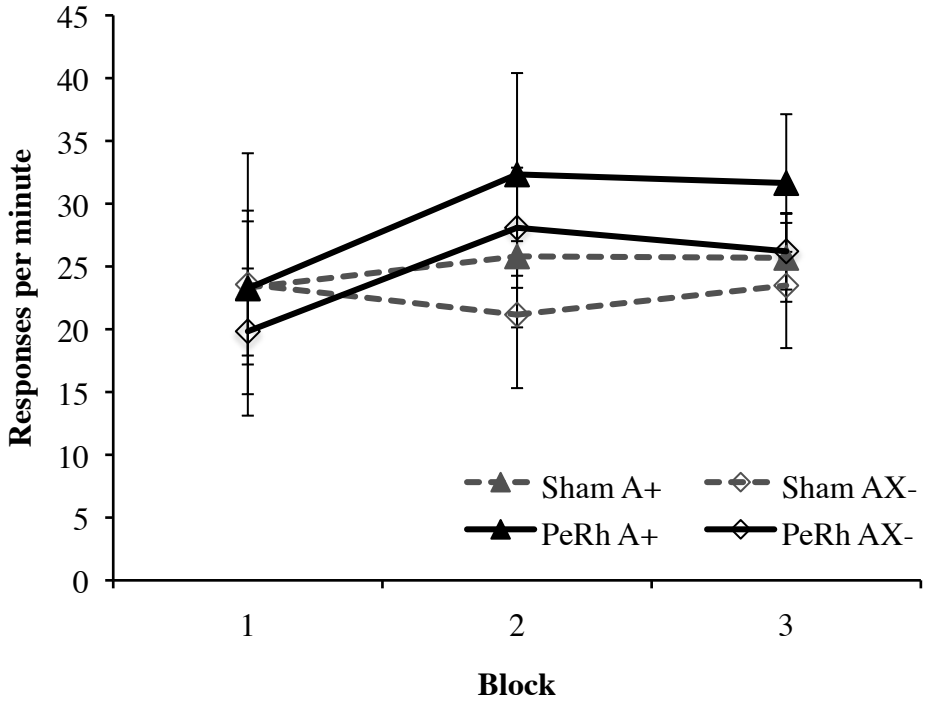


Figure 60. Responses per minute and standard error of the mean for A+ and AX- (feature-negative discrimination).

General Discussion

The aim of the present experiment was to investigate the effects of perirhinal lesions in rats on the ability to solve a feature-ambiguous discrimination. This was tested because of conflicting reports that the perirhinal cortex is important for ambiguous discriminations (e.g., Bussey et al., 2002), whereas other reports do not find a deficit (e.g., Aggleton et al, 2010; Clark et al., 2011). I used auditory and visual stimuli (like Campolattaro & Freeman, 2006a, 2006b) to test whether feature ambiguity was applicable across a range of stimuli.

Results from this experiment suggested that lesions to the perirhinal cortex did not impair acquisition of an ambiguous-feature discrimination. Results showed that there were no differences in responding between the sham group and the PeRh group. More detailed analyses showed that all rats were able to successfully solve the discrimination between reinforced and non-reinforced stimuli. When results were separated into a feature-positive and a feature-negative discrimination it was possible to see that rats were able to successfully solve the feature-positive discrimination, but not the feature-negative discrimination. Neither the sham group nor the PeRh group could successfully solve the feature-negative discrimination, suggesting that this finding was not based upon poor performance of those subjects with lesions.

These results suggest that the perirhinal cortex may not be involved in solving feature-ambiguous discriminations. These findings support those of Aggleton et al. (2010) and Clark et al. (2011), who reported that complex visual discriminations were not affected by lesions to the

perirhinal cortex. These results do not support the ideas of the representational-hierarchical model (Cowell et al., 2010a, 2010b), which states that perirhinal cortex is important for resolving feature ambiguity in complex visual discriminations. However, because the present experiment used auditory as well as visual stimuli, the model may have difficulty accounting for the present results anyway. Even if the model were to incorporate processing from other sensory areas, results from the present experiment conflict with predictions it would make.

This experiment found that rats with perirhinal lesions were able to perform normally in ambiguous-feature tasks, suggesting that perirhinal cortex has a role in discriminating novelty and familiarity, but not in discriminating rewarded and nonrewarded stimuli.

Appendix 2: Experiment 15. Textural Recognition

The aim of this experiment was to test rats' discrimination of textures. It was conducted in the same way as a standard object recognition experiment (Experiment 1); but, instead of objects, floor textures were used.

Method

Subjects.

Eight male Lister-hooded rats (*Rattus norvegicus*), supplied by Charles River (UK), served as subjects. Rats were pair-housed in identical cages that had plastic bases and steel bars. Cages contained sawdust, paper bedding, and a cardboard cylinder for environmental enrichment. Rats were kept in a room with lights on a 12-hr light cycle with an 0700 onset. The temperature in the holding room and the experimental room was 20°C ± 2°C with a humidity of 50 %.

On the day prior the test, rats weighed between 460 and 530 g, with a mean of 483.75 g, and had free access to food and water throughout the experiment. Rats had previously been used in Experiment 1 so were already familiar with the arena.

Apparatus.

The apparatus used were four identical white rectangular walled high-density polyethylene boxes (Mini Mobile, supplied by Slingsby, Shipley, UK). Each arena measured 60.0 cm (h) x 40.0 cm x 45.0 cm. A sheet of white acrylic was placed in each box to provide a flat floor. A black wooden frame supported a FireWire camera (Fire-I, Unibrain, Athens, Greece), which was fixed 90.0 cm over the centre point of the

floor of the arena. The view of each camera included the entire floor of its corresponding arena, and the lower portion of each wall. The camera was connected to a computer that was used to run AnyMaze video tracking software (Stoelting Co., Illinois, USA). This tracked the position of the rats' heads in the arena, so was used to record time spent in pre-specified zones where the objects were placed. Two lights were also positioned on the wooden frame (90.0 cm above the arena floor), each consisted of a circle of six light-emitting diodes (LEDs). These arena lights were on throughout the experiment. As well as the arena lighting, ceiling-mounted fluorescent strip lamps lit the room where the apparatus was held.

There were two floor textures used. One textured floor covered half the arena floor, so that two could be placed in the arena at one time. One texture (40.0 cm x 25.0 cm) consisted of copper pipes, secured to a wooden plank at each end. There were eight pipes, each had a diameter of 1.5 cm, and there was a gap of 1.5 cm between pipes. The second texture was a mesh floor (40.0 cm x 25.0 cm), made of mesh wire laid over white Perspex. The mesh had a square pattern; each square was 0.5 cm x 0.5 cm.

Procedure.

During the sample stage, rats were exposed to one type of floor texture. Two identical floors were placed on the arena floor so that they covered the entire floor area (Appendix 3F). Half of the rats (n = 4) had the pipe texture, and the other half (n = 4) had the mesh texture. After five minutes of exposure, rats were returned to their home cage. The arena and floors were cleaned with an ethanol solution and paper towels. After ten minutes, rats were placed back into the arena, which now contained a copy

of the sample floor, and a novel floor. The position of the novel floor was counterbalanced, so for half the rats (n = 4) it was on the right side and for the other half (n = 4) it was on the left side. The test stage lasted for five minutes.

Data collation and analyses methods.

The measurement used was the duration of time that rats spent in a pre-specified zone that was placed over the centre of the floor. Each zone was rectangular (30.0 x 15.0 cm) and covered an area 442.5 cm^2. To ensure rats were only counted in one zone at a time, there was a 5 cm gap from the edge of each floor stimulus to the zone, this ensured that measurements were clear. Results are reported in percentage of time in the zone and in a discrimination ratio. The discrimination ratio was calculated by subtracting the time spent in the zone with the familiar stimulus (A) from the time spent in the zone containing the novel stimulus (B), divided by the time spent in A and B summed together. This gives a ratio that can range between one and minus one, where zero indicates no discrimination between stimuli. Data from the sample stage and test stage were reported.

Results

Sample stage.

In the sample stage, rats spent a similar amount of time in each side of the arena, left side M = 24.77 %, SEM = 3.05, right side M = 31.09, SEM = 2.49. This was confirmed by a t-test, $t(7) = 1.37$, $p = .214$, $\eta_P^2 = .21$.

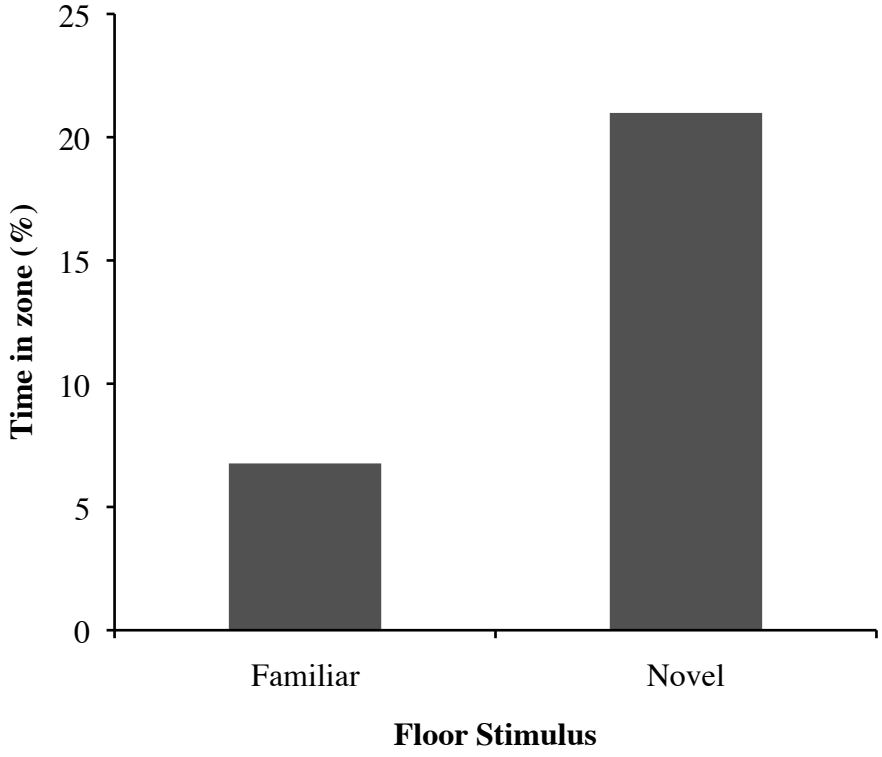


Figure 61. Mean time in zone (%) of familiar and novel floor.

Test stage.

Data from the first two minutes of the test stage were used. In the test stage, rats spent more time on the novel floor than the familiar floor (Figure 61). This was confirmed by a *t*-test, $t(7) = 3.19$, $p = .015$, $\eta_P^2 = .59$. The discrimination ratio was .482, this was significantly different from zero, $t(7) = 3.96$, $p = .005$, $\eta_P^2 = .69$. These results indicate that rats were able to discriminate between familiar and novel floor textures.

Discussion

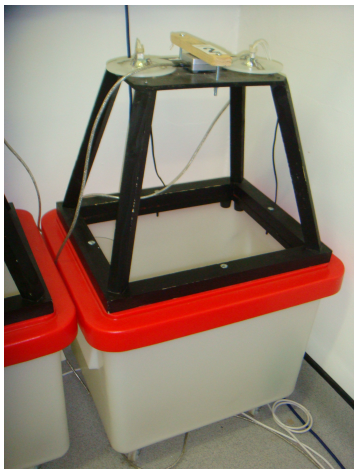
The aim of the present experiment was to test rats' discrimination of novel and familiar textures. Results showed that rats spent more time on the novel floor than the familiar floor, indicating discrimination. This may indicate that recognition memory involves other sensory stimuli as well as visual.

This experiment can only suggest the use of rats' perceptions of tactual stimuli. The experiment was conducted in a well-lit room, so the rats were able to use visual cues in addition to the tactile cues. A limitation with using the automatic tracking is that it does not work very well in dim light, so lights were left on in order to record data. To solve this, it might be possible to use red lights, as long as they were bright enough. Winters and Reid (2010) tested rats' discrimination of objects by texture; rats sampled stimuli and were tested in a red-lit room. Results showed that they did show discrimination. However, in red light using only visual stimuli, they were unable to discriminate stimuli.

The experiment demonstrated the potential of using textural stimuli in recognition experiments.

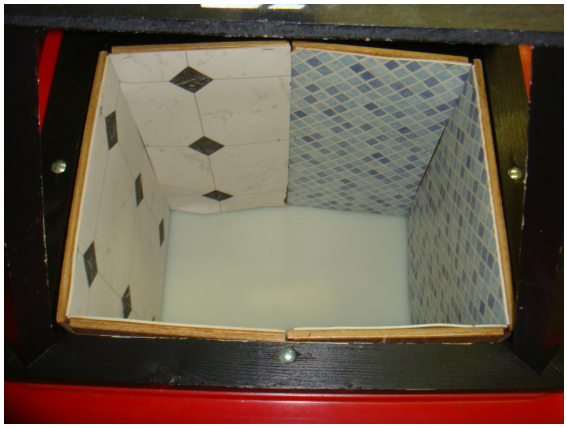
Appendix 3. Photographs of Apparatus and Stimulus Configurations

a). Photograph of arenas used in Chapter 2 and 3.



b). An example of stimulus set up for Chapter 2, Experiment 1 and Chapter 3, Experiment 6.

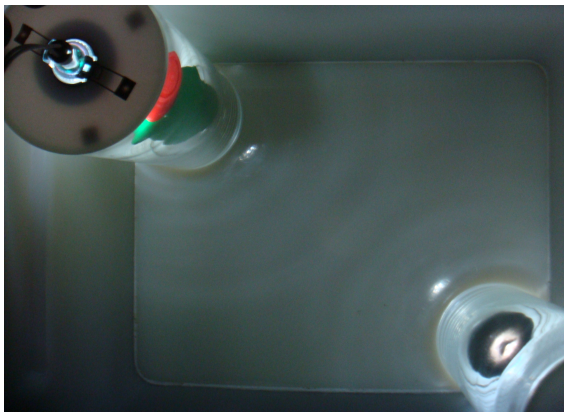
Sample stage



Test stage

c). An example of apparatus set up for visual object recognition and multiple stimulus presentations (Chapter 3).

Sample stage (normal light)



Test stage (arena lights)

d). An example of object recognition set up (Chapter 2, Experiment 1)

Sample stage

Test stage

e). An example of apparatus and stimuli set up for Chapter 2, Experiment 3.

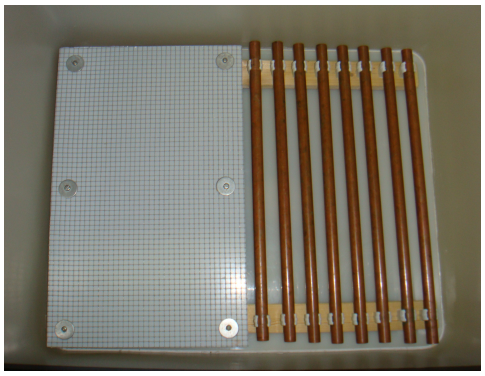
Stage 1, Trials 1 and 2

Stage 2

Test

f). An example of floor textures as used in Experiment 15 (Appendix 3).

Sample stage



Test stage

Appendix 4. Experiment Designs

Table 1
Experimental designs in Chapter 2. Priming object recognition.

	Stage 1	Stage 2	Test
Experiment 1	PP	-	PQ
Experiment 2	PX QY	PP	PQ
Experiment 3	PX QY	XX	PQ
Experiment 4	PX QY	XX	PQ

Note. P, Q, X, Y = stimuli, either objects or contexts.

Table 2
Experimental designs in Chapter 3. Spacing in object recognition.

	Sample	Test
Experiment 5	AA	AB
Experiment 6 Massed or spaced exposures	AA	AB
Experiment 7 Massed and spaced exposures	AA	AB
Experiment 8 Massed and spaced exposures, with either a long delay or a short delay between sample and test stage	AA	AB

Note. A, B = stimuli. These were objects in glass vases.

Table 3

Experimental designs in Chapter 4. Generalisation of familiarity.

	Preexposure	Conditioning	Test	Post test
Experiment 9	Nothing	Clicker -> Shock	Tone	Clicker
	Tone			
	Tone/Clicker 140			
	Tone/Clicker 280			
	Tone/Clicker 420			
Experiment 10 Perirhinal lesions	Clicker	Tone -> Shock	Clicker	Tones
	Tone/Clicker			
Experiment 11 Scopolamine in preexposure	Clicker	Tone -> Shock	Clicker	Tone
	Tone/Clicker			
Experiment 12 Scopolamine in conditioning	Clicker	Tone -> Shock	Clicker	Tone
	Tone/Clicker			
Experiment 13 Scopolamine in preexposure or conditioning	Clicker	Tone -> Shock	Clicker	Tone
	Tone/Clicker			

Table 4
Experimental design of ambiguous-feature experiment (Appendix 1).

Experiment 14	A+ B- AX- BX+

Note. A, B = tone or clicker, X = light. + = food, - = no food.

Table 5
Experimental design of floor recognition (Appendix 2).

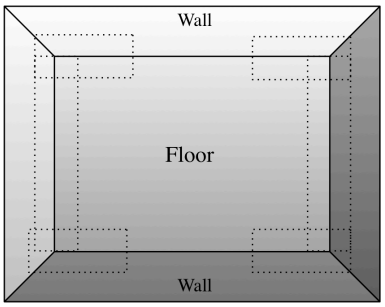
	Sample	Test
Experiment 15	AA	AB

Note. A, B = floor stimuli.

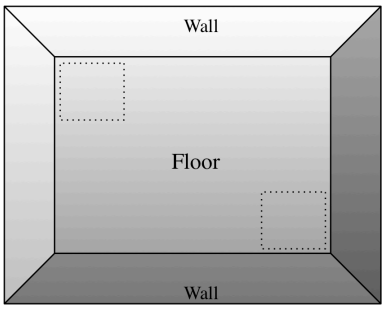
Appendix 5. Zone placement in arenas

The following are diagrams representing the zones used in experiments in Chapter 2 and 3. The diagrams give a birds-eye view of the arena, which is similar to the camera view. The zones are represented by the dotted lines. Sizes in relation to the arena are approximate.

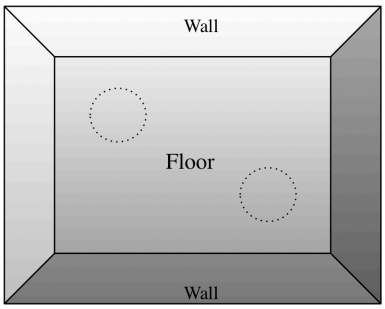
a) Zones for Experiment 1, group Context.



b) Zones for: Experiment 1, group Object; Experiment 2, group Context; Experiment 4.



c) Zones for Experiment 2, group Object, Experiment 3



d) Zones for Experiment 5, 6, 7, and 8.