EMPIRICAL AND METHODOLOGICAL INVESTIGATIONS INTO NOVELTY AND FAMILIARITY AS SEPARATE PROCESSES THAT SUPPORT RECOGNITION MEMORY IN RATS AND HUMANS

Magali H. Sivakumaran

A Thesis Submitted for the Degree of PhD
at the
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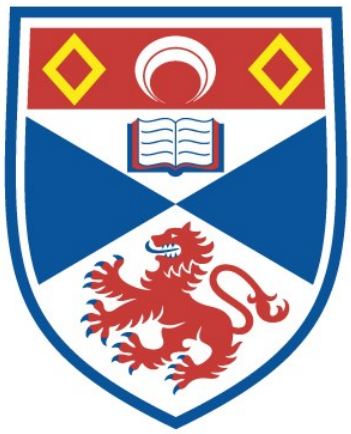

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Empirical and Methodological Investigations into Novelty and Familiarity as Separate Processes that Support Recognition Memory in Rats and Humans.

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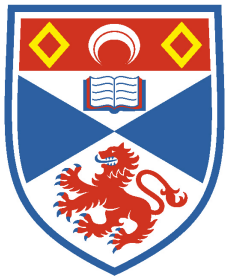

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ABSTRACT

There is a prevalent assumption in the recognition memory literature that the terms "novelty" and "familiarity" ascribe to differing extremities of a single memory strength continuum. The aim of the current thesis was to integrate experimental methodologies across human and rodents to further investigate novelty processing at both a cognitive and neural level, and assess its potential dissociation from familiarity processing. This dissociation was questioned at a cognitive level in human participants in Experiments 1 to 3 and at a neural level in rats in Experiment 4 and 5. Participants were found to differentially assess novelty and familiarity when making confidence judgements about the mnemonic status of an item (Experiment 1). Additionally, novelty and familiarity processing for questioned items were found to be dissimilarly affected by the presence of a concurrent item of varying mnemonic status (Experiment 2 and 3). The presence of a concurrent familiar item did not impact novelty processing in the perirhinal cortex (Experiment 4 and 5), yet disrupted the neural networks established to be differentially engaged by novelty and familiarity (Experiment 5). These findings challenge the assumption that the terms "novelty" and "familiarity" relate to a single recognition memory process. Finally, to allow integration of the findings from the human and rodent experiments, the relationship between measures of recognition memory obtained from spontaneous object recognition (SOR) task in rats and recognition memory measures estimated from signal-detection based models of recognition memory in humans was investigated (Experiment 6 and 7). This revealed that novelty preference in the SOR was positively correlated to measures of recognition memory sensitivity, but not bias. Thus, this thesis argues for the future inclusion of a novelty as a dissociable process from familiarity in our understanding of recognition memory, and for the integration of experimental methodologies used to test recognition memory across species.

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1. CHAPTER ONE: GENERAL INTRODUCTION

1.1 Chapter Overview

Knowing whether or not perceived material has been previously encountered is a skill regularly taken for granted. Yet this ability, termed recognition memory, is of considerable importance: it determines adaptive behaviour by allowing recognition of any differences in the environment, and subsequent implementation of the cognitive and behavioural modifications that may be necessary to respond to these. Indeed, recognition memory has been given significant attention within the memory research field. Our understanding of the cognitive processes and neural structures that underlie recognition memory is not complete. The existence of a comprehensive insight into the normal functioning of recognition is, however, demanded to allow the exploration of the deficits of recognition memory seen in both normal healthy aging and in clinical settings, such as those central to the dementias. Achieving such a comprehension relies upon two important components: firstly, a detailed evidence-based description and definition of the theoretical components of recognition memory, and secondly, the reconciliation of evidence and practices from different experimental sources (such as the animal and human literature) targeting different levels of explanation of recognition memory processing (such as the cognitive and neural components supporting these).

Until recently, the intuitive focus in recognition memory research has often been on old items, i.e on Hits (*H*), the correct recognition of an old previously encountered item, and on Misses (*M*), the failure to recognise an old previously encountered item. However, there is another, little discussed, side to recognition memory: identifying the novelty of a never previously encountered item. The terminology used to identify responses to new items highlights this focus on recognition of old

items in recognition memory. Correctly identifying a new item as such is termed a Correct Rejection (*CR*; correctly rejecting that this is an old item) while failing to identify a new item as such is termed a False Alarm (*FA*; falsely identifying a new item as old). The oversight of the component of novelty identification is understandable given the ease in intuitively considering these more or less equivalent, such that these are opposite ends of a single continuum, or inverses of a single process (i.e low familiarity = high novelty and vice versa). The difference in nomenclature would then simply reflect the differing directions in which the process or continuum is being considered or discussed. However, there is a growing body of evidence proposing that this assumption may be incomplete or even incorrect. Recent research has indicated that the identification and processing of novelty and familiarity may involve differing brain structures, processes and/or networks (Albasser, Poirier, & Aggleton, 2010; Burke et al., 2011; Burke, Wallace, Nematollahi, Uprety, & Barnes, 2010; S. Daselaar, Fleck, & Cabeza, 2006). Furthermore, a subset of these has raised the possibility that the deficits in recognition memory seen in normal healthy aging may be due to issues in novelty detection rather than an inadequacy in a familiarity or memory signal (Burke et al., 2011, 2010). As such, while previously generally overlooked, novelty and its processing becomes a fundamental facet of recognition memory requiring further investigation.

In the mid-1990s Tulving and colleagues (Tulving, Markowitsch, Craik, Habib, & Houle, 1996) emphasised that novelty played an important role in memory. Their novelty-encoding hypothesis outlines that due to “novelty assessment networks in the brain” (Tulving & Kroll, 1995, p. 389), novel items are better encoded, and hence subsequently retrieved, compared to familiar ones. Thus, especially under incidental encoding conditions (Kormi-Nouri, Nilsson, & Ohta, 2005), novelty detection is of significant importance as it informs later memory. This hypothesis places novelty within the landscape of memory research but depicts its processing as a facet of long-term memory encoding, rather than a component of recognition memory in its own right. In order for the enhanced encoding of a novel item to take place, this item must first be identified as new through the use of recognition memory. As

implied by the novelty-encoding hypothesis, the consequences of identifying novelty are considerable.

Furthermore, the scope of the importance of novelty in recognition memory becomes clear when we acknowledge the broad range of forms which novelty can take: from the absolute novelty of things never previously experienced (item novelty, e.g. encountering a platypus for the first time), to the new, unfamiliar or unexpected configurations of old things (context novelty, e.g. the re-location of an item of furniture to a different room), and further still to the novelty of a changed old thing (change novelty, e.g. someone's new haircut). In a similar manner to recognition of old items or associations, most (if not all) of these aspects of novelty rely on some form of representation against which they may be assessed. Experiences are compared to past representations to detect never previously encountered or new configurations of old things, while experiences must also be compared to what is expected for detection of unexpected or change novelty. Certainly, all such representations, even the generation of future expectations, are built based upon what is known and understood about the environment, with such representations based on past experiences (Bower, 2000). Thus, in its similarity to the conceptualisation of the recognition of old things, it is argued here that novelty identification is best conceived as a component of recognition memory. It is important here to note a differentiation in these types of novelty. Change and context novelty evoke newness based on associations between familiar things. On the other hand, item novelty is based on the lack of a previous encounter with that item and is therefore a theoretically a more absolute form of novelty. This difference is highlighted here as it will be relevant when discussing both cognitive models of recognition memory and their supporting neural structures in later sections.

With the importance of novelty in mind, it is necessary to re-visit and question the assumption that novelty and familiarity are opposites of a single process. The ostensible supposition that novelty is simply a lack of familiarity, and vice versa, has been deeply rooted in theories of recognition memory research. This is so deeply

ingrained that it permeates even the theoretical models and methodological constructs used to study it. An overview of these and their considerations in the context of the above assumption will be presented, followed by a review of the data obtained in the human and animal literature pertaining to recognition memory processes based upon these, amongst other, models and methodologies. What is known about recognition memory will be explored both at neural and cognitive levels. This review informs the direction of the research presented in this thesis, which aims to investigate novelty processing within a recognition memory framework, attempting to bridge the recognition research from differing species and at differing levels of analysis.

1.2 The Assumption that Novelty is the Opposite of Familiarity in the Literature

The assumption that novelty is the opposite of familiarity is inherent both in the tasks used to study recognition memory in rodents, and in the dominant theories of recognition memory. These are outlined below within the context of this assumption.

1.2.1 The Spontaneous Object Recognition Task

Behavioural animal recognition memory tasks frequently exploit animals' predisposition to explore and orient to novelty. Because no instructions may be given to, and no verbal feedback collected from animals, inferences about cognitive processes must be made based upon observable behaviour. Numerous animal species including rats and non-human primates show an innate novelty-based behaviour called orienting: they will orient their attention towards, and spend more time exploring, a novel object compared to a familiar one. The assumption researchers have made is that this facilitates encoding information about a novel item to inform behaviour for future occurrences of this item (see the novelty-encoding hypothesis, Tulving et al., 1996 outlined in Section 1.1). It is this overt

behaviour that is exploited in much recognition memory research. Consequently, recognition is assessed behaviourally as a lack of this orienting response, i.e. a lack of an innate novelty-based response.

This is the case in the Spontaneous Object Recognition (SOR) task (Ennaceur & Delacour, 1988) used within much of the animal recognition memory literature. In its simplest form, an animal is shown two identical objects, AA, and following a delay, is shown a copy of this object and a new object, AB. If it orients preferentially to the new object B, an inference is made such that the animal has "recognised" the old object A, as displayed by its lack of orienting to it. On the other hand if the animal oriented to both objects equally, the lack of a preferential orienting response to the new object B is assumed to demonstrate a lack of recognition for the old object A (for a review of the SOR see Antunes & Biala, 2012; Kinnavane, Albasser, & Aggleton, 2015). Assessing recognition as an absence of a novelty-related behaviour in this way relies on the assumption that novelty and familiarity are inverses of the same process: the absence of one is the presence of the other. However, if novelty and familiarity-related cognitive and neural processes are not correspondent, but rather are separate processes which work together in recognition memory, then these tasks and their inferences are flawed: the presence of one doesn't preclude the presence of the other. Rather, animals' behaviour may result from one or the other, or an interaction of both processes.

Interestingly, in the human literature the forced-choice paradigm, in which a novel and a familiar item are presented to participants who have to identify which is old, and therefore which shares similarities with the SOR, is considered not to require identification of familiarity or novelty *per se* (Macmillan & Creelman, 2005). This is because items do not have to be identified as either old or new for completion of the task, rather participants can solve the task by identifying which it is *relatively* the most familiar. This is also true of the animal SOR, and thus caution must be implemented when interpreting that animals are "recognising" the old item as old and orienting to an item they consider new. The animal may consider both items old

or both items new, simply differentiating between these based on the level of memory strength. Regardless however, the assumption remains that novelty and familiarity are words pertaining opposite ends of to a single memory strength continuum.

This assumption is also present in the theoretical models of recognition memory. Currently, two schools of thought about the theoretical processes supporting recognition memory are prevalent in the literature. The first characterizes recognition memory in terms of a single process (the Unequal-Variance Signal Detection Theory model; UEV-SDT). The second advocates recognition memory is best characterised by two processes (the Dual-Process model – DP): familiarity and recollection. Extensive available literature and reviews explore the reasoning and comparisons between these models (for e.g. see Wixted, 2007; Yonelinas, 2002; see Section 1.3 for a more detailed discussion), and hence this will not be discussed in detail here. However, both of these integrate the assumption that novelty and familiarity are opposites of each other, as this is inherent to the signal-detection based model used to characterise the UEV-SDT and the familiarity component of the DP theory, and thus these model will be outlined and discussed below within the context of this assumption.

1.2.2 Single and Dual Process Models of Recognition Memory

Signal-detection theory (SDT) is well situated to both describing and assessing individual's recognition memory performance. Indeed, SDT allows an explanation of how decisions (in this case: is it familiar or not?) are made based upon a continuous variable (in this case memory strength or familiarity). According to single process models of recognition memory, stimuli that are encountered lead to varying levels of memory strength (or familiarity). This memory strength evidence is then assessed and compared to a threshold. If memory strength is large enough (i.e. above the threshold), the item is classified as familiar (often referred to as "old"). On the other hand, if memory strength is too low, falling short of the threshold, then the item is

classified as un-familiar (or new). Noise is introduced into this decision process as memory strengths for “old” and “new” items are variable. In this equal variance SDT model, these variations are considered to be normally distributed, such that frequency distributions of these lead to overlapping equal-variance Gaussian-distributions (see Figure 1.1a). The level of separation between the means of these distributions is termed sensitivity (*d'*). Sensitivity thus reflects how distant old and new items are from each other in memory along the memory strength continuum and is therefore considered to represent memory ability. The placement of a decision threshold (termed bias or criterion (*c*) in SDT) along the memory strength continuum will always lead to some errors, where errors may either be falsely recognising a new item as “old” (a False Alarm - *FA*) or failing to recognise an old item, mistakenly identifying it as “new” (a Miss - *M*; see Table 1.1). Here a clear link can already be seen between familiarity-based recognition and novelty assessment, where simply the placement of a criterion differentiates the decision to classify an item as familiar or novel.

Table 1.1: Contingency table of responses to objectively new and old items and the terms given to the classification of these responses.

		Response	
		"Old"	**"New"**
Item	**Old**	Hit (*H*)	Miss (*M*)
	New	False Alarm (*FA*)	Correct Rejection (*CR*)

While this is a simple model, empirical data challenges the equal-variance frequency distributions model. Indeed, numerous studies have demonstrated that the old items are related to a greater variability in memory strength than new items (e.g. Mickes et al., 2007; Yonelinas, 1994; see Koen & Yonelinas, 2010; Ratcliff et al., 1992 for a review). Resolving this robust finding to the equal-variance model of recognition memory can be achieved in two different way: (i) by allowing the old item distribution variance to change, leading to an UEV-SDT model (Figure 1.1b), or (ii) by suggesting a second component (termed recollection) responsible for the high confidence old decisions, leading to a DP model (Figure 1.1c).

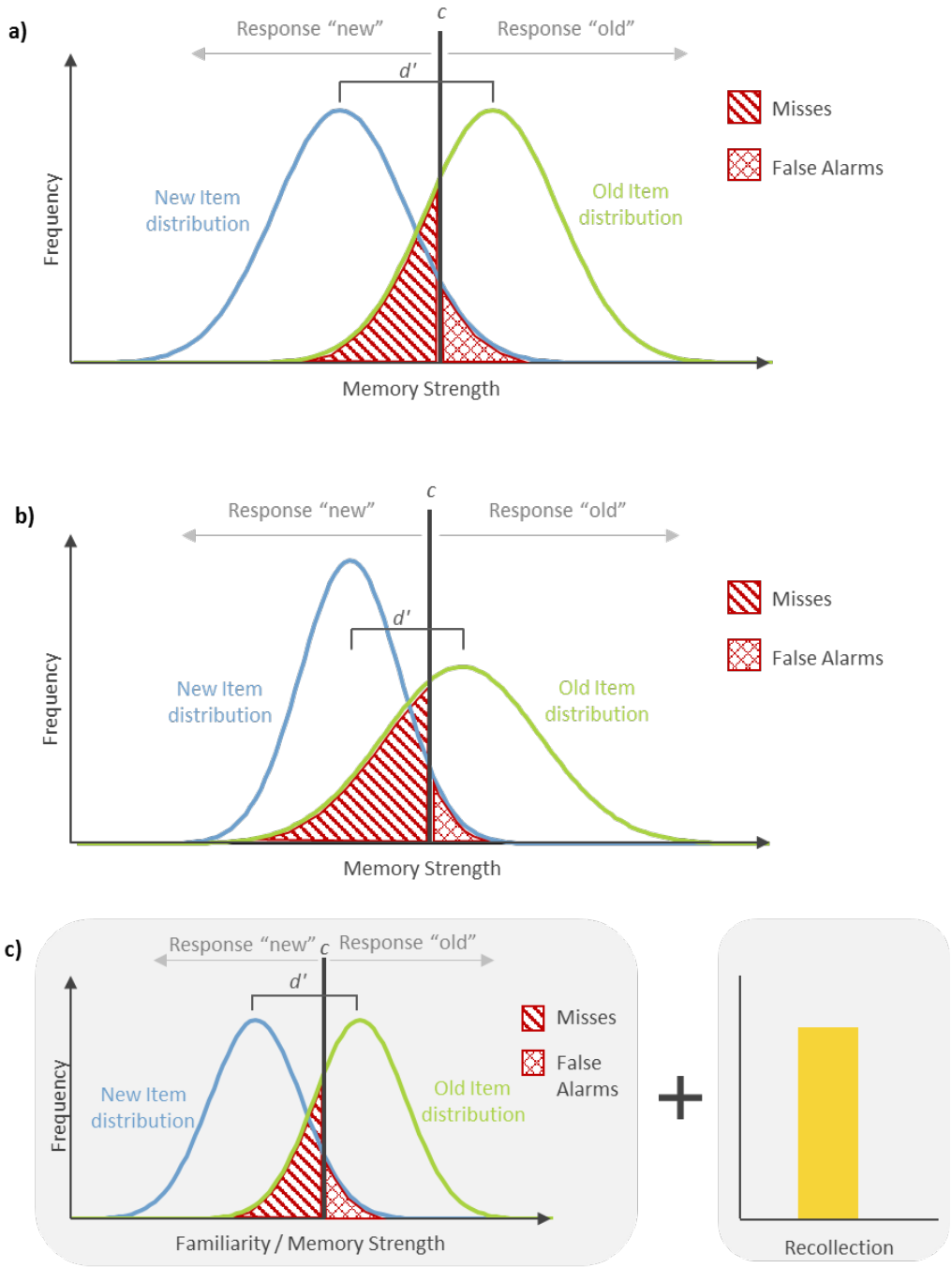

Figure 1.1: Models of Recognition Memory. a) Equal-Variance Signal Detection as a model for recognition memory supported by a single process representing the frequency distributions for objectively new (blue) and old (green) items. How well these items are differentiated in memory along the memory strength continuum is termed sensitivity (*d'*). A decision threshold (criterion – *c*) is placed along the memory strength continuum, with items falling above this being responded to as "old" and items falling below this being responded to as "new". This leads to errors, such that some objectively old items are called "new" (Misses - *M*) and some objectively new items are called "old" (False Alarms – *FA*). b) UEV-SDT model, where old item distribution variance is a free parameter. c) DP model with an EV-SDT familiarity process and a separate threshold recollection component.

Both of these have sound theoretical backing. As argued by Wixted (2007) in support of the UEV-SDT, old items can be conceptualised as new items to which memory strength has been added. At encoding, differing levels of memory strength can be added to different items, hence leading to a greater variance in the old item distribution (Wixted, 2007). Contrastingly, the DP model proposes that the greater old item variability is a product of having two processes contributing to this – both recollection and familiarity (Figure 1.1c), rather than the single process (familiarity) which supports new item memory strength. Unlike familiarity, recollection is a considered to be a dichotomous process: contextual/associative information about the previous encounter with an item is either recalled or not. If specific information about the previous occurrence of an item is recalled, then there is no doubt that this item was previously experienced, and hence leads to high confidence old responses. Thus, old items have an additional process contributing to high confidence old judgments, which in turn results in greater variance in the old item memory strengths. Of significant importance, these two theoretical processes supporting recognition memory correspond well to the human experience of recognition memory, as outlined below. As in Mandler's (1980) classic "butcher on the bus" example, most people will have experienced the phenomenon of meeting someone in passing (i.e. on the bus) who they feel they know, and yet are unable to identify. It is not until further "searching" in memory that the person is identified (i.e. he is the butcher) through remembering a detail about the person from a previous experience (i.e. the butchers apron). This would suggest that recognition memory is based on two processes: the feeling or assessment of familiarity, and the recollection of specific information surrounding what was recognised. This is precisely what the dual process model of recognition memory outlines, where familiarity and recollection are thought to be separate processes which are functionally independent at retrieval, and have distinctive features, as discussed in Section 1.3.1 of this thesis (see Yonelinas, 2002 for a discussion of this). It is important to note here that within the DP model framework, novelty recognition could be either recollection or familiarity dependent depending on the type of novelty referred to. Recognising novelty based on the context or associations of familiar items (e.g. the

new association of an item of furniture re-located to a new location) would be considered recollection dependent, while item novelty is purely based on whether an item was previous experienced or not, and therefore its recognition is considered to be based on the familiarity component of recognition memory. On the other hand, recognising novelty in all its forms is always based on memory strength in the UEV-SDT model.

Importantly, for the purpose of this thesis, under both the theoretical premise of single and dual process models of recognition memory, novelty for items is considered to be equivalent to the absence, or a very low level, of familiarity/memory strength, as depicted in Figure 1.1. Thus, the assumption that novelty and familiarity are inverses of the same process is inherent to both of these models, and consequently recognition literature more broadly.

The aims and context of the recognition memory research presented in this thesis were used to guide the consideration of which of these models should be used as the primary framework/backdrop against which to characterise recognition memory processes and develop this research. Broadly speaking, the research in this thesis aims to explore novelty processing in recognition memory, questioning its equivalence to familiarity processing, while bridging the animal and human recognition memory fields. As outlined below in Section 1.4.1 and 1.4.2 of this thesis, compared to the UEV-SDT model, the DP theory accounts for, and is supported by, a greater body of evidence from a variety of related fields such as cognitive psychology, neuroimaging, clinical psychology and animal neuroscience (see Yonelinas, 2002, for a review). This converging evidence from areas using different methodologies and with different assumptions, makes this model a good candidate. Furthermore, where possible when bridging fields, it is important to start form converging schools of thought and much of the animal neuroscience literature also favours the DP model (see Section 1.4.1 and 1.4.2 below). Finally, the DP model provides the constraint of having a process pure familiarity component. This allows for clearer predictions and tests when interrogating the novelty component of

recognition memory, and specifically the assumption that novelty and familiarity are words representing a single underlying memory process. For these reasons, the literature pertaining to the DP theory is used as the theoretical basis to define, describe and investigate the components of recognition memory in question.

A key assumption about the relationship between novelty and familiarity is that they reflect differing ends of a single memory strength continuum, and therefore this assumption is intrinsic to the familiarity component of the DP model of recognition memory. As such, to enable investigations into the processing of novelty, it is essential to characterise familiarity as it is currently understood, and subsequently establish what is known about the neural and cognitive processes supporting it, to enable investigations into the potential differences between this and novelty.

1.3 The Familiarity Component of Recognition Memory

As the aim of the current thesis is to investigate novelty processing, and the current assumption is that novelty reflects the opposite of familiarity processing, an understanding of novelty as it is currently considered in the literature is dependent upon the characterization of familiarity processing.

1.3.1 Characterising Familiarity

It is generally agreed that familiarity occurs faster than recollection. In two experiments, Hintzman and colleagues (1997; 1998) demonstrated that pure familiarity judgements (do you recognise this word?) were significantly faster (by about 100ms) than asking participants to recollect which modality the word was presented in (either seen or heard; Hintzman & Caulton, 1997; Hintzman, Caulton, & Levitin, 1998). Similar findings were observed by Yonelinas and Jacoby (1994). Identifying the modality of presentation or associated words requires recollection of details pertaining to the study phase, and is therefore often used as a measure of

recollection. On the other hand single item recognition is often used to test familiarity as this can be solved purely on memory strength.

Although, as Yonelinas (2002) indicates, many models make no predictions about the automaticity or controlled nature of familiarity and recollection, the speed at which familiarity information appears to become available would suggest a more automatic process in comparison to recollection. Moreover, this appears to reflect the human experience of recognition memory outlined in Section 1.2.2.

This is further supported by the research looking at the effect of divided attention, both at study and at test, on recognition performance. Divided attention during both of these impairs participants ability to recall recollection based source information significantly more than it affects participants familiarity based recognition of single items (Troyer, Winocur, Craik, & Moscovitch, 1999). Castel and Craik (2003) also demonstrate that divided attention at study appears to reduce future recollection significantly more so than future familiarity-based recognition judgements. Anderson, Craik & Naveh-Benjamin (1998) demonstrated a similar finding for divided attention conditions at tests, where such divided attention caused a greater impairment in free recall (Experiment 1) than in familiarity based recognition (Experiment 4). This data set must be interpreted with caution as no effect of divided attention at test was found for cued recall (Experiments 2 and 3). As this requires recall based on association, deficits would be expected based on the dual process model of recognition memory if divided attention at test impairs recollection. This discrepancy may be due to the fact that participants were asked to overtly recall words rather than responding by forced choice, making this a recall rather than a recognition task. Nonetheless, data on divided attention conditions both at encoding and retrieval endorses the notions that recollection is more cognitively demanding than familiarity, which in turn supports the hypothesis that familiarity is a more automatic process than recollection.

Familiarity is also more susceptible to interference than recollection. Hockley (1992) demonstrated stable recognition of word pairs (based on associations between words and hence recollection) across 2-8 intervening items in a continuous recognition task, whereas item recognition (familiarity based) declined gradually across the same intervening intervals. This was demonstrated across three experiments using both a forced-choice task (participants indicated whether the word or pair was old or new) and when participants were asked to select the old item or pair from those presented on the screen. Yonelinas and Levy's (2002) study showed similar results in a continuous recognition paradigm. Single words were presented on screen to participants. Study words were presented either in red or green, with participants instructed to create an association between the word and the colour (e.g. as cited in the original study associating the word mountain presented in red with a volcano). Test item words were presented in white with a colour prompt ("??red??") and participants indicated "yes" if the test item was presented in the colour matching the prompt or "no" if the test item was either presented in the other colour or was not previously seen during the experiment. The number of intervening items differentially influenced item and associative recognition memory. Item recognition accuracy showed linear decrease as the number of intervening items increased from one to thirty-two, whereas recognition for word-colour associations remained constant over these intervening item numbers. These studies indicate that familiarity is more prone to interference for intervening stimuli than recollection at relatively short delays.

As previously outlined, novelty detection is most likely to relate to familiarity processing. The signal detection model depiction of familiarity comprises a clear novelty component, while our subjective experience of novelty assessment being mostly automatic suggests a parallel or interaction with this faster and more automatic component of recognition memory. Having characterised various aspects of this familiarity component, let us review what is established with respect to the neural and cognitive processes considered to support it.

1.4 Cognitive Processes and Neural Structures Supporting Familiarity

1.4.1 Evidence from Patients and Animal Lesions

Data from neuroscience across species has supported the DP model of recognition memory, suggesting dissociable neural correlates for familiarity and recollection processing (Aggleton, Albasser, Aggleton, Poirier, & Pearce, 2010; Aggleton, Brown, & Wan, 1999; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002; Nemanic, Alvarado, & Bachevalier, 2004; Norman & Eacott, 2005 see Brown & Aggleton, 2001; Eichenbaum, Yonelinas, & Ranganath, 2007; Yonelinas, 2002 for reviews). While some amnesic patients demonstrate both recollection and familiarity impairments (Hamann & Squire, 1997; Stark & Squire, 2000), others seem to only be impaired on the former (Aggleton et al., 2000, 2005; Baddeley, Vargha-Khadem, & Mishkin, 2001). Indeed these differences are explained by the amount of damage to the patients' medial temporal lobe (MTL) (Aggleton & Brown, 1999; Aggleton & Shaw, 1996). Patients with extensive MTL damage may present deficits as a result of damage to the hippocampus proper or from the surrounding parahippocampal cortex.

Unlike animal studies where neural lesions can be induced, the selectivity of lesions in humans are harder to control. Their induction is rarely controlled, and where this is the case, the target area is dependent upon the patient's needs rather than specific neural boundaries. However, mild temporary oxygen deprivation (hypoxia) leads to hippocampal damage while sparing surrounding neural structures (Yonelinas et al., 2002). Compared to amnesiacs with extensive MTL damage showing deficits in both components of recognition memory, hypoxia patients show selective recollection impairments but no deficit in familiarity (Yonelinas et al., 2002). This

conclusion was drawn from both Remember(R)/Know(K)[1] and single item recognition procedures, strengthening its claim.

While a number of case studies of amnesic individuals with a selective recollection impairment (due to hippocampal damage) have been reported (see Eichenbaum et al., 2007, for a review), only one lesion example (to my knowledge) exists of a patient with a selective familiarity impairment. Patient NB underwent a surgically induced unilateral amygdala lesion to relieve severe drug resistant epilepsy (Bowles et al., 2007). Post-surgery high-resolution MRI scans showed successful ablation of the left amygdala, with significant damage to the entorhinal and perirhinal cortex unilaterally, while fully sparing the hippocampus.

Although NB's overall recognition performance was within the normal range of the control group, she showed significant impairments specifically for familiarity-based recognition in comparison to control. In an R/K paradigm without time constraints, NB made significantly fewer "know" responses than controls, while the opposite was true of "remember" responses. These results could have been obtained if NB has a remarkable recollection ability or uses encoding mechanisms to preferentially favour recollection (rather than simply not having access to familiarity). Bowles and colleagues (2007) ran this study again but forced participants to encode items at study on a short time scale. Creating association for future recollection is time demanding, so a forced time constraint was aimed at reducing NB's (and other participants') ability to make these. In this time-constrained task, NB's performance still showed a selective familiarity deficit.

Importantly, Bowles and colleagues (2007) also demonstrated that NB's impairment is not a metacognitive one in which she is unable to appraise her recognition

[1] Remember(R)/Know(K) tasks consist of asking participants to judge, for a specific stimuli, whether contextual information from the study phase is recalled ("remember" response) or whether they have a feeling the stimuli was presented but can't explicitly remember seeing it ("know" response). "Remember" responses are thought to reflect recollective experiences, whereas "know" are thought to reflect feelings of familiarity.

memory feeling. NB participated in another time dependent single item recognition task. In this design, the ability to recognise a previously presented word is necessary, but without the need to appraise memory. Participants are not asked whether they "remember" seeing or "know" that they saw this word, rather participants simply indicate whether they recognise the word. Even without this appraisal component, NB showed an accuracy deficit only when she was made to respond quickly (400ms vs 2000ms). As previously outlined, a characteristic of familiarity is that it is available faster than recollection. Thus this data strongly support the claim that NB's recognition impairment is one of familiarity processing, and is only apparent when she is unable to use recollection to compensate for this.

However, while these findings suggest a deficit in familiarity processing rather than metacognition, similar data would have been obtained if NB takes or needs longer to make recognition judgements. Her performance would drop significantly when forced to do this task under time constraints, whereas when given more time this would allow the processing required for recollection to occur, leading to greater "remember" responses than control.

Interestingly though, when rating her memory judgement confidence on a scale from "sure new" to "sure old" through "unsure new/old", NB made fewer middle confidence judgements than controls. This advances that her recognition is not supported by a continuous variable (such as that characterising familiarity), but rather depends on a more dichotomous process (such as that characterising recollection). Although such data appear to provide evidence for the independence of both the cognitive processes and neural structures underlying familiarity and recollection, NB's impairment is not a complete absence of familiarity. Furthermore, as noted by Bowles and colleagues (2007), it is important to note that NB's "familiarity impairment […] [does] not manifest […] as a phenomenological absence of the feelings of familiarity, but as a faulty discrimination process with reduced accuracy" (pp. 16386). This suggests that that the experienced feeling of familiarity is dissociable from the discrimination process referred to as familiarity sensitivity in the

DP model. However, this statement is made with some reserve as NB's neurological damage is not only unilateral, but spared some perirhinal and entorhinal cortecies on the lesion side. NB's intact right perirhinal cortex along with the remaining functional perirhinal and entorhinal tissue on the lesion side (Bowles et al., 2011) are likely to account for this aspect of NBs recognition memory presentation.

Nevertheless, human data pertaining to a selective familiarity deficit is rare and this unique case study provides invaluable human data speaking to the neurological underpinning of familiarity. Although very different in nature, reinforcement for the conclusions drawn from NBs case study is also obtained from a clinical populations of patients with temporal-lobe epilepsy (TLE). A subset of these patients experience déjà vu as part of their seizures, where déjà vu is "[a] clash between two simultaneous and opposing mental evaluations: an objective assessment of unfamiliarity juxtaposed with a subjective evaluation of familiarity" (Brown, 2004, p. 2). As individuals often experience this as an error in recognition memory, déjà vu is frequently studied within the context of recognition. Using an R/K paradigm, Martin and colleagues (2012) demonstrated that TLE patients exhibit a familiarity impairment. Interestingly, the TLE sample tested was subdivided into two groups: a set of TLE patients who report déjà vu as part of their seizures (TLE+) and a group of TLE patients who do not report déjà vu as part of their seizures (TLE-). The familiarity impairment was seen in both patients groups when compared to the control group, but a recollection impairment was only seen in TLE- patients. Hence, TLE+ patients appeared to have a selective familiarity deficit rather than the more global recognition deficit seen in TLE- patients. When assessing the patients' structural neurology scans, the TLE+ patients were found to have more focal reductions in rhinal (perirhinal + entorhinal) cortex volume as compared to the TLE- and control group, which is likely to account for the differences in recognition deficits.

In support of the human studies discussed above, substantial animal data also highly implicates the perirhinal cortex in item- (and therefore familiarity-) based recognition (as reviewed by Aggleton et al., 1999; Brown, Barker, Aggleton, &

Warburton, 2012; Murray & Bussey, 1999). Perirhinal lesions have been widely shown to impair item recognition using delayed-non-match-to-sample (Meunier, Bachevalier, Mishkin, & Murray, 1993; Mumby & Pinel, 1994; Nemanic et al., 2004) as well as SOR tasks (Aggleton et al., 2010; Barker, Bird, Alexander, & Warburton, 2007; Ennaceur, Neave, & Aggleton, 1996). On the other hand, and contrarily to hippocampus lesion animals (Langston & Wood, 2010; Mumby et al., 2002) perirhinal lesion animals appear to perform similarly to controls in recollection-based tasks identifying new configurations of familiar items in place or context (Aggleton et al., 2010; Eacott & Norman, 2004).

However, the picture painted by data from perirhinal cortex lesions is not perfectly clear. Ennaceur and colleagues (1996) demonstrated a spontaneous object recognition deficit only after longer intervals (>1 minute) between sample and test phases, which is not typically explained by the theoretical understanding of familiarity processing (this may however be reconciled when taking into account interference, see Section 1.4.3 of this thesis). Perirhinal cortex lesioned rats also appear to only demonstrate a deficit in the SOR when items are visually similar (Bussey, Saksida, & Murray, 2002, 2003) or after visual interference (McTighe, Cowell, Winters, Bussey, & Saksida, 2010; see Section 1.3.3 for a detailed discussion). Barker and colleagues' (2007) perirhinal lesion rats showed a very different behaviour to controls when familiar objects were swapped in location. Whereas control rats spent more time exploring the familiar objects that had swapped location (i.e. in the place they had never occurred before) the perirhinal lesion rats preferentially explored the familiar object that had not changed location. Thus, they were able to differentiate between the familiar objects based on location, but show an abnormal orienting response.

The difficulty in interpreting some of the results from these tasks is distinguishing whether animals are using familiarity or recollection to solve them. Based on the dual process theory and as suggested by the human research, it is assumed that single object/item recognition is solved using familiarity, whereas recognition based

on associations between an object and its location or context is solved using recollection. There is, however, no indication that recollection is not also used for single item recognition. An elegant task has recently emerged from Eichenbaum's research team to tackle precisely this issue while also significantly improving the ability to compare and contrast human and rodent recognition memory. These individuals have developed a behavioural paradigm for rats from which the contributions of familiarity and recollection can be parcelled out. Given the scope of this thesis, only the study pertaining to familiarity processing will be explored, but this behavioural procedure has also be used to assess the hippocampus' role in recollection (Fortin, Wright, & Eichenbaum, 2004; Sauvage, Fortin, Owens, Yonelinas, & Eichenbaum, 2008).

This behavioural task is based on the Delayed Non-Match To Sample (DNMS) task. Rats are presented with ten odours at study. At test, the rats are presented with these ten odours intermixed with ten new odours. Rats learn over a series of long training procedures that digging in new odour cups is rewarded while rewards for old odours are available in the empty cup at the back of the cage. Once the rat has learnt the above, differing levels of difficulty and reward are introduced. Cups have five different edge heights. Digging in higher cups is significantly more uncomfortable than digging in shallow cups (i.e. cup height is used as a proxy for difficulty). However, if the rat gets it right and digs in the higher cup, the reward gained is significantly larger. In this way the rat's confidence in its recognition judgement can be assessed based on its decision to dig or not. This is then comparable to the confidence responses given by human participants.

Using this test procedure Favorik and colleagues (Farovik, Place, Miller, & Eichenbaum, 2011) demonstrate an extremely convincing lack of familiarity component for their lesioned animals as compared to controls. The recollection component was however no different between the two groups. Their result suggest a clear differentiation of familiarity and recollection and their supporting neural architecture. However, these results are collected from a group of amygdala rather

than perirhinal lesion rats. The authors argue that this was done as they wished to spare "the flow of […] information from perirhinal cortex to hippocampus" (p. 1416), such that amygdala lesions should disrupt the perirhinal cortex's familiarity processing while sparing the ability for the perirhinal cortex to relay object information to the hippocampus. This argument is not without justification, and indeed there is a current debate in the literature as to whether the familiarity deficit seen in perirhinal cortex lesions is not in fact due to deficits in visual processing (see Section 1.4.3). Nevertheless, given the data pertaining to the perirhinal cortex's role in familiarity processing, it is a shame that a perirhinal cortex lesion group was not available for comparison, and that this behavioural paradigm has not been tested with such a group. It should be noted here however that patient NB's (in whom familiarity deficits were recorded) surgery, although affecting the perirhinal cortex, was primarily a complete unilateral amygdala ablation. Hence, her deficits may be attributed to this amygdala damage that Farovik and colleagues (2011) demonstrate is sufficient to cause such deficits.

The extensive lesion and clinical data presented above support the notion that, while other areas may be implicated, the perirhinal cortex has a significant role in hosting familiarity processing. Further insight into how such processing may be achieved is available from neuroimaging techniques.

1.4.2 Evidence from Electrophysiology and Neuroimaging Studies

Unlike lesion studies, neuroimaging methods such as functional magnetic resonance imaging (fMRI) and single unit recordings of neurons enable recognition to be tested in intact healthy participants or animals. Difficulties in assessing whether familiarity and/or recognition are/is used by animals during tasks means the little neuroimaging data pertaining to this issue available need to be carefully considered.

In an elegant within-subject paired-viewing task, Wan, Aggleton and Brown (1999) placed rats in front of a screen in such a manner that items displayed on the right of the screen, and thus the right visual field, could only be viewed by the rat's right eye

and vice versa. This allowed them to simultaneously present single familiar and novel items to individual eyes. The hemisphere contralateral to the eye to which the stimulus was presented then interprets initial processing of visual information. Protein markers of neural activity from *c-fos* immediate early gene expression can then be imaged for different neural structures within an intact brain responding to visual stimuli. Using this experimental setup, Wan and colleagues (1999) showed lower *c-fos* expression for the perirhinal cortex contralateral to the eye viewing the familiar item as compared to that of the perirhinal cortex contralateral to the eye viewing the novel item. These results suggest greater perirhinal cortex activation for novel items, which is then reduced for familiar items (where single item recognition is assumed to be familiarity based).

Single unit recording studies in both rats and primates support these findings. Numerous studies in monkeys and rats have indentified neurons in the perirhinal cortex which alter their firing pattern based on the novelty/familiarity of an item (e.g. Roloff, Muller, & Brown, 2016; Xiang & Brown, 1998; Zhu, Brown, McCabe, & Aggleton, 1995; Zhu, McCabe, Aggleton, & Brown, 1996). A significant number of these neurons in the perirhinal cortex (≈25%) show an exponential decreased activation as stimuli are repeatedly presented (Brown & Aggleton, 2001; Roloff et al., 2016; Xiang & Brown, 1998). Although recent studies have failed to replicate this finding, demonstrating that perirhinal cortex neuronal activity is mediated by the presence of objects but not by their mnemonic status (Burke et al., 2012; Deshmukh, Johnson, & Knierim, 2012), this has been explained to be the results of a methodological artefact (Roloff et al., 2016). Indeed, in a within-subjects experiment, neuronal activity in the rat perirhinal cortex was found to be mediated by the mnemonic status of items when tested using a paired-viewing task in which items are presented on screen (as outlined earlier in this Section, see Aggelton & Brown, 1999), but not when tested using a standard SOR task in which 3D objects are explored (Roloff et al., 2016). There are notable differences between these methodologies. Most notably in the paired-viewing task the activity was averaged across multiple stimuli (> 20), each seen only briefly and associated with a reward.

Contrastingly in the SOR the single novel object was presented and explored for a significant amount of time. Indeed multiple trials will increase both the sensitivity of the measures to detecting a signal, and the statistical power allowing its detection. As such, based on the combined evidence in the literature and their findings, the authors suggest that these results demonstrate the lack of detectability of this mnemonically driven change in the neural activity in perirhinal cortex based on the task used, rather than an absence of this activity in the SOR per se. Thus these results support the finding that neurons in the perirhinal cortex support item recognition.

The hippocampus does not appear to be as important in coding for such information with less than 1% of neurons showing a stimulus repetition decrease, where these were also only observed at chance levels (Brown & Aggleton, 2001; Kumaran & Maguire, 2007). Crucially, various studies (often only assessing visual stimuli) have characterised the perirhinal cortex neuronal activation showing that it has all the qualities necessary for a reliable familiarity detection system: (i) responses are extremely rapid, (ii) they differentiate between stimuli well and (iii) they show long lasting (>24hrs) single trial learning (Brown & Aggleton, 2001; Xiang & Brown, 1998). Furthermore, these neurons appear to respond automatically as their activation is seen even in anaesthetised rats (Zhu et al., 1995).

Similarities can be seen between the data obtained from single unit recordings in animal and human fMRI data. Within fMRI studies, of most interest here are those showing a double dissociation of activity in the hippocampus and perirhinal cortex during recollection and familiarity judgements (e.g. Daselaar et al., 2006; Daselaar, Fleck, Dobbins, Madden, & Cabeza, 2006). These studies show an increase in hippocampal Blood-Oxygen-Level-Dependent (BOLD) signal related to recollection along with a decreased in perirhinal cortex BOLD signal as familiarity increases (Daselaar et al., 2006; Daselaar, Fleck & Cabeza, 2006). A review of event-related fMRI studies in humans shows that this decrease in perirhinal BOLD signal with increased familiarity, paralleling the animal neuroimaging data, is reliable (Brown &

Aggleton, 2001). Importantly, plotted against confidence ratings the relatively linear BOLD activity seen in the perirhinal cortex matches that which is expected under the assumption that familiarity is a continuous variable.

Interestingly, the finer grained exploration of the perirhinal cortex enabled by neuroimaging techniques has pointed to neurons with differing roles. Xiang and Brown (1998), using single unit recordings in macaques, demonstrate a triple dissociation between neurons coding for familiarity, novelty and recency. Similarly, Daselaar, Fleck and Cabeza (2006) show a double dissociation between familiarity and novelty related fMRI BOLD signal at retrieval, while Habib, McIntosh, Wheeler & Tulving (2003) showed this dissociation for material at encoding. These findings importantly support the putting into question that familiarity and novelty are simply inverses of each other, rather these may in fact be differing converging processes. Before this is discussed in greater detail in Section1.5 of this thesis, any other roles the perirhinal cortex is proposed to have and any other structures which may also be responsible for familiarity processing need to be considered.

1.4.3 The Perirhinal Cortex – Debated Role

As seen from the multitude of studies presented, there is overwhelming evidence that the perirhinal cortex plays a significant role in recognising single items, with the extent and specification of this still being explored. However, there is a continuing debate about whether this should be characterised as a visual processing function rather than a mnemonic one (Murray & Bussey, 1999). The perirhinal cortex's anatomical position at the apex of the ventral visual processing stream with significant reciprocal connections to the medial temporal lobe places it at a potential junction between perceptual and mnemonic areas. The deficits seen in animals with perirhinal cortex lesions can be interpreted in either light. If the perirhinal cortex is the sole processor of familiarity, then its lesion would eliminate familiarity based recognition. Without this, animals would show a deficit in SOR tasks as they are unable to differentiate between the items at test based on familiarity. However, if the perirhinal cortex were responsible for the visual representation of whole objects

(Murray & Bussey, 1999), its absence would abolish an animal's ability to bind item's visual features into a cohesive object. This would mean that perirhinal cortex lesion animals would only have access to the use of un-bound visual features of objects (e.g. angles and colours), where these un-bound features, by virtue of being portions of objects, are likely to be shared by many objects. Again this would lead to the same behavioural SOR deficits, but this time caused by an inability to visually differentiate between test items. Indeed, there is evidence that perirhinal cortex lesion animals show deficits in visual discrimination tasks (Buckley, Booth, Rolls, & Gaffan, 2001; Bussey et al., 2002, 2003).

In a visual perception oddity task (chose the odd stimuli from an array of six), perirhinal lesion macaques showed a deficit when the discrimination required the use of combinations of features or whole object representation (e.g. for a whole face) but not when this depended on simple un-bound features (such as colour) (Buckley et al., 2001). Perirhinal cortex lesion animal's level of impairment on visual discrimination tasks is correlated to the amount of feature overlap between the stimuli used (Bussey et al., 2002, 2003). Demonstrating a role for the perirhinal cortex in visual perception does not however negate that the deficit seen during SOR tasks is also a mnemonic one.

McTighe and colleagues tested this using a modified SOR task (McTighe et al., 2010). In this task rats were presented with two identical objects in a sample phase (e.g. AA) and, after a one-hour delay, two identical objects in a test phase which were either the same as those presented in the sample phase (AA - familiar) or never previously presented objects (BB - novel). Rats were either placed in a dark room or in a holding cage for the delay duration. The rat's exploration rates for these objects were then compared across these different testing session conditions.
Their results showed that rats with perirhinal cortex lesions were only impaired if they were presented with other visual inputs in the delay between the sample and test phase (i.e. if they spent the delay in the holding cage). Curiously, this impairment manifested itself as a reduced exploration of the novel objects (i.e.

responding to novel objects as if they were familiar) rather than an enhanced exploration of the familiar object (which would suggest forgetting). The authors argued that this pattern of results is best explained if the perirhinal cortex is responsible for processing visual representations of whole objects rather than familiarity because a lack in the ability to process familiarity would lead to treating all objects as novel (i.e. forgetting). On the other hand, the novel objects in the test phase are likely to share visual features with the holding cage environment the rat was exposed to between the sample and test phases. Therefore, without whole object representations, relying only on these component features, novel objects appear familiar, as their component features are now familiar. The strength of this argument could be enhanced in a study where the specific visual features seen during the delay between same and test, and their overlap with the test objects' are manipulated.

Other research groups have recorded similar results. For example, Burke and colleagues investigated the role of the perirhinal cortex in rats and macaques using aged animals (Burke et al., 2011, 2014, 2010). As recognition memory declines in healthy aging, significant changes in the neurophysiology of the perirhinal cortex are apparent (for a discussion of these see Burke, et al., 2014). Aged animals are therefore presumed to be an ecologically valid model for perirhinal deficits. During a standard SOR task, aged rats were shown to lack a preferential orienting response. Careful examination of exploration times showed this was again due to treating the novel object as familiar rather than responding to the familiar object as if it were novel (Burke, et al., 2010). In a follow up study, aged rats and macaques were significantly impaired on object discrimination tasks only when the objects to be discriminated shared a large proportion of visual features.

Although these results are in line with both the findings and conclusions drawn by McTighe and colleagues (2010), there are some reservations about the use of aged rats. All the results in these studies can be attributed to age-related lower-level visual impairments (e.g. at the purely physical level of the eye). Some consideration was given to this such that rats were tested on visual Morris water maze tasks and

found to have normal performance. However, the visual discrimination needed for this performance is much less demanding than identifying very subtle differences in objects. Furthermore, Aggleton and colleagues (2010) demonstrate that their perirhinal lesion rats had a marked impairment on object discrimination, yet were able to learn and perform a task in which visual discrimination of features was necessary.

There is also one other important factor in the behavioural data that requires attention. Both aged and perirhinal lesion rats showed similar exploration times to control rats for the two novel objects presented during the sample phase (Burke et al., 2011, 2010; McTighe et al., 2010). If indeed the SOR deficit seen in the test phase was due to visual interference, then aged and perirhinal lesion rats should also show false recognition for the items seen during the sample phase, exploring these significantly less than the control rats do. This is especially true for the study run by McTighe and colleagues (2010), as the holding cages used between sample and test phases are presumably similar to the animals' home cages in which they would have been prior to the sample phase. Romberg and colleagues (Romberg et al., 2012) however, having shown the same pattern of results at McTighe and colleagues (2010) in a mouse model of Alzheimer's disease, maintain that the holding cage, while not devoid of visual interference, provides diminished levels of this compared to the home cage. They argue that the holding cage would then provide a small amount of "protection", and the darkened room a much greater level of "protection" from the visual interference the animals are exposed to in their home cage. Following on from this, the authors argue that the objects at sample provide sufficient interference to disrupt recognition at test, with this being "rescued" when a period devoid of visual information precedes this. However, this still does not account for the similar levels of exploration during the sample phase.

This view has been refuted by behavioural data showing that perirhinal lesion rats do not show an overall gradual decline in object exploration when tested on a series of consecutive SOR tasks in a bow-tie maze (Albasser et al., 2015), which presumably

provide significant levels of visual interference. Furthermore, these rats return to higher levels of exploration of novel objects even after numerous repetitions of familiar objects (Olarte-Sánchez, Amin, Warburton, & Aggleton, 2015). In this study, contrasting the results reported by McTighe and colleagues (2010), perirhinal lesion rats spent more time orienting to novel than familiar objects as long as these were not presented simultaneously. When perirhinal lesion rats were presented with a novel and a familiar item concomitantly, they explored the novel item as if it were familiar. However, if two novel objects were presented, these same rats spent significantly more time exploring these than if two familiar objects had been presented. Orlate-Sanchez and colleagues (2015) propose that the differences seen with the results from the study by McTighe and colleagues (2010) may be the result of the extent of the lesions in this later study.

Overall, when a novel and familiar item are presented concurrently, the total amount of time lesion and control rats spend exploring objects at test is not different (Orlate-Sanchez, et al., 2015). These data suggest that perirhinal lesion rats are able to recognise the presence of novelty but are unable to attribute this to a given object (Albasseur, et al., 2015; Orlate-Sanchez, et al., 2015). When faced with a novel and familiar object, lesion rats will spend the same extent of time exploring the objects as control rats, but with no discrimination, as if exploring the right amount given the presence of novelty but unable to direct that exploration to the correct source. Theoretically, as argued by Albasser and colleagues (2015), these data suggest that novelty signals are generated out-with the perirhinal cortex, where the perirhinal cortex allows binding of this novelty signal to a perceived whole object. Under this theory, while not directly responsible for novelty or familiarity, the perirhinal cortex plays a crucial role in recognition memory by hosting the processing which determines which items present are novel or familiar. These findings can suitably be incorporated with much of the familiarity processing research presented throughout this introduction.

While this section has outlined that a significant amount of data is available speaking to the questioning of the perirhinal's role as either perceptual or mnemonic, the patterns observed do not provide a definitive conclusion. The data presented here, rather, inplies that in all likelyhood the perirhinal cortex's function incorporates components of both of these. This has been proposed before, with the presentation of the rhinal cortex as a form of "gatekeeper" to the medial-temporal lobe and declarative memory system (Fernández & Tendolkar, 2006). In this view, activation from whole object representations lead either to a feeling of familiarity or not, where the less an item is perceived as familiar, the more resources are allocated for its encoding and the more effective information transfer to the hippocampus becomes (Fernández & Tendolkar, 2006). The anatomical positioning of the perirhinal cortex makes it an ideal candidate for such a function. Furthermore, the "gatekeeper" hypothesis integrates well with the "novelty-encoding" hypothesis described in Section 1.1 of this thesis, as encoding is prioritised for novel items. Indeed, it is easy to fall captive of human constructs and named concepts such as 'memory' and 'perception', trying to understand cognitive and neural functions within the borders of these boxes. However, is there really a defined boundary between these two? When does perception start becoming memory? Based on the data presented here, the perirhinal cortex is likely involved in aspects of both, where these are likely to work in complimentary ways.

1.4.4 The Prefrontal Cortex and Recognition Memory

While the perirhinal cortex's role in familiarity and/or novelty processing is fairly well established, the prefrontal cortex has also been implicated in recognition memory (Yonelias, 2002). Schacter and colleagues (Schacter, Curran, Galluccio, Milberg, & Julianna Bates, 1996) report extensive recognition memory tests in patient BG who suffered an infarction in his right frontal lobe. BG is noted to suffer from high rates of false recognition (Schacter et al., 1996). This was generated by a much larger number of "remember" responses (approx. 48% more) than controls in a set of R/K experiments. In itself this would suggest a deficit in recollection and not familiarity. However, when tests of recollection memory (associative recognition) were used,

BG's scores did not significantly differ from control subjects'. This pattern of results is highly suggestive of an impairment in the interpretation or meta-memory judgement of familiarity-related recognition memory signals rather than in the processing of familiarity itself.

Dias and Honey (2002) also argue that the prefrontal cortex's role is one of responding to novelty or familiarity rather than supporting these processes directly. Rats with lesions to the medial prefrontal cortex showed less orienting to a novel light displayed in the same location as a previously experienced different light (Dias & Honey 2002). This is suggested to occur as, lacking their medial prefrontal cortex, the rats were unable to inhibit the familiarity response generated by the perirhinal cortex due to the overlap between the two light source stimuli, despite the novelty of the light. Indeed, these selected studies suggest that that the prefrontal cortex's function is one of higher order cognitive processes such as attention or error and conflict monitoring, rather than causing primary memory impairments. Indeed, following a review of the literature Morici and colleagues (2015) conclude that the prefrontal cortex is required and engaged when recognition cannot be solved based on single item recognition. These higher order cognitive functions no doubt play a substantial role in recognition memory, but are at a level of analysis above that at which the familiarity and recollection components of memory are discussed in this thesis.

1.5 Are Novelty and Familiarity the Same Process?

A problem with the current recognition memory research with respect to this question is the discord between how recognition memory is conceptualised and approached and what the available data is saying. As discussed throughout this first chapter, both in the methodology used to study it and in the theoretical assumptions made, the field of recognition memory sees novelty as an absence, or low level of, a memory strength signal. High novelty is then synonymous to low/no familiarity. In

this respect the words “novelty” and “familiarity” ascribe to different ends of the same, single, recognition memory process.

This suggestion is appealing as it is adheres to the principles of plurality and parsimony. Assuming a single process can explain all familiarity and novelty assessment behaviour, a second would be unnecessary and redundant. This single process could be coded for at a neural level as a signal that increases or decreases with increased familiarity, the level of the signal would then indicate the level of novelty/familiarity. As described in Section 1.4.2 of this thesis, such a signal has been observed in perirhinal cortex neurons: these show high firing rates for novel (i.e. low familiarity) items which decreases as the animal’s exposure to this item (in duration and/or number of occurrences) increases (Brown & Aggleton, 2001; Kumaran & Maguire, 2007; Xiang & Brown, 1998; Zhu & Brown, 1995).

Despite its intuitive attraction, and although it has pervaded recognition memory research for decades, this conceptualisation of novelty and familiarity and its neural processing requires questioning in light of a body of evidence that hints at a differentiation between novelty and familiarity processing. For instance, using single unit records in macaques, Xiang and Brown (1998) characterise both novelty and familiarity neurons in the perirhinal cortex. The novelty neurons showed significantly higher firing rates for the first occurrence of novel stimuli (that had not been seen in the previous 2 months) than familiar stimuli (that were seen daily). On the other hand the familiarity neurons had relatively low responses to familiar items (seen daily) but higher rates of firing to novel items (that had not been seen in the previous 2 months) *both* during their first and second presentation of the day. This differentiation may be better captured by the terms ‘novelty neurons’ and ‘relative-familiarity neurons’ as the information gained from the familiarity neurons is the relative novelty or familiarity of items within a given set (regardless of the number of times these are presented). Similarly, this could be thought of as relative familiarity within a timeframe: the information gained from the familiarity neurons indicates whether the stimuli are novel or familiar across days of experimental testing sessions

rather than across trials within a single day's testing session. Regardless of how this is seen, these results add noise to the clear picture that familiarity is supported by response suppression neural activity in perirhinal cortex.

Furthermore, differentiation between novelty and familiarity is also seen in human neuroimaging research. Using fMRI Daselaar, Flek and Cabeza (2006) identify both linear increases (in parahippocampal cortex) and decreases (in perirhinal cortex) in BOLD response with recognition confidence. As participants rate item familiarity with greater confidence (presumably having a stronger feeling of familiarity), the BOLD response in perirhinal cortex decreases linearly, neatly paralleling the animal single unit data. The authors term this a "novelty" response – greater BOLD response for items that are considered novel, with reduced response as items are considered more familiar. On the other hand, as participants rate item familiarity with greater confidence, the BOLD response in parahippocampal cortex increases linearly. The authors term this a "familiarity" response – greater BOLD responses for items judged to be more familiar. While neurological regions don't work independently, it is unlikely that the BOLD responses seen in the perirhinal and parahippocampal cortecies reflect an inhibitory functional connectivity between these two regions (Lauritzen, 2005; Lee et al., 2010), with local field potentials obtained from inhibitory post-synaptic potentials often thought to result in negative BOLD responses (Lauritzen, 2005; Lee et al., 2010). This double dissociation, from another experimental field using different methods also adds further reason to question the understanding that novelty and familiarity are inverses of each other.

Finally recent research has identified that while some individual brain areas' neural activation (as measured by the protein marker Fos) does not differ between rats exploring novel and familiar objects, how they operate within a neural network is affected by the memory status of the objects the rat is exploring (Albasser et al., 2010). No differences in *c-fos* expression levels were seen in dentate gyrus (DG), lateral entorhinal cortex (LEnt), or many subregions of CA1 and CA3. Yet structural equation modelling suggests the integration of these is qualitatively different when

rats are presented with old and new objects. Rats having been presented with novel objects in a Bow-tie maze variant of the SOR (see Figure 1.2) showed parahippocampal to hippocampal connectivity mediated by the perforant pathway (i.e. Te2<->PRh -> LEnt **->** DG **->** CA3 **->** CA1) while those presented with familiar objects in the same task showed parahippocampal to hippocampal connectivity mediated by the temporo-ammonic pathway (i.e. Te2<->PRh -> LEnt **->** CA1). This falls in line with the novelty-encoding hypothesis, where novel items lead further encoding/processing into the hippocampus (Fernández & Tendolkar, 2006). While these results don't preclude that novelty/familiarity identification is based on a single neural process (indeed c-*fos* expression levels in caudal perirhinal cortex showed differentiation between the novel and familiar groups), they do highlight that novelty and familiarity processing differs at a network level. With respect to the question of whether novelty and familiarity are a single process, it becomes apparent that the concept of a "process" can be considered at various levels: identification of novelty/familiarity? How novelty/familiarity are treated by the MTL? The metacognition of novelty/familiarity? If anything, these results remind us that looking at individual neural areas in isolation is rather simplistic and that higher levels of analysis, such as a network approach, need to be considered given that they offer insight from differing angles.

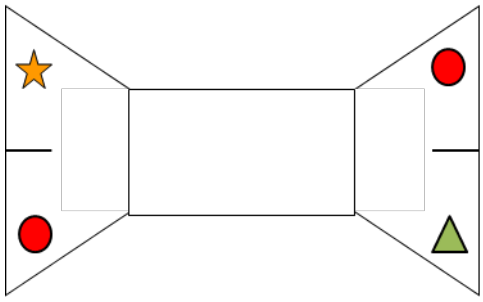

Figure 1.2: Bow-Tie Maze – a variant of the spontaneous object recognition (SOR) task.

In summary, the question of whether novelty and familiarity are words referring to the inverse of the same process can be addressed at differing levels of analysis, potentially with different outcomes. However, given the data currently available, this

question is still up for debate at most (if not all) of these levels. Given the importance and breadth of novelty (as outlines in Section 1.1), the relevance of this question to the assumption of the recognition memory field, and the suggestion that this assumption may be flawed, further consideration and exploration of novelty/familiarity processing is of significant importance.

1.6 Conclusion & Thesis Overview

The above review informs the motivations behind and direction of the research presented in this thesis. Firstly, it highlights the presence of a central assumption that novelty and familiarity are words pertaining to differing ends of a same memory strength continuum in both animal and human recognition memory research (Section 1.2). After characterising this component (Section 1.3.1), it reviews data outlining the expression of familiarity deficits seen in patients and rodents with neural lesions, along with that from neuroimaging studies that suggest the perirhinal cortex as the neural seat of familiarity processing (Section 1.4). Finally, some data is presented questioning the validity of the deeply rooted assumption about the transposable nature of novelty and familiarity. To shed further light on novelty processing and its relationship to familiarity, it is important to explore this from differing methodologies, consolidating the human and animal recognition memory fields, to allow investigation of both cognitive and neural aspects.

Hence, this thesis aimed to integrate experimental methodologies across humans and rodents to further interrogate novelty processing at cognitive and neural levels, and assess whether it is dissociable from familiarity. As depicted in the subsequent flow chart (Figure 1.3, page 36), this research was conducted within two strands. Firstly, the questioning of novelty and its dissociation from familiarity at varying levels of analysis in humans and rodents (Strand 1, Chapters 2 – 4, see Section 1.6.1 below). Secondly, to allow assimilation of the results obtained from these

investigations, the relationship between the memory indices derived from human and rodent research was explored (Strand 2, Chapter 5, see Section 1.6.2 below).

1.6.1 Strand One: Questioning Novelty and its Dissociation from Familiarity at Varying Levels of Analysis in Humans and Rodents

This strand resulted from the human (Daselaar et al., 2006) and animal research (Xiang & Brown, 1997; 1998) outlined in Section 1.5 suggesting that novelty and familiarity may be dissociable processes. This was questioned in two ways in this thesis. Firstly, do people attend to these in incongruent ways (Experiment 1)? Secondly, can these processes be isolated? To answer this question a behavioural paradigm that puts these into opposition/conflict is tested to assess their impact, if any, upon one another. This was tested both in humans, looking at the cognitive processing of these (Experiment 2), and in rodents, to explore the neural structures which may support these (Experiment 3). Putting novelty and familiarity in opposition in a behavioural paradigm was done with the intention of isolating these components to allow further imaging of these using fMRI in humans and potentially modulation of these using optogenetics in rodents to better understand their interaction and role in recognition memory. However, when trying to assimilate the results from the human and animal paradigm it was apparent that there is a lack of understanding as to the link between the memory indices derived from human and rodent recognition memory research. As such, before further investigation into novelty processing is possible, the relationship between these indices needed to be explored.

1.6.2 Strand Two: Investigating the Relationships Between the Memory Indices Derived from Human and Rodent Research

The recognition memory index obtained from SOR tasks in rodents (the discrimination index DI) is derived based on exploration times of novel and familiar objects (see Section 1.2.1). These are compared and sometimes taken in the context of total exploration time. In both forms these are taken as a measure of recognition memory. On the other hand, in the human literature, along with crude methods of

accuracy, measures of individuals' sensitivity (*d'*) and bias (*c*) are derived using signal detection theory to characterise their recognition memory (see Section 1.2.2). To allow comparison between recognition memory components and performance across species, it is imperative to understand what aspect of human recognition memory is captured by, or corresponds to, the DI derived from animal research, as investigated in Strand 2. This was directly tested in humans (Experiment 6) and rodents (Experiment 7), to further bridge these two fields and allow the future development of a cohesive understanding of recognition memory at various levels of processing.

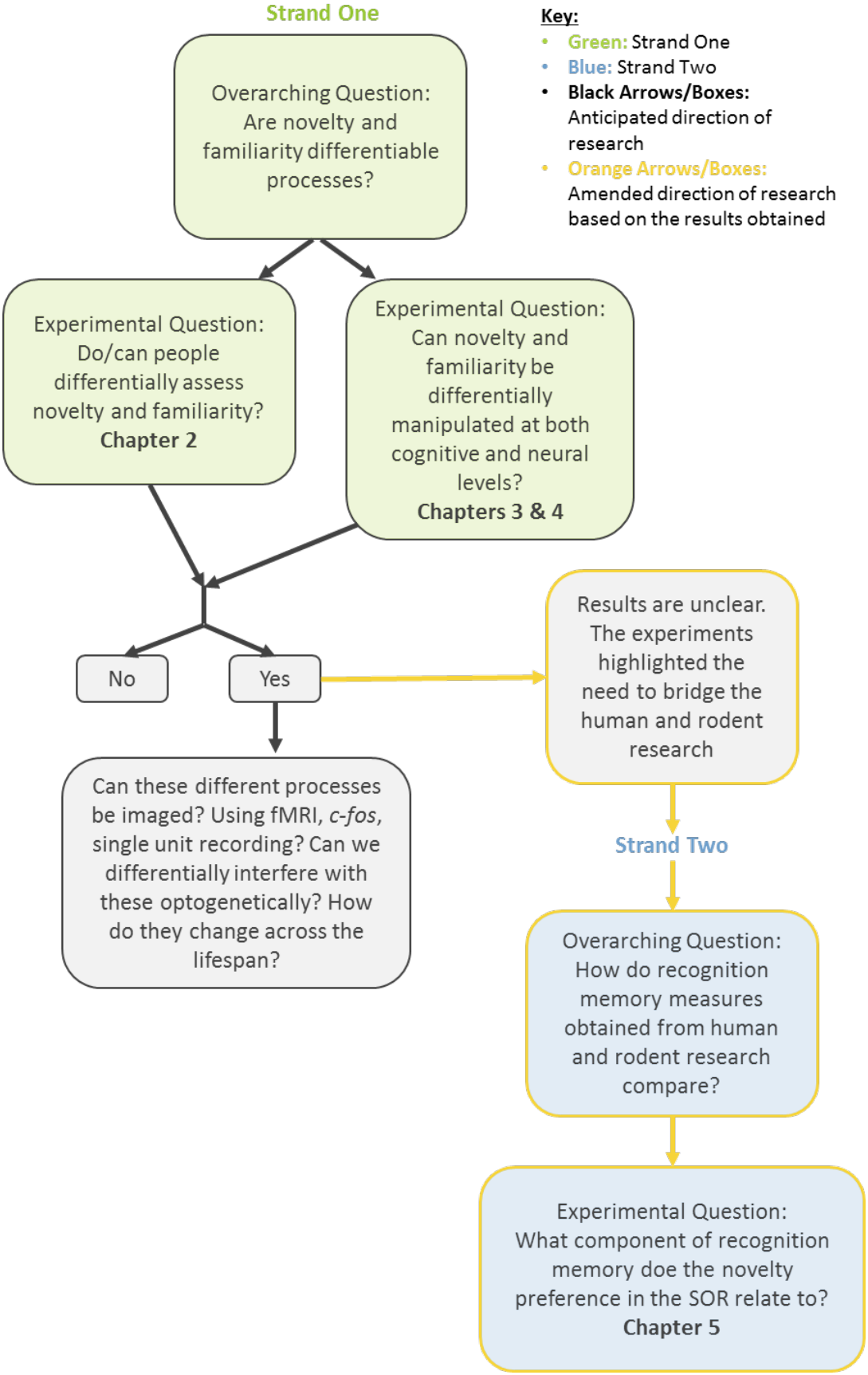

Figure 1.3: Thesis Outline. Anticipated direction of research for the current thesis, and amendments to this based on the results obtained.

2. CHAPTER TWO: DIFFERENTIAL INTERROGATIONS OF NOVETLY AND FAMILIARITY

2.1 Introduction Experiment 1

Both correctly identifying what *has* and *has not been* previously encountered are important facets of recognition memory. Within the recognition memory field, it is often assumed both implicitly and explicitly, that these two components are reflected in a single process. This single process providing feelings of familiarity is differentiated from recollection, which provides unequivocal evidence of previously having encountered a stimulus through conscious remembering of contextual/associative information about the event. Under this assumption, novelty and familiarity are terms ascribed to either end of a memory strength continuum, whereby novelty is considered simply to result from an absence of familiarity. Indeed, a recent study has suggested that high confidence novelty judgments arise from the evaluation of an absence of recollection, rather than a novelty signal per se (Bowman & Dennis, 2016). However, evidence from single unit recordings in macaques (Xiang & Brown, 1998), from examination of recognition memory neural network in rats (Albasser, Poirier & Aggleton, 2010), from studies of recognition deficits in healthy aging (Burke, et al., 2010; 2011), and from fMRI studies in humans (Daselaar, Flek & Cabeza, 2006) has questioned this lack of a distinction between the processing of novelty and familiarity (see Section 1.5 of this thesis).

Reconciling the dominant theoretical approach which assumes a single process for novelty and familiarity, with the emerging evidence of their possible distinction is therefore important. This will allow a more complete understanding of recognition memory and its deficits. Indeed, research has started to suggest that recognition difficulties apparent in healthy aging are due to novelty rather than familiarity processing deficits (Burke, et al., 2010; 2011).

Thus, the aim of this experiment was to investigate the potential dissociation between novelty and familiarity processing. A simple approach for initial investigation into this question is to ask whether people typically assess novelty and familiarity in the same manner. If novelty and familiarity describe opposing ends of a continuum of memory, with the only distinction between them a quantitative one – the amount of memory evidence recovered – then and any top-down assessment of this, should be unaffected by specifically questioning one or the other. Alternatively, if novelty and familiarity are qualitatively distinct levels of evidence and are able to be differentially interrogated, the amount of memory evidence recovered, and any top-down assessment of this by participants, may vary when these are individually questioned. This is exemplified below using Figure 2.1.

Assume the task at hand is to identify familiar items. Here we have two scenarios: either the source of memory strength information is singular and graded, such as a memory strength signal (Figure 2.1a) or it arises from the two separable (but interacting) components of familiarity and novelty (Figure 2.1b). In both scenarios, the assessment of what is identified as familiar depends upon the placing of a threshold (represented by the black lines in Figure 2.1a & b) within the area of uncertainty along the memory gradient, with items to the right of this being identified as new, and items to the left being identified as old. Now consider the task at hand is to identify items as high confidence new, low confidence new, low confidence old or high confidence old. If the source of memory strength information is singular, placing the threshold between high and low confidence new will be undertaken in the same manner as placing that between high and low confidence old, because the same (only) source of information is used to undertake this (Figure 2.1c). Contrarily, if memory strength arises from familiarity and novelty, then the familiarity component may be relied upon more heavily in deciding where to place the threshold between high and medium confidence old (Figure 2.1d), whereas the novelty component may be relied more heavily in deciding where to place the threshold between high and low confidence new (Figure 2.1d).

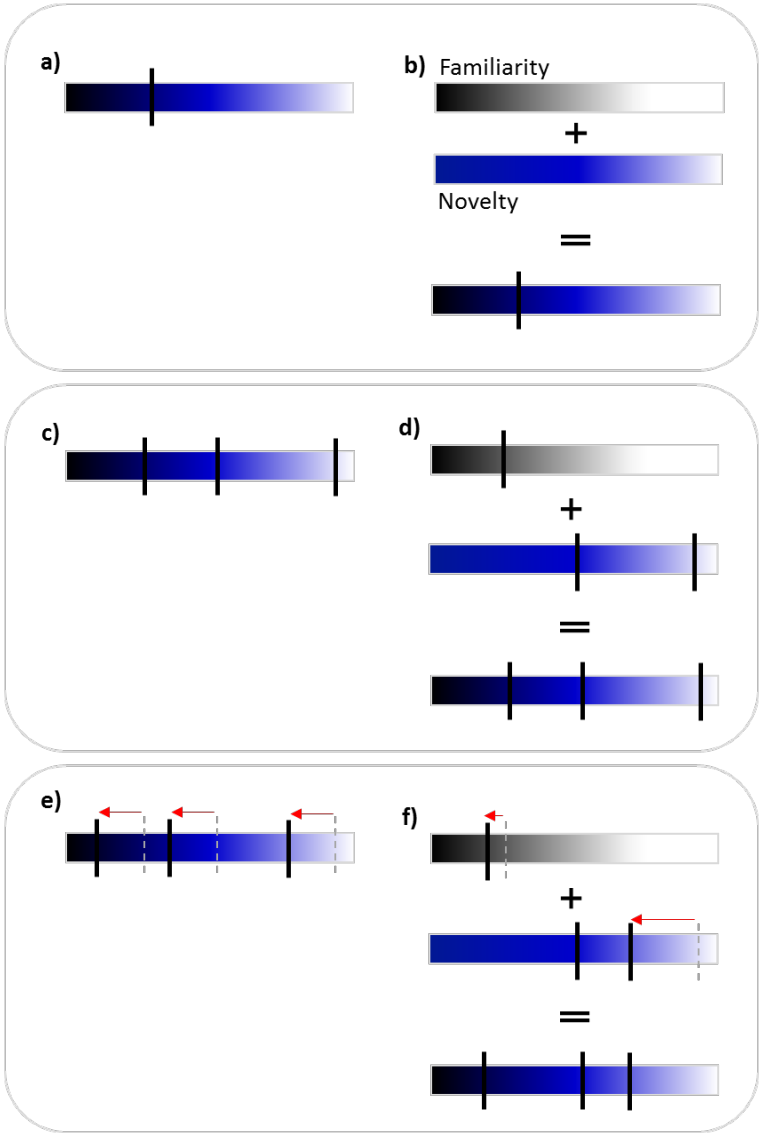

Figure 2.1: Illustration depicting how old/new assessment may vary based on the number of processes providing evidence for memory strength. a) and b) depict the same location of a decision threshold between old and new, irrespective of whether evidence for memory strength is singular (a) or composed of novelty and familiarity (b). c) and d) again depict the same location of three decision thresholds between high and low confidence old and new judgements for a single (c) and a combination (d) of sources of evidence for memory strength, with the different contributions of these sources towards the different thresholds (d). When evidence for memory strength is provided by a single source, any changes in its assessment occurs in the same manner across all thresholds (e), whereas thresholds can be individually altered by interrogating the different familiarity and novelty sources of memory strength (f). Threshold changes are shifts in bias, occurring as a result of top-down goal-directed processes.

If the goal of the task then changes, such that incorrectly identifying an item as old is more problematic than incorrectly identifying an item as new, threshold placement is amended to reflect this (red arrows in Figure 2.1e & f). Here if memory strength is provided by a singular source, all thresholds are amended in the same manner (Figure 2.1e). Contrarily, if memory strength has a familiarity and a novelty input, the different thresholds can be amended independently depending on which source of memory strength is primarily interrogated or given importance to (Figure 2.1f & g). In summary, if novelty and familiarity represent a single process, then assessments (bias) must occur and be amended similarly across the memory strength spectrum. Contrarily, if these are separable, then interrogation of memory strength could occur differently for novelty and familiarity judgments, and thus amendments in the assessment of memory strength may differ between these.

Framing single-item recognition tasks as interrogations of “old?” or “new?” causes participants to differentially assess their memory evidence, shifting their bias/criterion (Mill & O’Connor, 2014). Specifically, these shifts in criterion reflect that participants are less inclined to endorse a “new” recognition decision (becoming more liberal with their old responses) when they are specifically responding to the question “new?” compared to “old?”, and less inclined to endorse an “old” decision (becoming more conservative with their old responses) when responding to the question “old?” compared to “new”. In their reluctance to endorse a response emphasized by the test question, participants improve the accuracy of their responses to the emphasized question. Mill & O’Connor (2014) inferred from this that participants were shifting their criterion so as to better achieve the goal of getting the emphasized response correct, and thus that these results demonstrate a top-down criterion shift. Indeed, for two out of the three experiments presented by Mill & O’Connor (2014), the effect of emphasizing either novelty (“new?”) or familiarity (“old?”) was limited to participants’ bias (willingness to endorse either a “new” or “old” judgement under conditions of uncertainty), with sensitivity (the ability to discriminate old from new) left unaffected. This is consistent with previous experiments demonstrating an effect of test question on memory assessment in the

absence of a difference in sensitivity (Bruno & Rutherford, 2010; Hicks & Marsh, 1999).

Thus, Mill & O'Connor's (2014) results suggest that test question emphasis implicitly provided a top-down goal to more accurately identify the emphasized response, while sparing any differences in the bottom-up driven memory strength and subsequent sensitivity. However, it is unknown whether this arises from top-down processes leading to the differential interrogation of separate novelty and familiarity signals, or from top-down processes affecting the assessment of a single processes. When "old?" is questioned, participants may deduce that the experimenter is interested in the ability to correctly identify items which are "old" and hence may be more careful, needing greater levels of evidence (a more conservative bias), before identifying items as "old". Assuming participants only have access to a single source of evidence, then this should be the same for both "oldness" and "newness" – participants should be more conservative about identifying items as familiar if "oldness" is emphasized, and more conservative about identifying items as new if "newness" is emphasized. However, if novelty and familiarity provide separate sources of information which may be differentially assessed, top-down processes may differentially be applied to these, such that corresponding responses may differ across the memory strength spectrum (Figure 2.1).

To investigate this, in Experiment 1 participants were asked to rate how old or new previously studied stimuli felt to them on a 6-point scale. This six-point scale allows us to test five different criteria (or threshold) placements – those between high, medium and low confidence old and new judgements. It was hypothesised that if participants are relying on and attending to a single process to make decisions about novelty and familiarity, then shifts in criterion placements due to question emphasis will occur in an identical manner across both questions (Figure 2.2a). Alternatively, if differing sources of evidence support the memory strength continuum and can be differentially questioned, then these may be reflected in differences in the manner in which memory strength is assessed (Figure 2.2b). The interest here lies in

questioning any potential differentiation between novelty and familiarity. As acknowledged in the opening paragraph to this chapter, recollection is considered to be a separate process, contributing to recognition memory through the provision of unequivocal evidence of previous exposure to an item. Therefore, the contribution of recollection to recognition judgements is considered to be limited to high confidence old judgements, and participants' assessment of their recognition memory based on this will be differentiable to that based on memory strength. Hence, when investigating differences in biases due to question emphasis, only responses between medium confidence old and high confidence new are of interest.

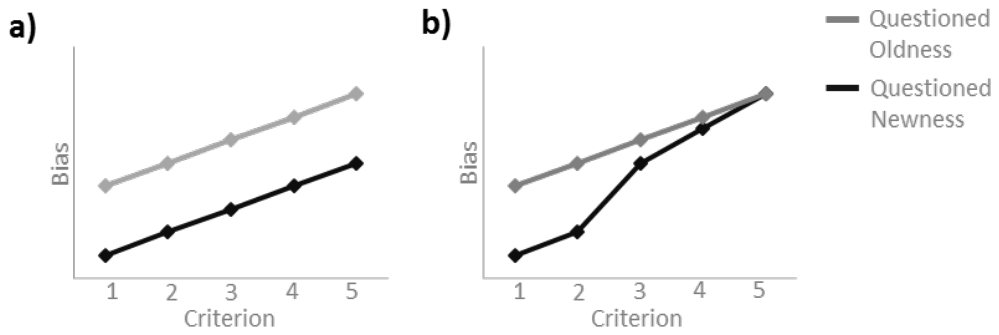

Figure 2.2: Illustration of hypothetical differences in criterion shifts as a result of a (a) single or (b) dual processes underlying memory strength. (a) All criterion are altered in an identical manner when a single process supports memory strength, or (b) the criterion are altered differentially when two processes support memory strength.

2.2 Materials and Methods

2.2.1 Participants

Data was collected from 43 self-reported native English speaking participants, each compensated £5 for their time. Twenty-two participants were excluded from the

analyses due to not reaching a minimum overall *d'* of 0.1[2] (n = 14), or due to failure to use more than a third of the confidence scale presented in either of the conditions (n = 8). It was established through systematic participant feedback that a significant proportion of the poor performance was due to misunderstanding the task instructions where participants were rating items based on their perceived linguistic age (i.e. how long ago a word has been in the dictionary) rather than their mnemonic "oldness" and "newness" (see section 2.2.3 below for detailed methods). Thus, the final sample consisted of 21 participants (48.84% of original samples; 18 females, mean age = 21.81, age range = 18-50 years). Informed consent was obtained in accordance with the University Teaching and Research Ethics Committee at the University of St Andrews (Appendix A).

2.2.2 Stimuli and Materials

For each participant, a stimulus set of 960 words was randomly sampled from a pool of 2199 singular, common nouns (e.g. injury, lane, vitamin) frequently appearing in the English language (minimum log Hyperspace Analogue to Language frequency $\geq$ 7.70, from the English lexicon Project, Balota et al., 2007). Using this minimum log Hyperspace Analogue to Language frequency threshold allows the exclusion of low frequency, and therefore distinctive, words known to strongly affect memory performance (e.g. Stretch & Wixted, 1998) which may mask the effects of interest from the task manipulations. The experiment was delivered and responses recorded using PCs running MATLAB (The MathWorks Inc., Natick, MA, 2000) and Psychophysics Toolbox (Brainard, 1997).

2.2.3 Design and Procedure

Participants' recognition memory was tested within-subjects under two experimental conditions: questioned Oldness and questioned Newness. A further

[2] As outlined in Section 1.2.2, *d'* is a measure of discrimination sensitivity, where *d'* = 0 reflects an inability to tell old items from new items, and therefore performance at chance. Hence, a *d'* threshold slightly above zero is used, e.g. Mill & O'Connor, (2014).

manipulation of the level of encoding of study material was integrated into the design.

After reading onscreen instructions, participants undertook four self-paced study-test blocks. Each study phase was comprised of a 120 trial incidental encoding task with intermixed levels of processing (LOPs). During these study phases, participants saw a series of single words presented centrally on screen. A question above the word indicated whether participants were being asked to count the number of syllables in the word ("SYLLABLES?"; shallow encoding, 50% of stimuli) or rate its pleasantness ("PLEASANTNESS?"; deep encoding, 50% stimuli) (Figure 2.3). These questions were pseudorandomly intermixed. The encoding questions were presented in different colours ("SYLLABLES?" in green, "PLEASNATNESS?" in blue) to help participants differentiate the questions more easily. Responses were made by keypress corresponding either to the number of syllables counted (1 – 4+) or the pleasantness rating ("1" very unpleasant – "4" very pleasant) of the word. A 0.5 second interval consisting of a fixation cross at the center of the screen was presented between each word.

Figure 2.3: Schematic of the experimental Design. Participants undertook two types of study – test blocks, each type being undertaken twice. Both study phases (ai; bi) comprised an intermixed shallow and deep encoding task during which 120 words were presented (50% shallow, 50% deep encoding). For a given test phases (aii; bii) participants were either asked to rate the oldness (aii) or newness (bii) of the presented words. 240 words were presented during each test phase, of which 120 had been studied (60 shallowly and 60 deeply encoded).

A test phase immediately followed each study phase. At test participant were again presented with a series of 240 single word stimuli consisting of 120 targets (studied items; 60 deeply encoded and 60 shallow encoded) and 120 lures (new items), in a random order. A question above the word indicated whether participants were being ask to rate how old ("OLDNESS?") or new ("NEWNESS?") the presented world felt to them on a scale of 0% (low confidence) to 100% (high confidence), in 20% increments (Figure 2.3). Here participants were asked to respond with regards to their mnemonic feeling of "oldness" or "newness" for words based on the study phase. This was emphasized verbally to participants after early debriefing highlighted that many participants were rating "oldness" and "newness" based on the perceived linguistic age of the words, i.e. how long a word had been in the dictionary. Responses were again made by keypress ("1" = 0%, "2" = 20%, "3" = 40%,

"4" = 60%, "5" = 80%, "6" = 100%). All words were presented with a 0.5 second fixation cross interval, while the test question and response scale remained on screen. Test phases were blocked such that the question presented was the same throughout a give test phase. Again the questions on screen were differentially coloured ("OLDNESS?" in red, "NEWNESS?" in yellow) to help participants differentiate these. Blocks were presented in an OLDNESS-NEWNESS-OLDNESS-NEWNESS sequence for 50% of participants, and in a NEWNESS-OLDNESS-NEWNESS-OLDNESS sequence for the remaining 50% of participants.

2.2.4 Calculations

Overall performance was investigated using sensitivity (*d'*) and bias (*c*) parameter estimates from the equal variance signal-detection model (Macmillan & Creelman, 2005). As *d'* and *c* estimates are dependent upon the z-scores of hit (*H*) and false alarm (*FA*) rates (see Equations (4) & (5) below), these are undefined for errorless responding (where *H* = 1 and *FA* = 0, the z-score of either being infinite). Thus, *d'* and *c* were calculated using hit and false alarm rates corrected for errorless responding (Snodgrass & Corwin, 1988). These adjusted hit (*H'*) and false alarm (*FA'*) rates were calculated as follows, based on the number of hits (*H*), misses (*M*), correct rejections (*CR*) and false alarms (*FA*):

$$H' = \frac{H + 0.5}{(H + M + 1)} \quad (1)$$

$$FA' = \frac{FA + 0.5}{(FA + CR + 1)} \quad (2)$$

Adjusted correct rejections are calculated as follows:

$$CR' = 1 - FA' \quad (3)$$

Sensitivity (*d'*) and bias (*c*) were subsequently calculated as follows:

$$d' = z(H') - z(FA') \tag{4}$$

$$c = -0.5 \times [z(H') + z(FA')] \tag{5}$$

When there are differences in *d'* the same absolute criterion can reflect different levels of bias, as depicted in Figure 2.4. Under these circumstances, to compare *c* across differing conditions resulting in different *d'*, a relative value of *c* (*c'*), scaled to *d'* is calculated as follows (Macmillan & Creelman, 2005, p.33):

$$c' = \frac{c}{d'} \tag{6}$$

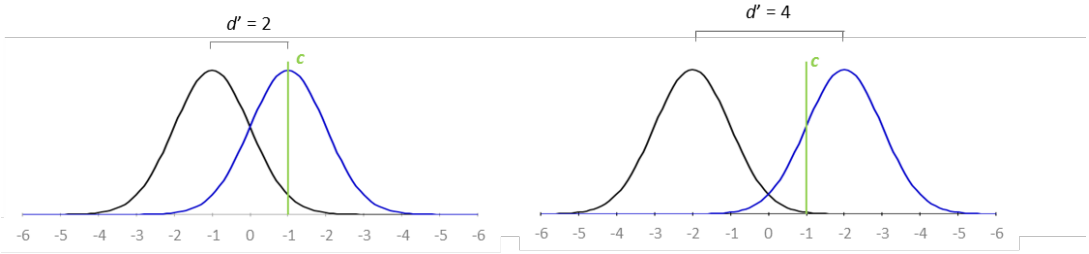

Figure 2.4: Relationship between sensitivity and bias. Diagram depicting how the same absolute criterion (*c*) reflects different levels of bias for different levels of sensitivity (*d'*).

2.2.5 Data Analysis

Participants' performance was investigated relative to chance by submitting the parameter estimate of sensitivity (*d'*) to a one-sample t-test, with zero as the value of comparison. Participants' overall bias (*c*) was also assessed using a one-sample t-test, with zero as the value of comparison, where *c* = 0 shows optimal bias.

The effects of test question and LOP on *H'* were tested statistically using a 2 (test question: oldness vs newness) x 2 (LOP: shallow vs deep) repeated measures ANOVA.

For any given block, target items presented at test were either shallowly or deeply encoded during the study phase (due to the intermixed design). However, all lure items presented at test in the same block were unable to be divided into "shallow" and "deep" LOPs as these were not seen at study. Thus, given the intermixed study design, a single *CR*' was available for use in the analyses of both shallow and deep LOPs. Hence, the effect of test question on *CR*' were investigated statistically using a paired sample t-test.

Statistically, the effects of test question and LOP on *d*' and *c* were tested using a 2 (test question: Oldness vs Newness) x 2 (LOP: shallow vs deep) repeated measures ANOVA.

As participants were asked to give confidence responses to items on a six-point scale, five criterion parameters between high, medium and low confidence old and new judgements (see Table 2.1) can be estimated. As such, participants' response frequencies for each confidence level were used to fit an equal variance signal detection model. For each participant, the five criterion parameters (*c*1-*c*5; Table 2.1) and sensitivity (d'_{fit}) were estimated from the model (Mill & O'Connor, 2014; Harris, 1998). To clearly discern the fit estimate of sensitivity from the un-fit measures of sensitivity (*d'*), where used, it is referred to as d'_{fit}. It is conventional to zero-center criterion on the intersection of the old and new distribution, however the data were fit to a model zero-centered to the center of the new-item distribution. Thus, to align criterion parameter estimates obtained from the model to those conventionally used, $d'_{fit}/2$ (the intersection of the old and new distribution) was subtracted from the criterion parameter estimates obtained from the model. All *c*1-*c*5 parameters are presented in this aligned form. *c*3 is that placed between (low)

old and new judgments (Table 2.1), and thus is the fit equivalent to the un-fit *c* parameter estimate calculated using Equation (5) (see Section 2.2.4).

Table 2.1: Table of participant response options and superimposed arrows depicting the location of the five measures of bias obtained.

		Confidence					
		High new	Medium new	Low new	Low old	Medium old	High old
Rating	Oldness	0%	20%	40%	60%	80%	100%
	Newness	100%	80%	60%	40%	20%	0%
		*c*1	*c*2	*c*3	*c*4	*c*5	

To ascertain that the d'_{fit} and *c*3 paramters obtained from fitting participant responses to an equal variance model corresponded well to the *d'* and *c* calculated from un-modelled responses, these were correlated using Pearsons' correlations.

Effects of test questions on the *c*1 - *c*4 parameters extracted from the fit model were analysed using a 2 (test questions: oldness vs newness) x 2 (LOP: shallow vs deep) x 4 (criterion: c1 vs c2 vs c3 vs c4) repeated measures ANOVA. *c*5 parameter estimates were excluded from the analysis as this would be highly contaminated by any potential influence of recollection, as outlined in the Introduction (Section 2.1). Where a significant main effect of criterion was obtained, Bonferroni corrected pairwise comparisons were used to identify where differences occurred. Following a significant test question x criteria interaction, main effects were examined using Bonferroni corrected pairwise comparisons, and simple effects were examined using t-tests.

An α threshold of 0.05 was adopted for all statistical analyses reported.

2.3 Results

Participants had a mean overall accuracy of 0.82 (SD = 0.05), with a mean adjusted hit rate (*H'*) of 0.83 (SD = 0.07) and a mean adjusted correct rejection rate (*CR'*) of 0.82 (SD = 0.07). Participants' overall sensitivity was significantly above chance (*d'*: M = 1.92; SD = 0.37) and bias was not significantly different from the optimal, neutral placement (0; *c*: M = -0.005; SD = 0.23) as demonstrated by two single-sample t-test performed on *d'* and *c*, $t_{(20)} = 23.90$, $p < 0.001$, d = 10.69, and $t_{(20)} = -0.096$, p = 0.924, d = 0.021 respectively.

2.3.1 Analyses of Hit and Correct rejection rates

Adjusted hit and correct rejection rates were firstly examined to question the effects of LOP and test question on correct identification of targets and lures. Figure 2.5 depicts the mean *H'* and *CR'* rates for items in both the questioned Oldness and questioned Newness conditions. *H'* rates (proportion targets correct) were lower for shallowly encoded compared to deeply encoded targets, and are lower for targets in the questioned Newness compared to the questioned Oldness condition, as confirmed by the significant main effects of LOP, $F_{(1,20)} = 37.0$, $p < 0.001$, $\eta_p^2 = 0.649$, and test question, $F_{(1,20)} = 5.45$, p = 0.030, $\eta_p^2 = 0.214$, in a repeated measures ANOVA (Figure 2.5). No significant interaction is observed between LOP and test question, $F_{(1,20)} = 0.211$, p = 0.651, , $\eta_p^2 = 0.010$. Participants have higher *CR'* rates (proportion correct lures) in the questioned Oldness condition compared to the questioned Newness conditions, as confirmed by a paired-samples t-test, $t_{(20)} = -2.48$, p = 0.020, d = -0.541 (Figure 2.5).

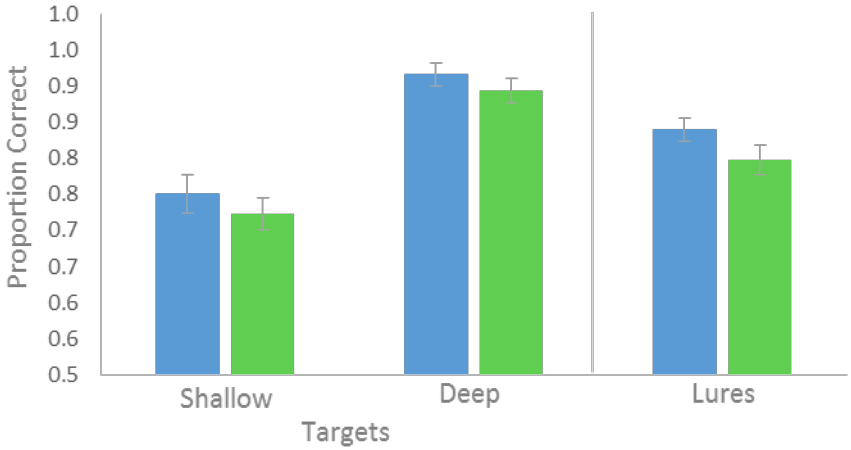

Figure 2.5: Mean adjusted proportion of correct responses to targets (*H'*) and lures (*CR'*) for questioned Oldness (blue) and Newness (green) conditions and shallow and deep LOPs. Error bars represent SE.

2.3.2 Analyses of sensitivity (*d'*) and bias (*c*)

Mean *d'*s for each test question condition and LOP are presented in Figure 2.6a. Participants have a greater *d'* following a deep as compared to a shallow LOP, as well as a greater *d'* when responding to the test question Oldness? as compared to Newness?, as confirmed by significant main effects of LOP $F_{(1, 20)} = 41.64$, $p < 0.001$, $\eta_p^2 = 0.676$ and test question $F_{(1, 20)} = 16.77$, $p = 0.001$, $\eta_p^2 = 0.456$ in a repeated measures ANOVA (Figure 2.6a). No significant interaction is observed between LOP and condition, $F_{(1,20)} = 0.037$, $p = 0.849$, $\eta_p^2 = 0.002$.

Given the differences observed in *d'* outlined above, to enable comparison of *c* between conditions and LOPs, *c* was scaled to *d'* on a participant by participant basis (see Section 2.2.4). Mean *c'* for each test condition and LOP are presented in Figure 2.6b. *c'* is significantly lower following a deep as compared to a shallow LOP, but is unaffected by test question, as confirmed by a significant main effect of LOP, but no such main effect of test question in a repeated measures ANOVA, $F_{(1, 20)} = 37.05$, $p < 0.001$, $\eta_p^2 = 0.649$, and, $F_{(1, 20)} = 0.720$, $p = 0.406$, $\eta_p^2 = 0.025$, respectively. No significant interaction is observed between LOP and condition, $F_{(1,20)} = 0.431$, $p = 0.519$, $\eta_p^2 = 0.003$. The difference in *c'* between the LOPs reflects the differences in *H'*

between these LOPs (Figure 2.5), as the *CR'* are shared between the shallow and deep *c* parameters for each condition.

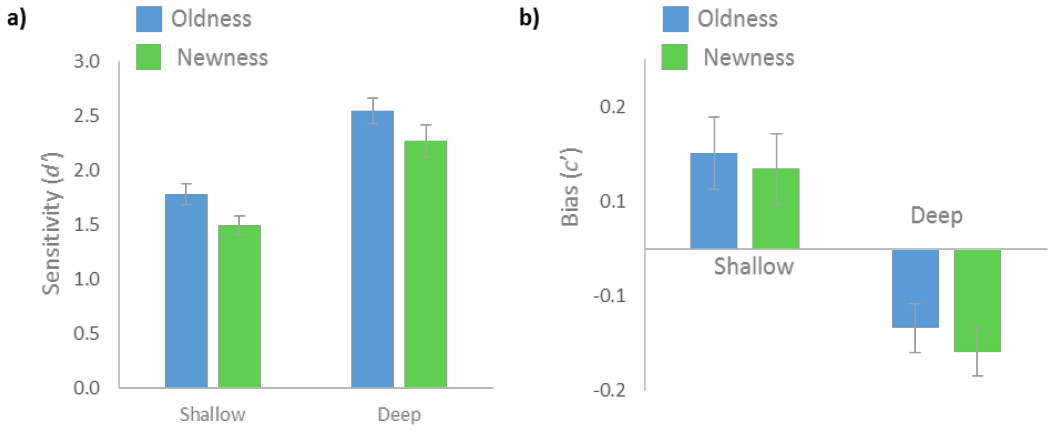

Figure 2.6: Mean (a) sensitivity (*d'*) and (b) bias (*c*) under the questioned Oldness (blue) and questioned Newness (green) conditions for deep and shallow LOPs. Error bars represent SE of the mean.

2.3.3 Analyses of fit bias (*c1 – c4*)

Fit estimates of d'_{fit} and *c*3 parameters were compared to un-fit *d'* and *c* estimates. Mean and standard deviations for these are presented in Table 2.2. As expected assuming the data from the fit and un-fit model corresponded well, these were found to be highly positively correlated (Table 2.2).

Table 2.2: Mean and standard deviations for fit and un-fit parameter estimates of sensitivity (*d'*) and bias (*c*), along with their inter-correlations, as calculated using Pearson's correlation.

Parameter	Condition	LOP	un-fit M (SD)	fit M (SD)	correlation r
d' / d'fit	Newness	Shallow	1.50 (0.40)	1.52 (0.40)	0.990**
		Deep	2.28 (0.15)	2.38 (0.75)	0.982**
	Oldness	Shallow	1.78 (0.45)	1.79 (0.46)	0.974**
		Deep	2.55 (0.54)	2.66 (0.62)	0.961**
c / c3	Newness	Shallow	0.13 (0.26)	0.13 (0.27)	0.999**
		Deep	-0.26 (0.29)	-0.30 (0.33)	0.988**
	Oldness	Shallow	0.16 (0.32)	0.15 (0.33)	0.998**
		Deep	-0.22 (0.29)	-0.27 (0.34)	0.984**

*Note: ** denotes significance at p < 0.001*

Paralleling the analyses on *c* above, to enable comparison of *c*1-*c*4 parameters between conditions and LOPs, these were scaled to d'_{fit} on a participant by participant basis (see Section 2.2.4, page 47). Mean *c'*1-*c'*4 parameter estimates for each test condition and LOP are presented in Figure 2.7a. To better visually represent these results within the context of recognition memory as a whole, mean *c'*1 - *c'*4 and d'_{fit} values for each test question and LOP are presented as modelled by an equal variance signal detection theory model in Figure 2.7b&c.

A 2 (test questions: oldness vs newness) x 2 (LOP: shallow vs deep) x 4 (criterion: *c*'1, *c*'2, *c*'3, *c*'4) repeated measures ANOVA was conducted. Mauchly's test revealed that the within-subjects effect of criterion violated the assumption of sphericity, $\chi^2_{(5)}$ = 90.96, p < 0.001, and thus all further analyses involving this factor were greenhouse-Geisser corrected. The repeated measures ANOVA revealed significant main effects of LOP, $F_{(1, 20)}$ = 30.74, p < 0.001, η_p^2 = 0.606, and criterion, $F_{(1.125, 22.495)}$ = 59.48, p < 0.001, η_p^2 = 0.748, but no main effect of test question, $F_{(1, 20)}$ = 2.78, p =0.111, η_p^2 = 0.013 (Figure 2.7a). The shallow LOP lead to more conservative responses (M = 0.09, SD = 0.17) than the deep LOP (M = -0.10, SD = 0.12). Pairwise comparisons confirmed that *c'*1, *c'*2, *c'*3 and *c'*4 all significantly differed from each other, p < 0.001 for all comparisons. There was a significant test question x criterion interaction, $F_{(1.34, 26.77)}$ = 4.06, p = 0.043, η_p^2 = 0.169, and a significant LOP x criterion interaction, $F_{(1.20, 23.95)}$ = 36.36, p < 0.001, η_p^2 = 0.645 (Figure 2.7a-c). All other interactions were non-significant.

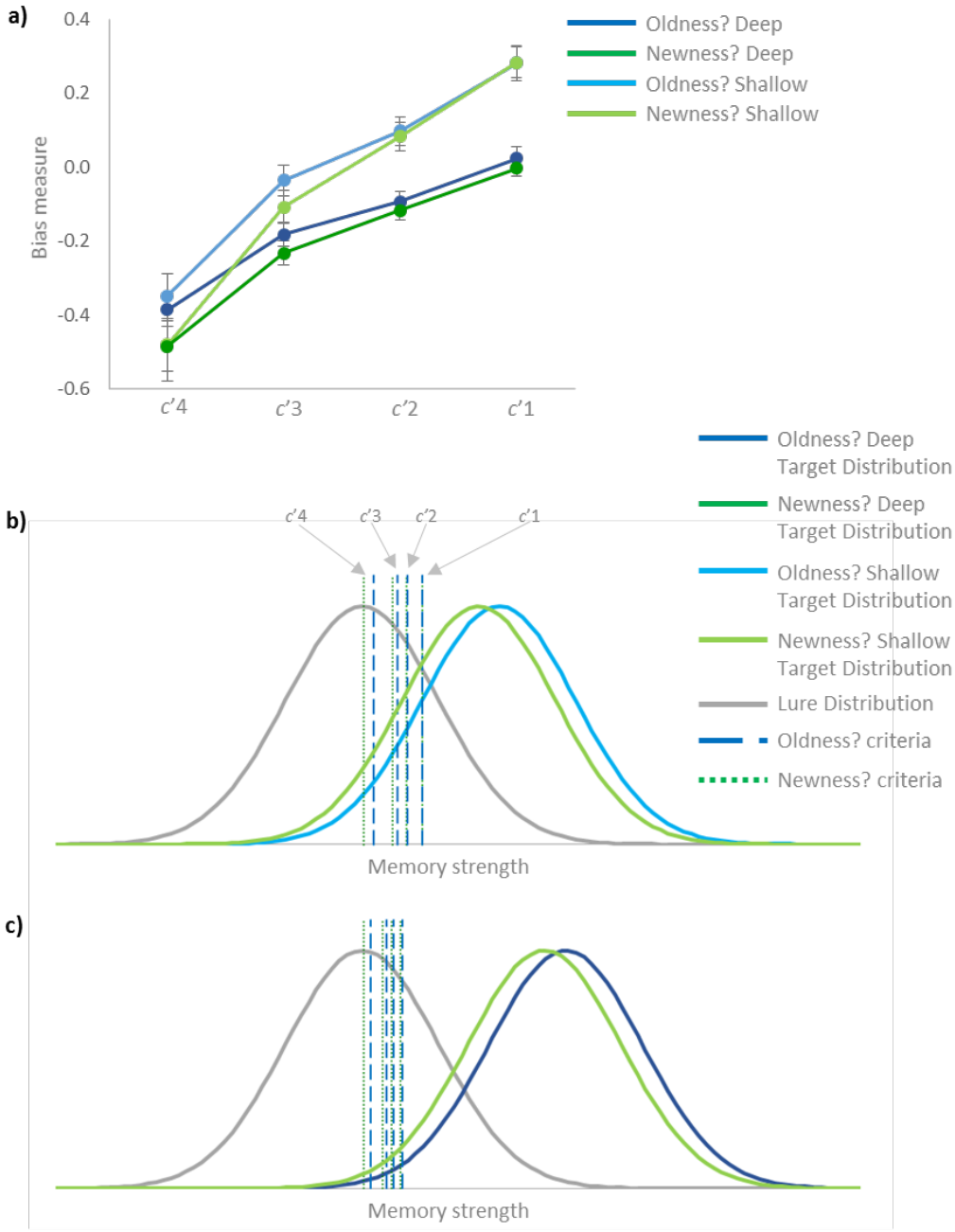

Figure 2.7: Figures depicting the interaction between bias and test question condition. a) Mean scaled bias measures and their relationships for the 4 criteria between high confidence new and medium confidence old judgements under conditions of questioned Oldness and Newness, and for shallow and deep LOPs (*c*'1 = criterion between high confidence new and medium confidence new, *c*'2 = criterion between medium confidence new and low confidence new, *c*'3 = criterion between low confidence new and low confidence old, *c*'4 = criterion between low confidence old and medium confidence old). b) & c) Signal detection models showing the average lure and target distributions with scaled mean criterion 1-4 placements for b) shallow and c) deep LOPs for both questioned Oldness and Newness conditions. The figure illustrates the test condition x criterion interaction.

Looking at Figure 2.7, it is clear that these significant interactions are predominantly dependent upon differences in *c'*4. The test condition x criteria interaction (Figure 2.7) was interrogated through the examination of simple main effects, presented in Table 2.3. The differences in each of *c'*1 – *c'*4 between the two test conditions did not reach significance (Table 2.3).

Table 2.3: Simple main effects for the condition x criteria interaction. Paired samples t-test results comparing the criterion parameters between the Oldness and Newness test conditions.

Criterion	Paired samples t-test	Cohens' d
***c'*1**	$t_{(20)} = 1.990$, p = 0.060	0.43
***c'*2**	$t_{(20)} = 1.866$, p = 0.077	0.41
***c'*3**	$t_{(20)} = 0.746$, p = 0.464	0.16
***c'*4**	$t_{(20)} = 0.569$, p = 0.576	0.12

*Note: * denotes significance at p < 0.05*

The LOP x criteria interaction was interrogated by looking at simple main effects, presented in Table 2.4. A deep LOP lead to significantly lower *c'*2 – *c'*4s, with the effect of LOP abolished for *c'*4 (Figure 2.7a, Table 2.4).

Table 2.4: Simple main effects for the LOP x criteria interaction. Paired samples t-test results comparing the criterion parameters between the shallow and deep encoding conditions.

Criterion	Paired samples t-test	Cohens' d
***c'*1**	$t_{(20)} = 6.791$, p < 0.001*	1.48
***c'*2**	$t_{(20)} = 6.212$, p < 0.001*	1.36
***c'*3**	$t_{(20)} = 5.340$, p < 0.001*	1.17
***c'*4**	$t_{(20)} = 0.645$, p = 0.526	0.14

*Note: * denotes significance at p < 0.05*

2.4 Discussion

Recognition memory performance was investigated under conditions emphasizing novelty or familiarity, to question for a dissociation in the processing of these. Indeed, participants' recognition memory performance was altered by differently asking them to rate either how old or new a word felt during a memory task. Participants' had a higher sensitivity when asked to rate oldness rather than newness, and of most interest, altered their bias across their ratings of memory strength differentially in response to these questions.

Consistent with Mill & O'Connor's (2014) second experiment in which participants made single-item recognition judgments in response to the questions "old?" or "new?", participants had a greater sensitivity when familiarity compared to novelty was emphasized. These results suggest that the test question emphasis impacted the amount of memory evidence recovered during recognition, making it easier for participants to discern novel and familiar objects under conditions emphasizing familiarity. This is challenging to reconcile to an understanding that a single process contributes to memory evidence. Indeed, if a singular process underlies memory strength, then this should remain constant regardless of test question emphasis. The role of recollection requires consideration here. Certainly, the greater levels of memory strength for old items when oldness is emphasized can be ascribed to a greater engagement of recollection in this condition (Bowman & Dennis, 2016; Kumaran & Maguire, 2007, 2009). Thus alone this difference in sensitivity for the differing test questions does not provide compelling evidence of a differentiation between novelty and familiarity.

However, participants were also found to differentially assess their memory evidence (reflected in shifts in bias/criterion) in response to the questions "oldness?" and "newness?", even when the effects of recollection were parceled out by excluding *c*5 criterion from the analysis. Contrastingly to the results obtained by Mill & O'Connor (2014), this effect of test question on bias was not apparent in the bias

for old/new judgments. As opposed to the experiment in Mill & O'Connor (2014), here participants rated their confidence in their recognition judgments. Thus it was established that emphasizing oldness or newness differentially affected participants' bias in making high and medium confidence new responses (i.e. low and medium confidence old responses). Bias shifts did not occur in the same manner across emphasis. Rather, although no main effect of test question was observed, the interaction suggests that participants were less inclined to endorse greater confidence novelty responses when newness was emphasized, but were not more conservative about giving greater confidence old responses when oldness was emphasized. This suggests that the counter-emphasis bias demonstrated in Mill & O'Connor (2014) is driven by a shift in bias at the top end of the lure distribution (high confidence new decisions).

These results do not support a straight-forward goal-directed counter-emphasis bias, where participants are reluctant to endorse the recognition decision emphasized by the test question (Mill & O'Connor, 2014). If this were the case, bias shifts would be expected to be mirrored for old and new responses, where this is not supported given the interaction between test question and criterion placement. Rather these results are consistent with an interpretation in which participants are differentially evaluating distinct sources of evidence, one for novelty and one for familiarity, to make their recognition judgements when these are individually emphasized. Assuming the same top-down goal oriented processes are engaged across test question, then the differences observed arise from differences in the interrogation of sources contributing to the memory evidence signal. Interpreted in this manner, the results suggest a differentiation between novelty and familiarity exists.

In the context of these results, the limitation of the stimuli used is considered. Specifically, these were common English nouns, and hence will have been associated with significant levels of familiarity from pre-experimental exposure. As participants are reluctant to endorse an emphasized decision to improve accuracy for that response (Mill & O'Connor, 2014), participants may show greater reluctance to

endorse high confidence new judgments for words with a significant level of associated familiarity when newness is specifically emphasized. While this can be easily tested through repeating this experiment using stimuli never previously encountered, if the stimuli were the cause of the interaction effects seen in bias, it is surprising that these differences would not also be apparent for the bias for old/new judgements. Indeed, if word familiarity was affecting participants' identification of high confidence new items, to improve accuracy for these under conditions of questioned newness (as suggested by the counter-emphasis bias), then it is surprising that it is not also affecting their identification of new as compared to old items more generally. Thus, while this highlights an important consideration of the use of familiar stimuli in investigations of novelty processing, this is not considered to explain the pattern of results obtained.

Although not speaking to the main aim of this experiment, the results demonstrate that participants also shifted their bias in response to the level of processing manipulation, with deeper levels of encoding leading to a more liberal bias (greater willingness to endorse an old decision). This result conflicts with a significant body of evidence showing that participants are reluctant to shift their criterion on an item-by-item basis, but rather persist with a consistent bias even under conditions encouraging shifts in bias (e.g. Han & Dobbins, 2008; Morrell, Gaitan, & Wixted, 2002; Stretch & Wixted, 1998). This is considered to reflect the significant cognitive demand required to amend criterion (Stretch & Wixted, 1998). Due to the intermixed design of the LOP manipulation, the results from the current experiment demonstrate bias shifts on an item-by-item basis. However, a single correct rejection rate was available for the calculation of bias for both shallow and deep LOP. Therefore, differences in bias between test conditions reflect the difference in hit rates between these (see Equation (5), page 47), which can be explained when recollection is taken into consideration. As such the shift in bias on an item-by-item basis, contradicting the current literature on this, is considered spurious and of minimal interest.

To conclude, the interaction between criterion placements along a scale of confidence and question emphasis of newness or oldness is considered to reflect a differentiation in novelty and familiarity processing. However, it is suggested that further investigations into these processes should be undertaken using never previously experienced stimuli (e.g. fractals; Miyashita, Higuchi, Sakai, & Masui, 1991). The simplicity of investigating whether participants are differentially able to interrogate and assess novelty and familiarity processes was an important initial exploration into the questioned differentiation of these. Following on from this, to further interrogate this distinction, an attempt should be made to specifically manipulate novelty and familiarity processing. Hence, using abstract stimuli, the next experimental chapter investigates whether novelty and familiarity processing can be differentially manipulated.

3. CHAPTER THREE: DIFFERENTIAL MANIPULATIONS OF NOVETLY AND FAMILIARITY AT A COGNITIVE LEVEL

3.1 Introduction Experiment 2

The previous experimental chapter outlines the investigation of potential differentiation between novelty and familiarity processing, through examination of participants' ability to differentially question and assess these. While demonstrating that participants assessed their memories dissimilarly under conditions emphasising novelty or familiarity, further investigations are required to ascertain whether the dissimilarities reflect differential processing of these. The present experiment aimed to obtain clearer evidence with regard to this question by systematically, and potentially differentially, interfering with novelty and familiarity processing. It is proposed that if these are terms referring to a single cognitive process or memory strength continuum, the presentation of items with contrasting mnemonic statuses will inevitably interfere with recognition. Specifically, presenting a concurrent novel item will hamper recognition memory judgments for familiar items, and vice versa, as the same memory strength signal will be in use for both items. Furthermore, assuming a single process, the effects of concurrent novelty on familiarity judgements should be equal and opposite to those of familiarity on novelty judgments. This was tested by examining recognition performance for targets and lures presented with concurrent items of various mnemonic statuses.

Presenting items of various mnemonic statuses parallels well with the recognition memory behavioral paradigms used in rodents. As detailed in Section 1.2.1 of the introduction, the spontaneous-object-recognition (SOR) task, in which rodents are presented with a previously seen and a novel object, is widely used. Their preferential exploration of the novel object is considered to demonstrate recognition of the familiar item (for a review see Antunes & Biala, 2012). Thus, in these tasks,

two items of competing mnemonic status are used. Recent research has shown that rats with perirhinal lesions, while known to demonstrate significant recognition deficits when tested using this version of a SOR task (Aggleton et al., 2010; Barker et al., 2007; Ennaceur et al., 1996), spend a similar amount of time to controls exploring two novel or two familiar objects presented concurrently (Orlate-Sanchez, et al., 2015; McTighe, et al., 2010; Romberg, et al., 2012). These results suggest that presenting items of competing mnemonic statuses interferes with item recognition memory, which is otherwise spared in the absence of this competition. This further suggests an interaction between novelty and familiarity processing. However, investigations have not been systematic enough for conclusion to be drawn about the nature of the interaction (such as whether this reflects a single or separable processes). Furthermore, in the standard SOR task, identification of novelty and familiarity is deduced based on relative exploration of the objects, and hence, using this methodology, an independent measure of these is not available. Thus, in the following experiment, the degree of mnemonic competition, and its effect on both the correct identification of targets and lures, is investigated to further question the nature of this interaction.

In the human literature, single item old/new paradigms dominate item recognition memory investigations (O'Connor, Guhl, Cox, & Dobbins, 2011). Multiple items, sometimes including a combination of novel and familiar items, are commonly presented in tasks of source-recognition (e.g. Horner, Bisby, Bush, Lin, & Burgess, 2015). However, performance in these tasks is not expected to be affected by interference of concurrent mnemonic items because source-recognition is dependent upon recollection, which is a binary informant - details of the study phase (e.g. a source) are either recalled or not – and is considered to be supported by distinct cognitive and neural substrates to familiarity (see sections 1.3 and 1.4 of the General Introduction).

Nevertheless, in a series of experiments aimed at questioning the validity of including a recollection component to recognition memory models, O'Connor and

colleagues (2011) have interrogated recognition memory for words presented in threes. In their three-alternative-forced choice task, in which participants answered a "which is old?" (previously see in the study phase) or "which is new?" question, participants were as accurate at identifying an old word presented alongside two new words as they were at identifying a new word presented alongside two old words. Given the symmetry of this design and the lack of a condition in which all items had the same mnemonic status, it is not possible to conclude whether these results reflect that concurrent items of competing mnemonic status interfered with recognition memory for targets and lures in the same manner and to the same extent, or whether there was no interference.

However, participants also undertook an oddity task in which they were cued to identify "which is different?". Here participants are still identifying an old word presented amongst two new words or a new word presented amongst two old words, but they are not required to identify whether it is old or new. Rather participant are simply responding as to which word feels most mnemonically different to the others. Under these conditions participants are reliably better at identifying a novel word amongst two old words than an old word amongst two new ones. Interestingly, these results suggest recognition interference due to concurrent items, with this being differentially disruptive for isolating new or old items among mnemonically conflicting ones. Once more, as the mnemonic status of concurrent items for lures and targets were not systematically manipulated, the details of the exposed interference cannot be examined.

Furthermore, it is important to note that the findings of the oddity task outlined above (O'Connor, et al., 2011) were obtained using word stimuli. As discussed in the previous experimental chapter's discussion section (Section 2.4), word stimuli have significant pre-experimental familiarity and this can confound the results obtained. Indeed, as outlined by O'Connor and colleagues (2011), the results from the oddity task can equally be conceptualized as demonstrating that participants found it easier to identify two old words as perceptually similar to each other than two new words.

Taken into consideration with the lack of an effect of triplet construction (1:2 or 2:1 old to new) on explicit recognition memory, and because of the use of word stimuli, it is tentatively argued here that the results from the oddity task reflect participants' use of mnemonic recency, rather than old/new recognition judgments. While the differentiation between familiarity and recency is not of central importance to this thesis, and hence will not be outlined in detail, evidence supports the differentiation of these (Aggleton & Brown, 2001).

The proposition here is that participants are using different strategies to undertake the oddity and the three-alternate-forced choice task. Specifically, as depicted in Figure 3.1a, all the words presented in the experiment will have a pre-experiment underlying level of associated memory strength (based on the frequency of encountering these ahead of experiment commencement; black line). The variation in this will be high. While presenting words during a study phase will serve to add a set amount of familiarity to each of these (Figure 3.1a, red arrow), the variation in the memory strength distribution for both studied and un-studies words is still large (Figure 3.1a). Contrarily, when in the test phase, words presented at study will have been experienced recently, within a short time frame of each other, in contrast to words experienced out-with the experiment (Figure 3.1b).

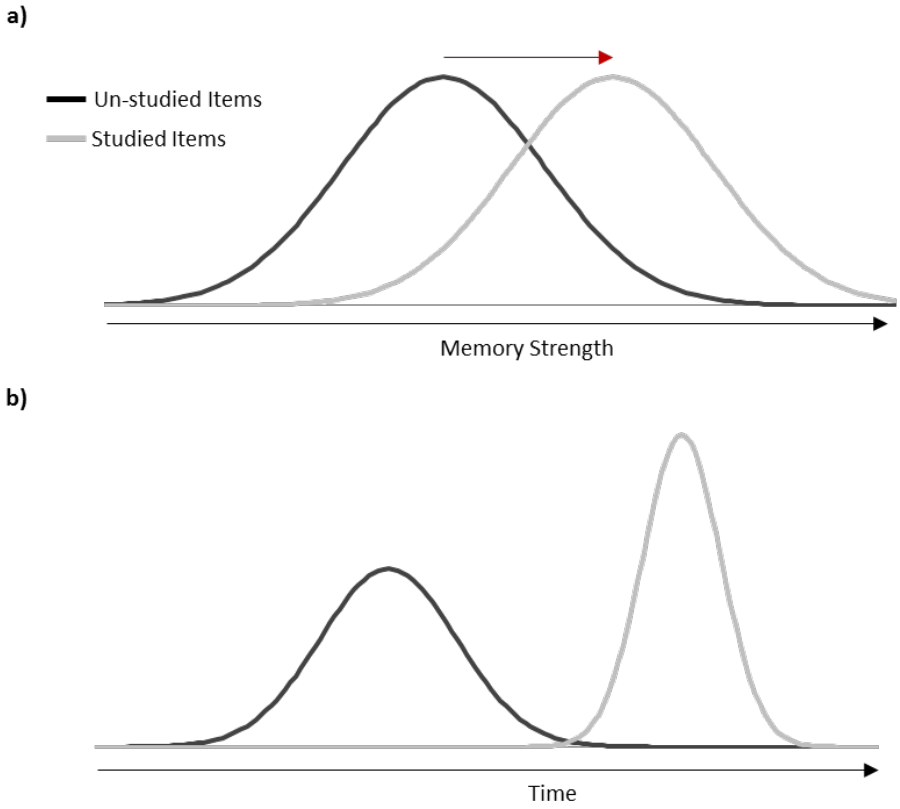

Figure 3.1: Frequency distributions for studied and un-studied items along a) a memory strength continuum and b) a time (recency) continuum.

Hence, studied words will be more similar to each other with respect to recency as compared to frequency of exposure, and will also be more different to un-studied items along this same dimension (Figure 3.1b). As such, recency is a more strategic basis upon which to identify the most "mnemonically similar" words in a triplet (and consequently the most "different" word). This would account for the greater facility for a new item to be identified amongst old ones than an old one amongst two new as old items are more similar to each other in recency than two new items. If the stimuli used in the experiment had never previously been encountered, the distributions of items along a frequency and a recency continuum would be similar, and hence recognition based judgments would be as optimal. Although a highly

tentative suggestion which requires empirical support before being accepted, this possibility further highlights the necessity to use completely novel stimuli for experiments including investigations into novelty and/or familiarity processing.

Based on the above discussed literature, the experiment reported below investigated the interaction of novelty and familiarity processing by varying the degrees of mnemonic competition between a questioned fractal image and a concurrently presented fractal image. Fractals are abstract geometric shapes in which the structure recurs at progressively smaller and smaller scales (e.g. a snowflake). These can be generated artificially, with their complexity allowing a diverse set to be obtained (Miyashita, et al., 1991), and their abstract nature ensures that these are difficult for participants to base upon previous representations. As such, these are considered appropriate stimuli to use in investigations of novelty and familiarity processing, addressing the issue of underlying pre-experimental familiarity. On account of the potentially small nature of the interference between novelty and familiarity processing, as hypothesised due to the lack of an experiential disruption when presented with a new item in our daily familiar lives, this experiment was delivered online to allow a large enough sample size for statistical power.

3.2 Materials and Methods

3.2.1 Participants

A total of 305 individuals completed the online experiment. Twenty participants who did not provide demographics were excluded, along with seventeen participants whose overall performance did not reach a minimum threshold of $d' > 0.1$ (see Section 2.2.1 of previous chapter). Hence, the final sample consisted of 268 individuals, 66.0% females (n = 177), 33.6% males (n = 90) and 0.4% undisclosed sex (n = 1) (mean age = 35.16, age range = 18–78 years). Forty-eight participants completed the experiment in French, while the remaining 220 participants

completed it in English. Participants were recruited from a link to the study on the laboratory website and through social networking sites (e.g. Facebook and Twitter). As an incentive to take part, participants were informed that they would receive feedback on their memory performance. Informed consent was obtained in accordance with the University Teaching and Research Ethics Committee at the University of St Andrews (Appendix B).

3.2.2 Stimuli

Given the interest in investigating novelty and familiarity processing, minimizing contaminating familiarity from previous out-of-experiment exposure was a requirement. The stimuli thus consisted of a set of 168 colour and greyscale fractals, measuring 480 x 360 pixels (12.7 x 9.5 cm), created using Chaos Pro Freeware (Pfingstl, 2004), (see Figure 3.2 for examples, and Appendix C for the full set).

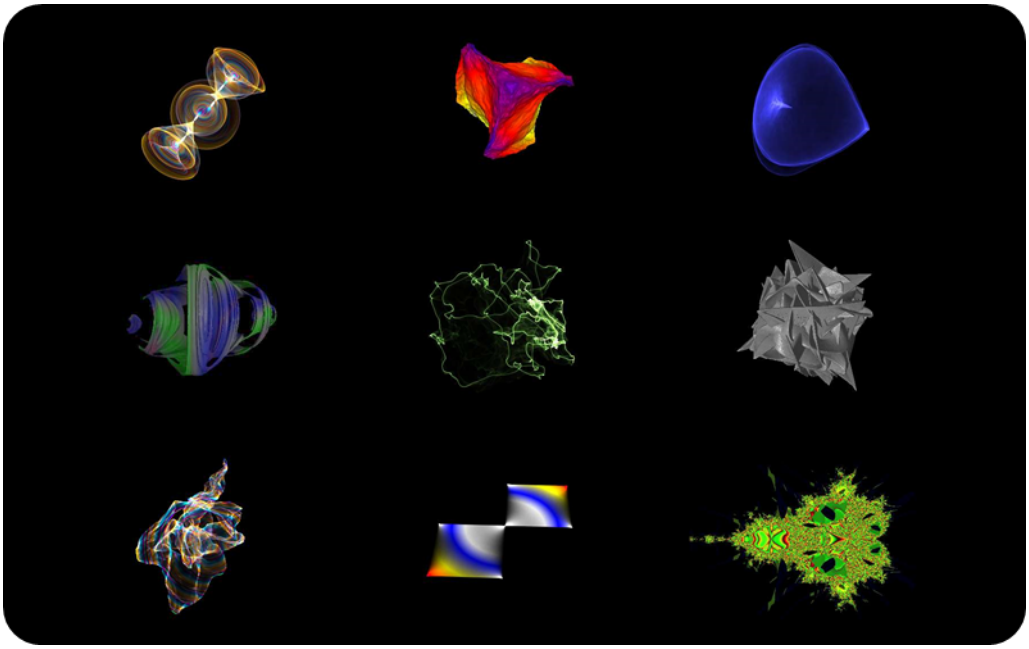

Figure 3.2: Nine exemplar fractals used as stimuli. These were presented measuring 480 x 360 pixels (12.7 x 9.5 cm). See Appendix C for the full set of 168 fractals.

For each participant, half of these were randomly selected to be old (presented both at study and at test), while the other half were designated new (N; only presented at test). Of the 84 old fractals, 24 of these were randomly selected to be High Familiarity (HF), with the remaining 60 being Low Familiarity (LF). The familiarity of the fractals was manipulated by altering the number of exposures to these in the study phase: High Familiarity fractals were presented three times while Low Familiarity ones were presented only once.

3.2.3 Materials

The experiment was programmed using JavaScript and presented to participants via their internet browser. To widen participation, the experiment was translated into French and delivered in exactly the same manner as that outlined in English below.

3.2.4 Design and Procedure

After reading onscreen instructions, participants started the single study-test block. The study phase consisted of a self-paced 132 trial incidental encoding task. Intermixed randomly within these 132 trials were three presentations of each of the 24 High Familiarity items, along with a single presentation of each of the 60 Low Familiarity items. For each item presented in the center of the screen, participants responded to the question “Does this image have purple in it?”, presented above the item, with either a “yes” or “no” response (Figure 3.3a). Responses were made using either the keyboard (1 = “yes”; 0 = “no”), if the participant was undertaking the experiment on a computer, or by pressing buttons below the item if they were undertaking the experiment on a touchscreen device (Figure 3.3a). A 0.1 second inter-trial-interval black screen preceded each trial.

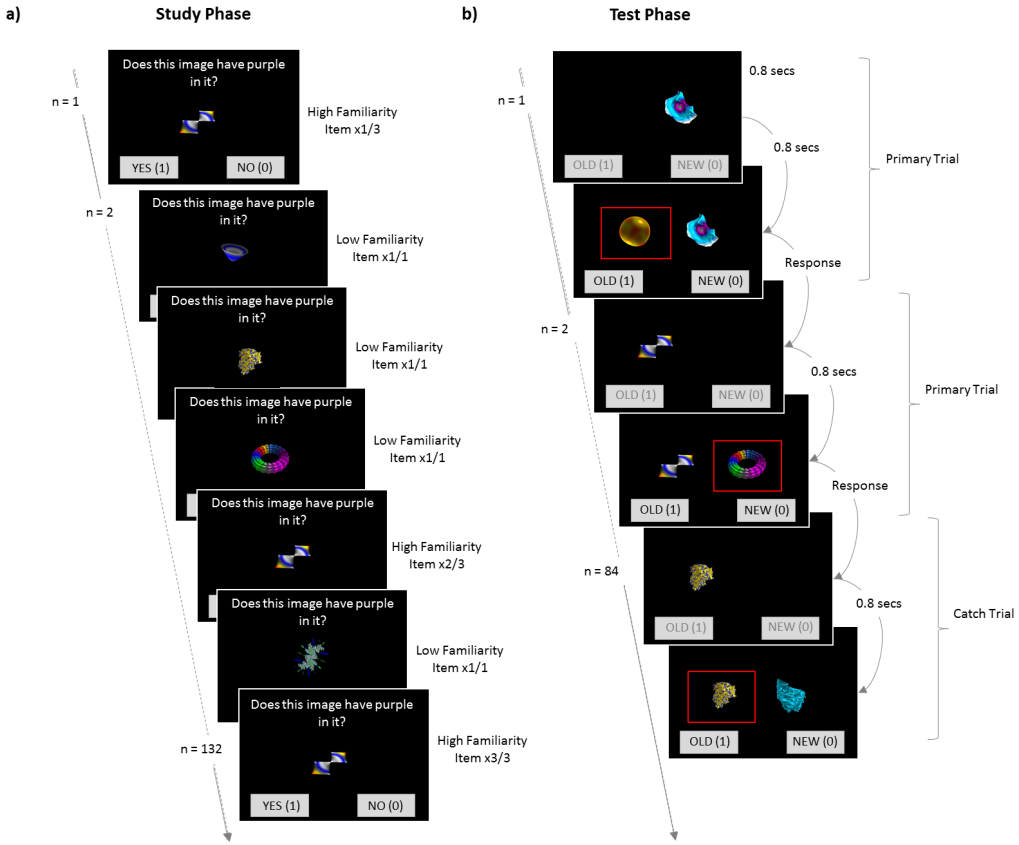

Figure 3.3: Experiment 2 experimental design. Examples of trials within a a) study and b) test phase.

The test phase was presented immediately following the study phase. At test, items were presented in pairs: one questioned item and one concurrent item. Questioned items were either N or LF, but not HF. This was implemented as minimizing experiment duration is crucial for online studies, and this experiment was designed to investigate familiarity and novelty processing rather than recollection. Recollection is known to contribute to high confidence old judgements, and as such, while HF items were used as concurrent items, recognition for these was not directly tested. Six test-pair conditions were based on the memory status of the two concurrently presented items (questioned items are underlined): (i) N-N; (ii) N-LF; (iii) N-HF; (iv) LF-N; (v) LF-LF; (vi) LF-HF (see Table 3.1).

Table 3.1: The six within-subjects test conditions undertaken by participants.

Condition	Questioned Item	Context Item	Number of Primary Test Trials	Number of Catch Test Trials	Total Number of Test Trials
N-N	New	New	20	4	24
N-LF	New	Low Familiarity	10	2	12
N-HF	New	High Familiarity	10	2	12
LF_N	Low Familiarity	New	10	2	12
LF-LF	Low Familiarity	Low Familiarity	10	2	12
LF_HF	Low Familiarity	High Familiarity	10	2	12

Note: Primary Test trials are the trials of interest, and consist of presenting a concurrent item first, followed by a questioned item. Catch trials are designed to ensure participants do not simply ignore the first item, such that questioned items are presented first, followed by the concurrent item.

The test phase consisted of 84 self-paced trials. At test a first item appeared either on the left or right of the screen. After a 0.8 second delay, a second item appeared on the other side of the screen. At the same time as the second item appeared, a red border appeared around one of the items, indicating to the participant that this was the questioned item. Presentation was staggered to encourage participants to pay attention to, and process, the first item presented on screen. The participants then responded by indicating whether the questioned item was “old” or “new” in the same way that “yes” and “no” responses were made in the study phase (Figure 3.3b). Keyboard and button responses were disabled until the second item and red border had been presented onscreen, such that participants could not respond before this. A 0.2 inter-trial black screen preceded each trial.

Primary trials of interest in which the questioned item appeared onscreen second (to allow participants time to attend to and process the concurrent item) occurred for 80% of the trials in each conditions. The remaining 20% of trials were catch trials in which the questioned item was that which appeared onscreen first (Table 3.1). This ensured participants did not simply ignore the first item presented onscreen. Questioned items were presented on the left and right hand side of the screen in a pseudorandom order. Of the 84 items questioned at test, 48 questioned items were N lures and 36 were LF targets. This allowed a 1:1 ratio of new and old item to be

presented on screen during the test phase, as differing numbers of old and new items can affect bias (Ratcliff & McKoon, 2007). A 0.2 second black inter-trial-interval screen preceded each trial.

3.2.5 Calculations

To investigate the effect of a concurrent item's mnemonic status on recognition memory, mean adjusted hits (*H*'), correct rejections (*CR*'), misses (*M*') and false alarms (*FA*'), along with the sensitivity (*d'*) and bias (*c*) parameters estimated from these (Macmillan & Creelman, 2005), were the measures of principal interest for this experiment. These were calculated for each participant as outlined in Section 2.2.4 (page 46) of the previous chapter.

3.2.6 Data Analysis

Participants' performance was investigated relative to chance by submitting the parameter estimate of sensitivity (*d*') to a one-sample t-test, with zero as the value of comparison. Participants' overall bias (*c*) was also assessed using a one-sample t-test, with zero as the value of comparison, where $c = 0$ shows optimal bias.

Following this, to question the effect of the mnemonic status of concurrent items on adjusted hit (*H*') and correct rejection (*CR*') rates, each was submitted to a separate one-way repeated measures ANOVA with the mnemonic status of the concurrent item (New - N, Low Familiar - LF, High Familiar - HF) as the within-participant factor. Any significance was further assessed using Bonferroni corrected Pairwise comparisons.

Measures of sensitivity and bias were also each submitted to a one-way repeated measures ANOVA with the mnemonic status of the concurrent item (N, LF, HF) as the within-participant factor. Any significance was further assessed using Bonferroni corrected Pairwise comparisons.

For all one-way repeated measures ANOVAs, where the data were found to violate the assumption of sphericity, as denoted by a significant Mauchly's test, Greenhouse-Geisser corrections were applied. Where this is the case it is explicitly stated within the reporting of the results.

A α threshold of 0.05 was adopted for all statistical analyses reported.

3.3 Results

The main focus of the present experiment was to investigate the effects of the mnemonic status of concurrent items on recognition memory. Thus, only results pertaining to primary test trials in which the questioned item was second were analysed. Furthermore, due to the nature of an online study where participants can easily concurrently engage in other tasks, mean hit and correct rejection response times (RTs) were calculated for each participant, and trials where RTs were under or above 3 standard deviations from the participant's mean RT for that response (hit or correct rejection) were excluded from further analyses. A mean of 0.98 trials were excluded per participant (SD = 0.69, rage = 0 - 3). This equated to a total of 262 trials being excluded across all participants (1.2% of all trials), 260 for being above three standard deviations above participant's mean RT, and 2 for being under.

3.3.1 Overall performance

Participants had a mean overall accuracy of 0.68 (SD = 0.07), with a mean *H*' of 0.50 (SD = 0.16) and a mean *CR*' of 0.80 (SD = 0.12). Participants were performing significantly above chance as demonstrated by a single-sample t-test performed on *d*' (M = 0.97, SD = 0.57), $t_{(267)} = 34.75$, $p < 0.001$, d = 2.12. Overall, participants had a conservative *c*, showing greater predisposition to calling items of ambiguous mnemonic status "new" than "old" (M = 0.48, SD = 0.41), as confirmed by a single-sample t-test, $t_{(267)} = 19.17$, $p < 0.001$, d = 1.17.

3.3.2 Analyses of Hit and Correct rejection rates

Mean *H*' and *CR*' rates for items paired with New, Low Familiarity and High Familiarity items are presented in Figure 3.4. Participants' *CR*' rates were greater than their *H*' rates. *H*' rates were not affected by the mnemonic status of the concurrent item (N, M = 0.51, SD = 0.19; LF, M = 0.50, SD = 0.19; HF, M = 0.50, SD = 0.19), as confirmed by a one-way repeated measures ANOVA with the mnemonic status of the concurrent item (N, LF, HF) as the within-participant factor, $F_{(2, 534)}$ = 0.481, p = 0.618. Contrastingly, the mnemonic status of the concurrent item differentially affected *CR*' rates, as demonstrated by a Greenhouse-Geisser corrected one-way repeated measures ANOVA with the mnemonic status of the concurrent item (N, LF, HF) as the within-participant factor, $F_{(1.927, 514.462)}$ = 7.694, p = 0.001, η_p^2 = 0.028. Bonferroni corrected pairwise comparisons showed that participants had higher *CR*' rates for lures paired with New (M = 0.80, SD = 0.12) compared to both High Familiarity (M = 0.77, SD = 0.17), and Low Familiarity (M = 0.78, SD = 0.15) items, p < 0.001, and p = 0.014, respectively. However, no differences were seen between *CR*' for lures paired with Low Familiarity and High Familiarity items, p = 0.808.

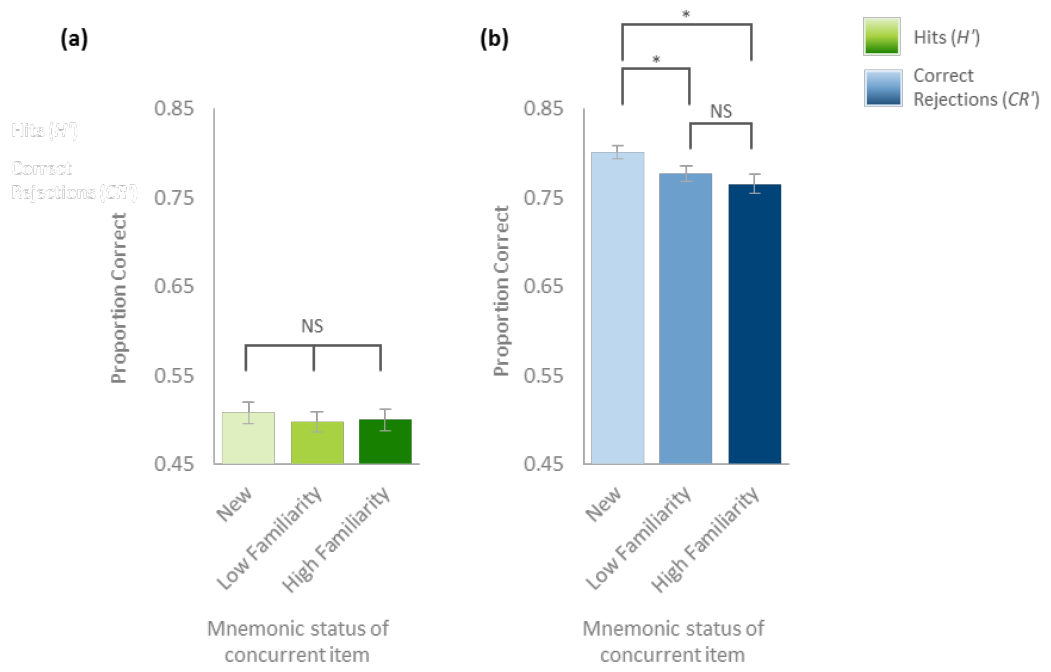

Figure 3.4: Decision accuracy for items paired with concurrent items of differing mnemonic statuses. Bars show mean proportion of targets and lures correctly identified when these were paired with concurrent items of varying mnemonic statuses. Error bars represent the standard error.

3.3.3 Analyses of sensitivity (*d'*) and bias (*c*)

As simply considering the rate of correct responding to targets (hits) and lures (correct rejections) does not capture the measure of participants' bias (*c*) or sensitivity (*d'*) - the combined effect of hits and correct rejections – these were also investigated. To question how the mnemonic status of concurrent items affects recognition memory sensitivity and bias, the data were collapsed for each participant across conditions based on the mnemonic status of the questioned item is underlined):

N-N	and	LF-N	=	concurrent N
N-LF	and	LF-LF	=	concurrent LF
N-HF	and	LF-HF	=	concurrent HF

Mean *d*' and *c* for items paired with New, Low Familiarity and High Familiarity concurrent items are presented in Figure 3.5a&b. The mnemonic status of the concurrent item affected participants *d*' as demonstrated by a one-way repeated measures ANOVA with the mnemonic status of the concurrent item (N, LF, HF) as the within-participant factor, $F_{(2, 534)} = 3.484$, p = 0.031, $\eta_p^2 = 0.013$. Pairwise comparisons confirmed that participants' *d*' was better for items presented with a concurrent New item (M = 0.97, SD = 0.57) compared to when presented with a High Familiarity item (M = 0.86, SD = 0.59), p = 0.043. Participants *d*' for items presented with a Low Familiarity concurrent item did not differ from that for items presented with either a New or High Familiarity item, p = 0.101, and, p = 1.000, respectively.

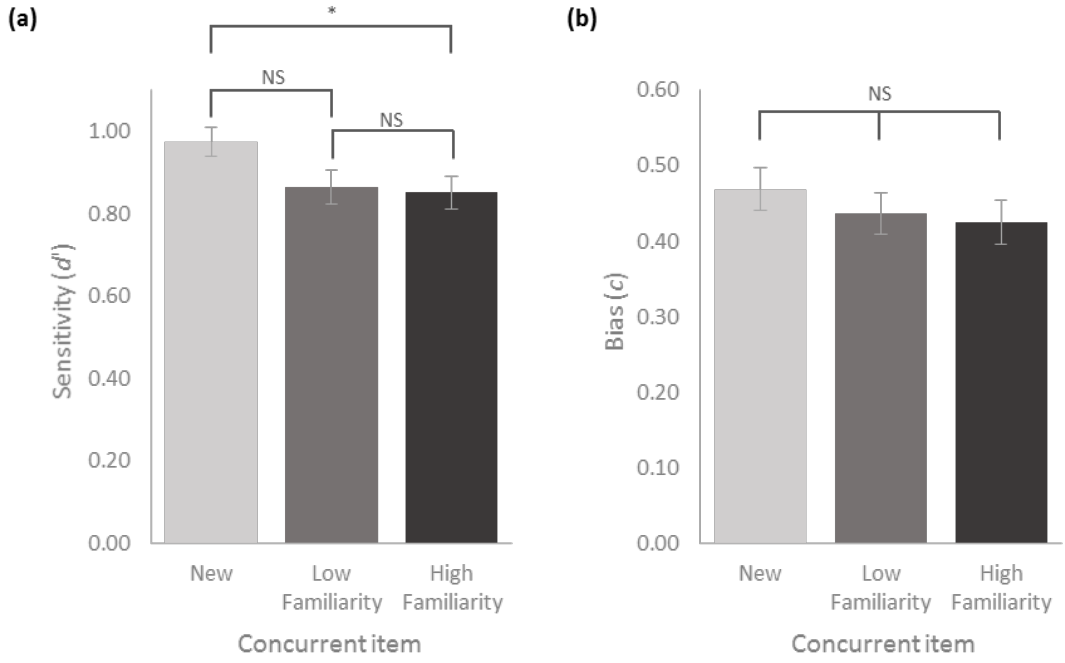

Figure 3.5: (a) Sensitivity and (b) Bias for items paired with concurrent items of differing mnemonic statuses. Bars show mean parameter estimates of (a) sensitivity (*d*') and (b) bias (c) under conditions where recognition is tested while concurrently presenting New, Low Familiarity or High Familiarity items. Error bars represent standard errors.

As illustrated in Figure 3.2b, the mnemonic status of concurrent items appeared to have no impact upon *c* (concurrent item: N, M = 0.47, SD = 0.45, LF, M = 0.44, SD =

0.44, HF, M = 0.43, SD = 0.48). This was confirmed by a Greenhouse-Geisser corrected one-way repeated measures ANOVA with the mnemonic status of the concurrent item (N, LF, HF) as the within-participant factor, $F_{(1.92, 512.98)} = 1.624$, p = 0.198.

Due to the differences in *d*', it is important to also consider *c* as scaled by *d*' to enable comparisons between the mnemonic pairs presented at test. The use of scaled *c* allows for bias relative to sensitivity to be considered. *c* was scaled to *d*′ on a participant by participant basis for items paired with a New, a Low Familiarity and a High Familiarity item using the following formula (Macmillan & Creelman, 2005, p.33):

$$c' = \frac{c}{d'} \quad (7)$$

Although participants with an overall *d*' < 0.1 were excluded from the analyses, a number of participants passed this performance threshold while their *d*' for items paired with a given mnemonic status (e.g. N) was zero[3]. As a *d*' of zero renders the calculation of *c*' impossible, participants for whom this was the case were excluded from the following analyses. As such *c*' was calculated for a subset of 190 individuals (70.9 % of the total sample), 65.1% females (n = 123), 34.9% males (n = 66) and 0.5% undisclosed sex (n = 1) (mean age = 35.06, age range = 18–78 years). Mean *c*' for items paired with New, Low Familiarity and High Familiarity concurrent items are presented in Figure 3.6.

Participants *c*' appears similar for items paired with New and low Familiarity items, but lower for items paired with High Familiarity items. This was supported by the one-way repeated measures ANOVA with the mnemonic status of the concurrent

[3] To ensure consistency across research in this thesis, exclusion criteria were maintained throughout all experiments.

item (N, LF, HF) as the within-participant factor, $F_{(2, 468)} = 3.039$, $p = 0.049$, although subsequent Pairwise comparisons (presented in Table 3.2) failed to reach significance.

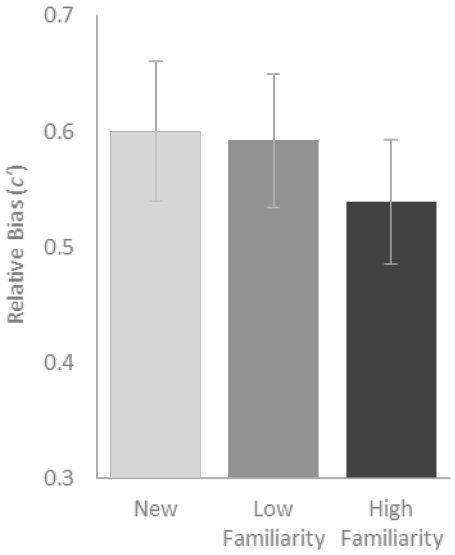

Figure 3.6: Bias for items paired with concurrent items of differing mnemonic statuses. Bars show mean parameter estimates of bias (*c*) relative to sensitivity (*d*') under conditions where recognition is tested while concurrently presenting New, Low Familiarity or High Familiarity items. Error bars represent standard errors.

Table 3.2: Mean differences in *c*' parameter estimates for items paired with New, Low Familiarity or High Familiarity items, as used for Pairwise comparisons.

	***c*' New**	***c*' Low Familiarity**	***c*' High Familiarity**
***c*' New**			
***c*' Low Familiarity**	0.061		
***c*' High Familiarity**	0.051	0.011	

*Note: * denotes significant differences as supported by Pairwise comparisons*

3.4 Discussion

The aim of this experiment was to investigate whether novelty and familiarity could be differentially influenced through the presentation of concurrent items of a competing mnemonics status, as suggested in O'Connor et al., (2011). The current experiment demonstrates a selective impact of mnemonic concurrent items on correct rejections, with no impact on hits. Identification of novel items was superior when these were presented alongside equally novel items compared to highly familiar items. Indeed, this finding parallels well with findings from the animal literature. Rats with perirhinal cortex lesions show a deficit in identifying an object as novel when this is paired concurrently with a familiar object, however are unimpaired (showing greater levels of exploration than for familiar items) for novel objects presented alongside a second novel object (Orlate-Sanchez, et al., 2015; McTighe, et al.,, 2010). Both in this experiment and in the rodent literature, due the lack of a baseline condition in which items are presented individually, it is not possible to ascertain the whether this difference reflects that novel concurrent items aid recognition of novel items, or whether highly familiar concurrent items hampers recognition of novel items.

The results from the current experiment are difficult to assimilate to the findings from O'Connor et al., (2011) as, consequentially to their triplet design, items were always presented with both a mnemonically equal and mnemonically conflicting item. As such their results reflect the effects of these individual mnemonic pairings and their interaction. Hence, the effect of individual mnemonic pairings is unknown, and cannot be compared to those observed in Experiment 2.

The difference in correct rejection rates was reflected in participants' sensitivity: participants' recognition ability was superior for items paired with a novel item than those paired with a highly familiar item. Thus, the mnemonic value of a concurrent item impacted the bottom-up evidence signal participants used to make their recognition memory judgements. While participants absolute bias was not affected by the mnemonic status of concurrent items, once bias was considered relative to sensitivity, participants were shown to be less conservative (more likely to endorse

an “old” decision) for items presented alongside a concurrent highly familiar item. This reflects a strategic shift where, when presented with a concurrently familiar item, participants become relatively more willing to wrongly identify a novel item as “old” compared to an old item as “new”. The reason for this shift is unclear. As items in Experiment 2 were presented with concurrent items of varying mnemonic statuses in an intermixed design, the differences in bias observed suggest participants are indeed amending their bias on an item-by-item basis. However, as outlined in in the discussion to Experiment 1, previous literature has demonstrated that participants are disinclined to do this, even under experimental designs promoting it (Stretch & Wixted, 1998; Han & Dobbins, 2008; Morrell, Gaitan & Wixted, 2002). Stretch & Wixted (1998) proposed this invariance in criterion placement reflects the cognitive demands necessary for such strategic shifts to occur. The results from Experiment 2 would thus suggest that the mnemonic status of concurrent items is of enough importance to outweigh the cognitive effort required for criterion shifts. However, in light of the body of evidence demonstrating participants’ reluctance to implement criterion shifts, these results require replication before this interpretation can be fully explored.

The interest within these results lies in the differential effect of varying concurrent mnemonic statuses on novelty and familiarity judgements. The results demonstrate an effect of presenting items of equal and conflicting mnemonic statuses only for the identification of novel items as such, with no effect of their presentation on the identification of old items. This supports the suggestion that novelty and familiarity processes are dissociable, as if these were a single process interference across the memory strength spectrum would occur in equal and opposite forms. Specifically, if there is an advantage of presenting an item of equal mnemonic status, this would be observed for both novel and familiar items. The results provide evidence towards the dissociation of novelty and familiarity processing within recognition memory.

It is important to consider here that these results occur under a methodological design in which the concurrent item did not initially appear on screen simultaneously

with the test item. The argued interference on recognition memory of a concurrent item of a differing mnemonic status has been presented and hypothesised as a one of interference of a competing memory strength. However, as items appeared on screen in a delayed manner, a lack of an effect of the concurrent item would not negate that novelty and familiarity processing disrupt each other when items of differing mnemonic statuses are presented simultaneously and recognition memory resources for both are competed for simultaneously. However, the results suggest interference occurs despite this delayed presentation in the methodology.

It is also important to consider these result within the context of the absolute recognition performance of participants as well as the relative effects of the test conditions discussed above. Firstly, although significantly above chance, participant's sensitivity was low (M = 0.97, SD = 0.57). This is best illustrated by the degree of overlap between the lure and target distribution when the data is plotted on an equal-variance signal detection model (Figure 3.7). This is also reflected in the low hit rates, where participants were only correctly identifying approximately 50% of old items as "old". Furthermore, participants were highly conservative, requiring a significant amount of evidence before identifying an items as "old" (Figure 3.7). Taken together, these results indicate that participants did not find the stimuli memorable.

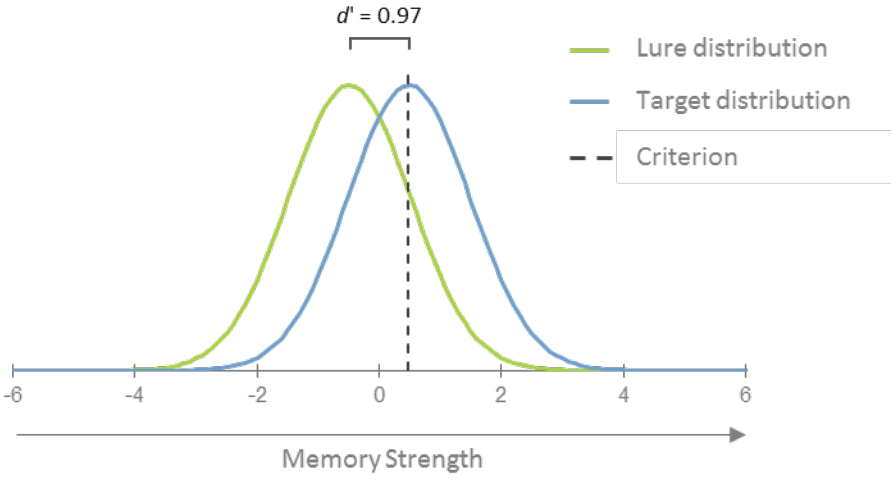

Figure 3.7: Signal detection representation of participants' overall sensitivity and bias.

In this respect, the choice of stimuli appears to have caused the opposite problem to the word stimuli: the fractals are too novel, such that after their presentation during the study phase they are not familiar enough to allow participants to display a typical recognition judgement performance. Given the effects present in this data are directly informative with respect to the overarching question of whether novelty and familiarity are dissociable processes, their validity is important. Hence, the following experiment aimed to determine if the results obtained are maintained when using more memorable stimuli.

3.5 Introduction Experiment 3

Experiment 3 was devised to address the issues arising from the experimental design of Experiment 2. Firstly, while Experiment 2 demonstrated differential interference resulting from concurrently presented mnemonic items on the identification of novel and familiar items, participants had difficulty recognizing fractals that had been presented at study. Thus, Experiment 3 aimed to determine if the results observed in Experiment 2 are maintained under conditions of superior recognition memory. Fractals were the stimuli of choice in Experiment 2 as these were complex abstract shapes with no associated pre-experimental levels of familiarity (see Section 2.4 for a further discussion). To satisfy this requirement of no associated pre-experimental levels of familiarity while raising recognition memory performance, Digimon and Pokémon characters were chosen as stimuli for the current experiment[4]. These have more distinctive features than fractals and consequently are more memorable. Characters were chosen from late and less publicized generations of Digimon and Pokémon to minimize pre-experimental familiarity. While there is a potential for these characters to be familiar to participants (where participants reporting high levels of out of experiment exposure to these will be excluded from the analyses) these stimuli are nonetheless notably less familiar than the common English nouns frequently used in recognition memory experiments.

Differences in participants' correct rejection rates between lures paired with a concurrent New item and those presented with a High Familiarity item were observed in Experiment 2. Due to the lack of a single item baseline condition, it was not possible to ascertain whether these differences resulted from concurrent novelty aiding identification of new items, or whether concurrent high familiarity hinders recognition of new items. As such, single item baseline conditions in which recognition for novel and familiar items presented alone was tested, were included

[4] The release of the game Pokémon Go (Niantic, Inc, 2016), which re-introduced Pokémon characters into popular culture, occurred on the 13th of July 2016, 19 months after this experiment's completion. As such, popularisation of these characters in 2016 did not affect the current experiment.

in Experiment 3. Furthermore, as the differences observed in Experiment 2 were restricted to being between conditions when items were presented with a concurrent New and a concurrent High Familiarity item, only these levels of familiarity were used in Experiment 3, omitting Low Familiarity items. This follow-up experiment was conducted in a more controlled environment, within a laboratory setting.

3.6 Materials and Methods

3.6.1 Participants

Participants consisted of 30 self-reported native English speaking participants 83.3% females (n = 25, mean age = 20.8, age rage = 18-28), who all reported that less than 15% of the stimuli appeared familiar and reached a minimum overall performance of $d' > 0.1$. Participants were each compensated £5 for their time. Informed consent was obtained in accordance with the University Teaching and Research Ethics Committee at the University of St Andrews (Appendix D).

3.6.2 Stimuli and Materials

A set of 432 colour Pokémon generation II-VI (© 1995-2016 Nintendo/Creatures Inc./GAME FREAK inc. Pokémon) and Digimon (© 1997-2008 Bandai) characters were selected from online databases ("Pokémon Wiki", n.d.; "Wikimon", 2005; see Figure 3.8 for examples)[5]. Images measured 200 x 200 pixels (5.29 cm^2). For each participant, these 432 items were randomly divided into four, such that each subset was used for one of the four experimental blocks. Of the 108 items in each block, 54 were randomly selected to be High Familiarity (HF) old items (presented three times

[5] These stimuli do not infringe any copyright regulations as they are used under the "Fair dealing" law, originating in Section 29 and 30 of the Copyright, Designs and Patents Act 1988. This states that "fair dealing with a literary, dramatic, musical or artistic work for the purposes of research for a non-commercial purpose does not infringe any copyright in the work provided that it is accompanied by a sufficient acknowledgement".

at study and at once test) and 54 were randomly selected to be new (N) items (presented only once at test).

The experiment was programmed using JavaScript and presented to participants via an internet browser within a laboratory setting.

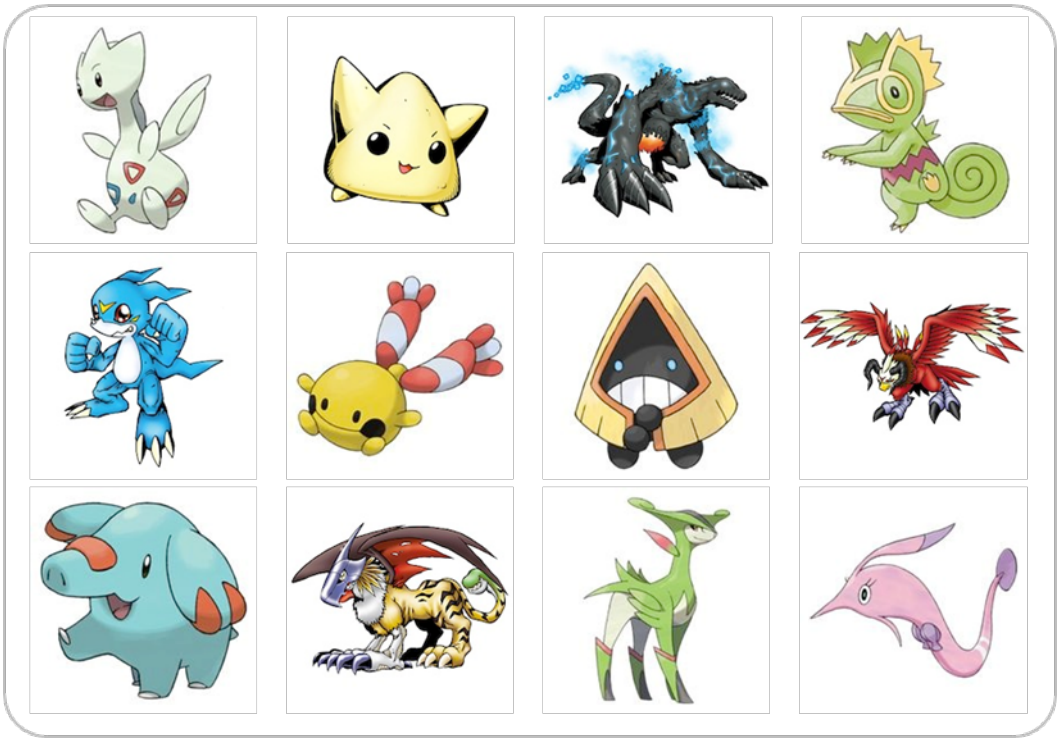

Figure 3.8: Twelve exemplar Pokémon and Digimon characters used as stimuli. These were presented measuring 200 x 200 pixels (5.29 cm^2).

3.6.3 Design and Procedure

After reading onscreen instructions, participants undertook 4 self-paced study-test blocks in succession. Each study phase consisted of a 162 trial incidental encoding task where items were presented serially, one at a time at the center of the screen (Figure 3.9a). For each item presented on screen, participants responded the question “Does this character have purple in it?”, presented above the item, with either a “yes” or “no” response. Responses were made by keypress (1 = “yes”; 0 =

"no"). A 0.5 second white inter-trial-interval screen preceded each trial. Intermixed randomly within the 162 items presented for a given study phase were three presentations of each of the 54 High Familiarity (HF) items (Figure 3.9a). These 54 items were then considered to be highly familiar for the subsequent test phase.

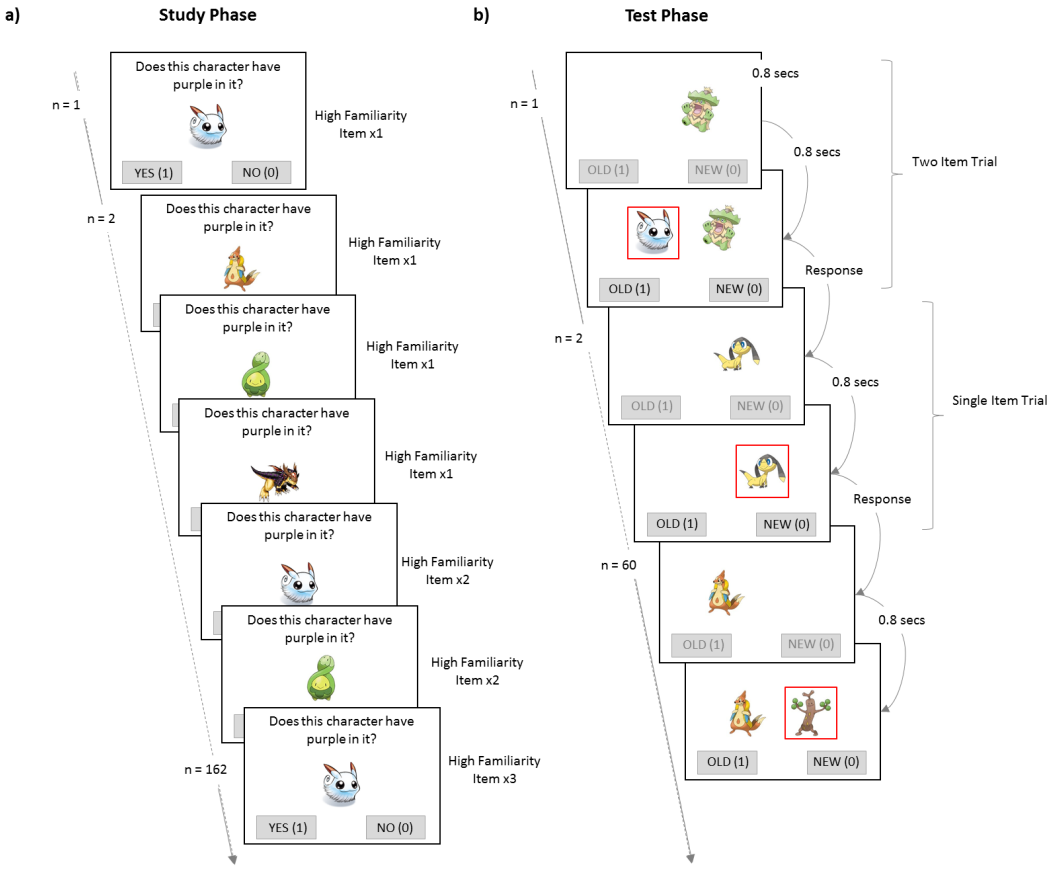

Figure 3.9: Experiment 3 experimental design. Example of a) a study phase and b) a test phase trials.

For each study phase a corresponding test phase immediately followed. Each test phase consisted of 60 intermixed trials from six conditions (Figure 3.9b). Items were presented either alone (single item: N or HF item) or in pairs (two item: N-N, N-HF, HF-N, HF-HF), where pairs included one questioned item (mnemonic status underlined) and one concurrent context item (Table 3.3).

Table 3.3: The six within-subject test conditions undertaken by participants, with the number of Primary and Catch trials presented in a given block for each condition.

Condition	Questioned Item	Context Item	Number of Primary Test Trials	Number of Catch Test Trials	Total Number of Test Trials
N-N	New	New	6	6	12
N-HF	New	High Familiarity	6	6	12
HF-N	High Familiarity	New	6	6	12
HF-HF	High Familiarity	High Familiarity	6	6	12
N	New	-	-	-	6
HF	High Familiarity	-	-	-	6

Note: Primary Test trials are the trials of interest, and consist of presenting a concurrent item first, followed by a questioned item. Catch trials are designed to ensure participants do not simply ignore the first item, such that questioned items are presented first, followed by the concurrent item.

At test an item appeared either on the left or right of the screen. If the trial was a two item trial, after 0.8 seconds a second item appeared on the other side of the screen. At the same time, a red boarder appeared around one of the items, indicating to the participant that this was the questioned item. If the trial was a single item trial, after 0.8 seconds, a red border appeared around the item already onscreen, indicating to participants that this was the questioned item (Figure 3.9b). The participants then responded by indicating whether the questioned item was "old" or "new" in the same way that "yes" and "no" responses were made in the study phase. A 0.2 second white inter-trial-interval screen preceded each trial.

For each test block, left/right positioning of the questioned item was counterbalanced within conditions and across trials. For two item trials, questioned items were presented second for 50% of the test trials (primary trials), with the remaining 50% of trials being catch trials, where the questioned item was the first to appear. This ensured participants did not ignore the first item presented onscreen, and provided a two item base line condition.

3.6.4 Calculations

To question the effect of a concurrent item's mnemonic status on recognition memory, mean adjusted hits (*H*'), correct rejections (*CR*'), misses (*M*') and false alarms (*FA*'), along with the sensitivity (*d'*) and bias (*c*) parameters which are estimated from these (Macmillan & Creelman, 2005), were the measures of principal interest for this experiment. These were calculated for each participant as outlined in Section 2.2.4 of the previous chapter.

3.6.5 Data Analysis

Participants' response times (RTs) to primary and catch trials were submitted to a paired samples t-test for comparison, to confirm an effect of order presentation.

Participants' overall sensitivity was compared to chance using a one-sample t-tests where the value of comparison was set to zero. In a similar manner, participants' bias was compared to optimum using a one-sample t-tests where the value of comparison was set to zero.

Following this, to question the effect of the mnemonic status of concurrent items on adjusted hit (*H*') and correct rejections (*CR*') rates, each was submitted to a separate one-way repeated measures ANOVA with the mnemonic status of the concurrent item (None - O, New - N, High Familiarity – HF) as the within-participants factor. Any significance was further assessed using Bonferroni corrected Pairwise comparisons.

Measures of sensitivity and bias were also each submitted to a one-way repeated measures ANOVA with the mnemonic status of the concurrent item (O, N, HF) as the within-participant factor. Any significance was again further assessed using Bonferroni corrected Pairwise comparisons.

For all ANOVAs, where the data were found to violate the assumption of sphericity, as denoted by a significant Mauchly's test, Greenhouse-Geisser corrections were

applied. Where this is the case it is explicitly stated within the reporting of the statistics.

An α threshold of 0.05 was adopted for all statistical analyses reported.

3.7 Results

Participants were significantly faster at responding to catch (M = 1.81 seconds, SD = 0.26) compared to primary (M = 1.95 seconds, SD = 0.22) test trials, as confirmed by a paired samples t-test, $t_{(29)} = 6.24$, $p < 0.001$, d = 1.14. This suggests that the delayed presentation allowed processing of the first item presented on screen. As RTs confirm different processing of the concurrent items presented during primary and catch trials, and as the aim of this experiment is to assess the effects of the concurrently presented item, only results pertaining to primary trials were analysed. In an identical manner to Experiment 2, individual trials were excluded from the analyses based on RTs. A mean of 3.70 trials were excluded per participant (SD = 1.15, rage = 2 – 6). This equated to a total of 111 trials being excluded across all participants (6.17% of all trials), with all these trials being excluded for being responded to slower than three standard deviations above participants mean RT.

3.7.1 Overall Performance

Participants had a mean overall accuracy of 0.82 (SD = 0.10), with a mean adjusted hit rate (*H*') of 0.71 (SD = 0.19) and a mean adjusted correct rejection rate (*CR*') of 0.92 (SD = 0.08). Participants overall sensitivity was significantly above chance (*d*'; M = 2.28, SD = 0.89), as confirmed by a single-sample t-test, $t_{(29)} = 14.20$, $p < 0.001$, d = 2.593. Overall, participants has a conservative bias (*c*; M = 0.49, SD = 0.37), as confirmed by a single-sample t-test, $t_{(29)} = 7.29$, $p < 0.001$, d = 1.331.

3.7.2 Analyses of Hit and Correct rejection rates

Firstly, to investigate how the mnemonic status of concurrent items affected recognition judgements of old and new items, participants' *H*' and *CR*' rates for items presented alone, paired with a New or paired with a High Familiarity item were examined (Figure 3.10).

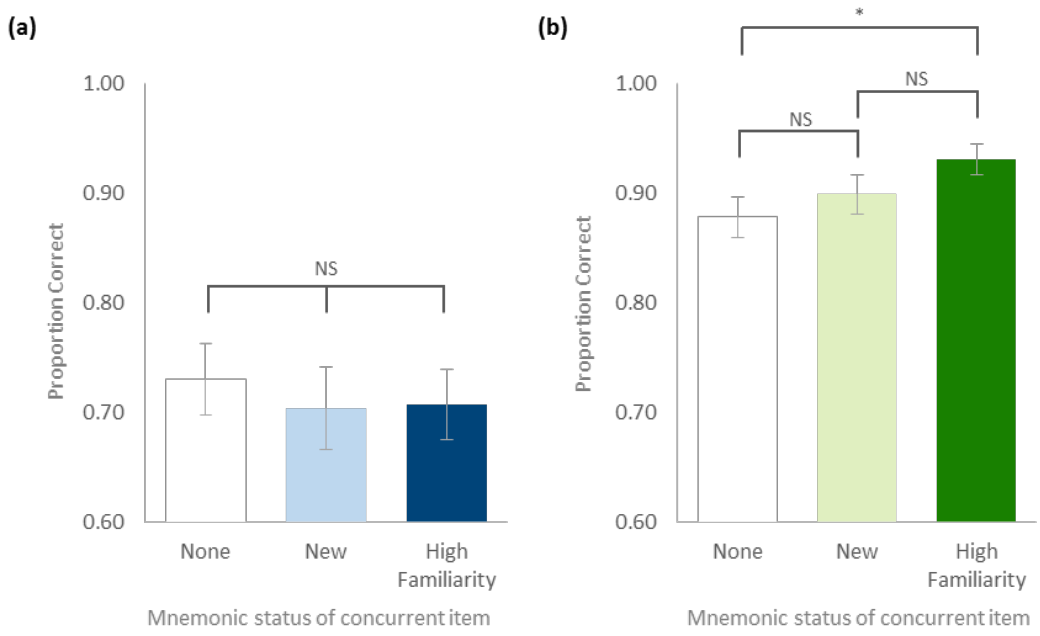

Figure 3.10: Decision accuracy for a) targets and b) lures presented alone or paired with concurrent items of differing mnemonic statuses.Bars show mean proportion of targets (blue) and lures (green) correctly identified when these were presented alone or paired with concurrent items of varying mnemonic statuses. Error bars represent the standard error.

H' rates were not affected by the mnemonic status of the concurrent item (O, M = 0.73, SD = 0.18; N, M = 0.70, SD = 0.21; HF, M = 0.71, SD = 0.18), as confirmed by a one-way repeated measures ANOVA with the mnemonic status of the concurrent item (O, N, HF) as the within-participant factor, $F_{(2, 58)} = 0.699$, $p = 0.501$, $\eta_p^2 = 0.024$. Contrastingly, the mnemonic status of the concurrent item differentially affected *CR*' rates, as also demonstrated by a one-way repeated measures ANOVA with the mnemonic status of the concurrent item (O, N, HF) as the within-participant factor,

$F_{(2, 58)}$ = 6.05, p = 0.004, η_p^2 = 0.173. Bonferroni corrected pairwise comparisons showed that participants had higher *CR*' rates for lures paired with a High Familiarity item (M = 0.93, SD = 0.08) than when the lures were presented alone (M = 0.89, SD = 0.10), p = 0.002. However, no differences were seen between *CR*' for lures presented with a New item and those presented either alone or with a High Familiarity item, p = 0.0479, and p = 0.229, respectively.

3.7.3 Analyses of sensitivity (*d*') and bias (*c*)

Participants' bias (*c*) and sensitivity (*d*') - the combined effect of *H*' and *CR*' – were also investigated. To question how the mnemonic status of concurrent items affected *d*' and *c*, the data were collapsed for each participant across conditions based on the mnemonic status of the concurrent items as follows (mnemonic status of questioned item is underlined):

N-N	and	HF-N	=	concurrent N
N-HF	and	HF-HF	=	concurrent HF
N	and	HF	=	None (O)

Mean *d*' and *c* for items presented alone, paired with New, and paired with High Familiarity concurrent items are presented in Figure 3.11. The mnemonic status of the concurrent item did not affected participants *d*' as demonstrated by a one-way repeated measures ANOVA with the mnemonic status of the concurrent item (O, M = 2.03, SD = 0.87; N, M = 2.10, SD = 0.90; HF, M = 2.30, SD = 0.81) as the within-participant factor, $F_{(2, 58)}$ = 2.44, p = 0.096, η_p^2 = 0.078.

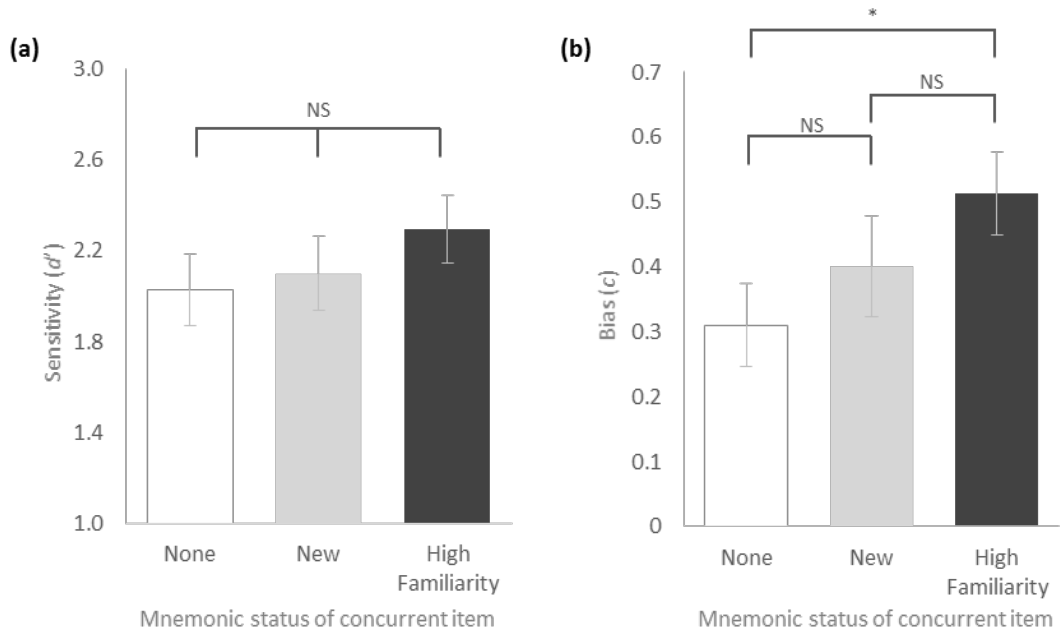

Figure 3.11: (a) Sensitivity and (b) Bias for items presented alone and paired with concurrent items of differing mnemonic statuses. Bars show mean parameter estimates of (a) sensitivity (*d*') and (b) bias (c) under conditions where recognition is tested while concurrently presenting no item (None), a New item or a High Familiarity item. Error bars represent standard errors.

As illustrated in Figure 3.11b, the presence of a concurrent item, and its mnemonic status appeared to influence participants' *c*. A one-way repeated measures ANOVA, $F_{(2, 58)} = 5.71$, p = 0.005, $\eta_p^2 = 0.165$, and corresponding Pairwise comparisons revealed that participants' were significantly more conservative for items paired with a High Familiarity concurrent item (M = 0.51, SD = 0.35), compared to when items were presented alone (M = 0.31, SD = 0.35), p = 0.005. However, there were no statistical differences in participants' *c* between items presented with a New concurrent item (M = 0.40, SD = 0.43) and either an item presented alone or with a High Familiarity item, p = 0.350, and p = 0.290, respectively.

Although no differences in *d*' were observed, for consistency, relative *c* (*c*') as scaled by *d*' for the differing conditions was also examined (see Section 2.2.4, page 46). This was calculated in an identical manner to that in Experiment 2. Similarly to Experiment 2, one participant (female, 25 years old) was excluded due to a *d*' of zero

for items presented alone. As such, *c*' was examined for a subset of 29 individuals, 86.2% females (n = 25) and 13.8% males (n = 4; mean age = 20.76, rage = 18-28 years). Mean *c*' for items presented alone, paired with a New or paired with a High Familiarity concurrent item are presented in Figure 3.12.

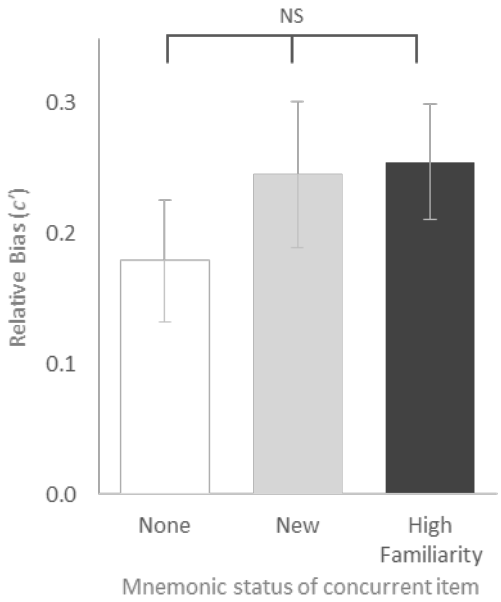

Figure 3.12: Bias for items presented alone or paired with concurrent items of differing mnemonic statuses. Bars show mean parameter estimates of bias (*c*) relative to sensitivity (*d*') under conditions where recognition is tested while concurrently presenting no item, a New item or a High Familiarity item. Error bars represent standard errors.

Although participants appear to have a more conservative *c*' for items paired with a concurrent item than for items presented alone, a one-way repeated ANOVA showed no significant difference in *c*' across the different conditions, $F_{(2, 56)} = 2.008$, $p = 0.144$, $\eta_p^2 = 0.067$. This differs from the differences in absolute *c* outlined previously.

3.8 Discussion

The aim of this experiment was to validate the findings from Experiment 2 under conditions of improved recognition memory performance. Participants did demonstrate better recognition for Digimon and Pokémon characters than fractals, however, dissimilarly to Experiment 2, the mnemonic status of concurrently presented items did not affect the correct identification of either old or new items. This reflected the absence of an effect of the mnemonic status of concurrent items on both memory sensitivity and bias. As such, the results obtained in Experiment 2 are not replicated under conditions of improved recognition memory.

The combined presence and mnemonic status of a concurrent item did however have differing effects on the correct identification of old and new items. The inclusion of a single item condition revealed that the old item recognition was unaffected by the presence of a concurrent item, while correct identification of a new item was aided when this was paired with a highly familiar item compared to when it was presented alone. However, presentation of a concurrent novel item had no effect on the correct identification of lures compared to when these were presented alone. The differences in the disruption occurred in the absence of an enhanced memory sensitivity for items presented with highly familiar concurrent items compared to those presented alone, but reflect a shift in bias. Indeed, participants are relatively more conservative (more inclined to endorse “old” decisions) under conditions when concurrent items are highly familiar than when these are presented alone, resulting in the observed higher correct rejection rate. Similarly to Experiment 2, it appears the combined presence and mnemonic status of a concurrent item drive participants to amend their criterion on an item-by-item basis. Similarly to Experiment 2, the interference observed occurred despite the delayed presentation of concurrent items onscreen compared to target items. This suggests that processing of novelty and familiarity is differentially disrupted by a concurrent item presented within a relatively short time frame (<1 second), despite these not being presented simultaneously.

3.9 Conclusion

The main interest in Experiments 2 and 3 was to question whether the processing of novelty and familiarity was differentially affected by the presence of mnemonically conflicting items. While the result of Experiment 2 did suggest a differentiation, this was established under conditions of modest recognition performance, and was not supported in Experiment 3. Furthermore, the differences observed in recognition performance for lures presented with novel or highly familiar items (Experiment 2) and for lures presented alone or with a highly familiar item (Experiment 3) were driven by strategic shifts in memory assessment (bias) rather than changes to the evidence levels questioned (sensitivity).

4. CHAPTER 4: DIFFERENTIAL MANIPULATIONS OF NOVETLY AND FAMILIARITY AT A NEURAL LEVEL

4.1 Introduction Experiment 4

Following investigations into the dissociation of novelty and familiarity at a cognitive level in Chapters 2 and 3 of this thesis, the current chapter investigates the potential dissociation of these at a neural level using neuroimaging techniques in rodents.

The perirhinal cortex has been heavily implicated in the processing of item novelty/familiarity within recognition memory. Its ablation leads to significant item recognition deficits (Aggleton et al., 2010; Barker et al., 2007; Ennaceur et al., 1996; Meunier et al., 1993; Mumby & Pinel, 1994; Nemanic et al., 2004; but see McTighe et al., 2010), while neuroimaging and single unit recordings in animals have repeatedly shown differences in activity within this region for novel as compared to familiar items (Wan et al., 1999; Xiang & Brown, 1998; Zhu et al., 1995, 1996). Indeed, single unit recordings in the perirhinal cortex demonstrate a decaying firing rate both over the duration that an item is presented, and across the number of exposures to an item (Brown & Xiang, 1998; Brown & Aggleton, 2001), consistent with a decaying novelty rather than familiarity signal (see Section 1.4.2, page 20).

Furthermore, using *c-fos* as an indication of neural activity, greater perirhinal engagement has been demonstrated in rats after presentation of a novel as compared to a familiar item (Wan, Aggleton & Brown, 1999; Zhu, et al., 1995; 1996; Albasser, Poirier & Aggleton, 2010). This is elegantly exhibited in both Zhu and colleagues' (1995; 1996) within-subject experiments. Here, rats' visual fields were divided such that each eye was only able to view the ipsilateral visual field. A novel and a familiar item (2D or 3D) were then presented simultaneously but in the

different visual fields, such that each eye viewed only either a novel or a familiar item. As visual information from a given visual field is initially processed by the contralateral hemisphere (Zhu, et al., 1996), this allows for differences in activation for novel and familiar items to be explored within-subjects. Under this design, controlling for rat alertness and eye movements, greater densities of active cells were observed in the perirhinal cortex of the hemisphere processing the novel as compared to the familiar item. No differences were observed in the *c-fos* expression in medial temporal lobe regions downstream of the perirhinal cortex such as the lateral entorhinal cortex or the hippocampus (Zhu, et al., 1995; 1996). The above outlined research demonstrates *c-fos* expression in perirhinal cortex under conditions of passive viewing. Increased *c-fos* expression for novel objects has recently been demonstrated in an experiment in which rats were behaviourally exploring novel and familiar objects (Albasser, Poirier & Aggleton, 2010). This was done between subjects using a bow-tie maze in which one group of rats explored a series of novel objects, while the other group explored highly familiar objects. In their study, Albasser and colleagues (2010) also demonstrated the engagement of qualitatively different networks for animals presented with novel and familiar objects (see Section 1.5). Potentially, this suggests that novelty and familiarity may be coded for at a network level in addition to the novelty and familiarity neural response in perirhinal cortex.

It is important to note that some data has argued against the necessity of the perirhinal cortex in object recognition (McTighe et al., 2010; Albasser, et al., 2011; Orlate-Sanchez, et al., 2015). Indeed, rats with lesion to the perirhinal cortex demonstrate normal levels of heightened exploration for two simultaneously presented items compared to two simultaneously presented familiar items (Albasser et al., 2011; Olarte-Sánchez et al., 2015). While displaying this recognition behavior, the same animals are impaired on classical versions of the SOR task in which a novel and a familiar item are presented concurrently (Orlate-Sanchez et al., 2015; Albasser, et al., 2011). However, the suggestion put forward by Orlate-Sanchez and colleagues (2015) is that a novelty and familiarity signal is still available to the rats, but that

these are unable to be bound to the presented objects, such that the rat is unable to identify which of the presented objects is novel and which is familiar. Following this interpretation, it is argued here that the perirhinal cortex is of significant importance for the processing of object novelty and familiarity, as a novelty or familiarity signal that cannot be bound to a specific object does not allow object recognition, rather it simply allows the detection of the presence of these.

The *c-fos* and single unit recoding data have supported the assumption in the animal literature that novelty and familiarity depend upon a single process, where the level of neural response in the perirhinal cortex is considered to code for the level of familiarity/novelty of an object (see Section 1.4.2 for more details). However, as discussed at length in this thesis (see Section 1.5), this assumption is under question based on both animal (e.g. Xiang & Brown, 1998) and human (e.g. Daselaar, Flek & Cabeza, 2006) experiments suggesting a potential differentiation between these processes. As novelty and familiarity may be dissociable at some levels of analysis but not others, it is important for this questioning to occur at multiple levels of analysis, from the neural structures and activity supporting novelty and familiarity, to the conscious experience of these. Thus, while this dissociation of novelty and familiarity processing was investigated at a cognitive level in Chapters 2 and 3 of this thesis, the aim of this experiment was to consider this differentiation at a neural level. Using the same framework as in the previous chapter, Experiment 4 investigates the effect of concurrently presenting two items with differing mnemonic statuses on the neural processing of novelty/familiarity, as measured by *c-fos* expression.

The immediate-early gene (IEG) *c-fos*, and the corresponding translation into the Fos protein, is used as an indirect marker of neural activity (Chaudhuri, 1997). Its expression in the hippocampus has successfully been used to identify and target neurons which are active in a given condition, such that these can be re-activated through experimental manipulation under different conditions (Liu et al., 2012; Ramirez et al., 2013). Furthermore, *c-fos* expression has been closely associated to

learning and memory (Herdegen & Leah, 1998; Herrera & Robertson, 1996; Kubik, Miyashita, & Guzowski, 2007; Tischmeyer & Grimm, 1999). Its expression is reliably documented in MTL regions (Herdegen & Leah 1998) including the perirhinal cortex (Seoane, Tinsley, & Brown, 2012), where disrupting *c-fos* expression in the perirhinal cortex impairs long-term (3+ hours) recognition memory (Seoane, Tinsley & Brown, 2012). Hence, *c-fos* is considered an adequate marker from which neural activity may be inferred in this experiment.

The following experiment (Experiment 4) used a classic SOR task to present rats in all experimental groups with a novel item paired with a concurrent familiar item. The level of familiarity of the concurrent familiar item was manipulated across experimental groups by systematically changing the number of exposures to the familiar item. Contrastingly, control rats were presented with two familiar items. Based on the vast literature designating the perirhinal cortex as the principal seat of novelty/familiarity processing (see Brown & Aggleton, 2001, for a review), examining the effect of concurrently presenting items with differing mnemonic statuses on perirhinal cortex activity was the main focus of this experiment. Based on the neurophysiology literature highlighted above (Xiang & Brown, 1998; Wan, Aggleton & Brown, 1999) whereby novelty induces increased neural response in the perirhinal cortex, it is hypothesised that greater perirhinal *c-fos* expression will be observed in experimental groups (presented with a novel object) than in the control group (presented with only familiar objects). Furthermore, as novelty and familiarity are assumed to be a single neural process, it is hypothesised that a concurrent familiar item will decrease the perirhinal activity seen for a novel item, and based on the perirhinal signal being graded rather than binary (Brown & Xiang, 1998; Brown & Aggleton, 2001), the magnitude of this decrease is expected to be inversely related to the level of familiarity of the concurrent item. However, if novelty and familiarity processing are dissociable at a neural level, perirhinal novelty-related activity may be unaffected by the presence of a concurrent familiar item. These alternatives are explored through the examination of the activity of the perirhinal cortex among other MTL structures.

In contrast to the majority of previous studies investigating the effects of novel and familiar objects on MTL *c-fos* expression, this will be done in the context of overt recognition behavior. Previous studies reliably demonstrating greater *c-fos* expression in the perirhinal cortex to novel items have done so by passively presenting 2D and 3D objects to rats while they placed their nose in a nose-poke hole (Zhu, et al., 1995; 1996). Thus, while differential *c-fos* expression was observed, this was done so in the absence of rats manifesting recognition of items as either novel or familiar. Indeed, this pattern of perirhinal cortex activity to novel and familiar items appears to be automatic as it is maintained in anesthetized rats (Zhu & Brown, 1995). Thus, the relationship between perirhinal activity and recognition behavior will also be investigated in this experiment.

4.2 Materials and Methods

4.2.1 Subjects

Subjects consisted of 24 naïve male Lister-Hooded rats (Harlan Olac Ltd, Bicester, UK) weighing between 290 and 395g at experiment commencement who were housed in groups of three. Six rats were assigned to each of the following testing conditions: High Familiarity (HF), Moderate Familiarity (MF), Low Familiarity (LF) and Control (C; see below for condition details). One rat from the control group did not perfuse due to equipment failure, hence only data pertaining to 5 control rats was available.

All rats were kept on a 12-hour light/dark cycle, with behavioural testing taking place during the light phase. To allow for greater motivation for the rats on the tasks, their food access was controlled such that their weights were maintained at approximately 90% of their free-feeding weight. Rats had *ad libidum* access to water in their home cages. Behavioural testing was undertaken over a period of two weeks. All procedures were carried out under the Project License numbers 70/8306 and 60/4069, and Personal License number 60/13883. All procedures were approved

by the Animal Welfare Ethics Committee of the University of St Andrews, and complied with national (Animal [Scientific Procedures] Act, 1986) and international (European Communities Council Directive of 24 November 1986 [86/609/EEC]) legislation governing the maintenance of laboratory animals and their use in scientific research was ensured.

4.2.2 Apparatus

All behavioural testing took place in a wooden 67cm square arena with 40cm high grey patterned walls and a dark blue floor. Behaviour was monitored live and recorded from an HP HD 4310 webcam. All objects were 3D easily cleanable household objects and toys of approximately the same size as a rat in one dimension. Objects were made of either plastic or metal, and fixed to the floor using Dual Lock Velcro (3M2, St. Paul, MN). To ensure that rats did not have an intrinsic preference for any of the given objects used (which may result in greater exploration for that object regardless of its familiarity or novelty), object exploration data for 5 objects from 21 rats in previous studies (Wilson, Langston, et al., 2013; Wilson, Watanabe, Milner, & Ainge, 2013) was compared. After greenhouse-Geisser correction following violation of the assumption of sphericity, as determined by significance of Mauchly's test, $\chi^2_{(9)} = 47.92$, $p < 0.001$, the one-way repeated measures ANOVA confirmed that no significant preference had been previously demonstrated by rats for any of the objects chosen, $F_{(1.69, 32.10)} = 0.424$, $p = 0.624$, $\eta_p^2 = 0.022$.

4.2.3 Behavioural Testing

Rats were handled by the experimenter daily for five days prior to any behavioural testing or habituation. During behavioural testing, rats were always brought into the testing room in home-cage groups and placed in a holding cage in the room. They were then tested separately.

Habituation. Rats were habituated to the testing box by being placed in the box, facing the back wall, by themselves and allowed to explore for 10 minutes. This was done on four consecutive days for each rat. All habituation occurred with no objects in the box.

Testing. Rats were randomly assigned to one of four conditions: High Familiarity, Moderate Familiarity, Low Familiarity and Control. Testing occurred on four consecutive days and rats were always placed in the box facing the back wall. All rats were presented with two objects on each day (Figure 4.1) and given 10 minutes to explore these on days 1-3, and 3 minutes on test day. The difference in the time allowed for exploration was implemented to ensure novel items presented on test day did not have the time to become familiar, as would be expected within a 10-minute trial.

Figure 4.1 depicts the experimental design. On Day 1 both of the items presented were new to the rat. On subsequent days (including Test day) one object was familiar, having been seen on the previous day, and one was new (Figure 4.1). Test day consisted of the same procedure whereby rats were presented with two objects, a familiar one seen on the previous day and a new object. Importantly we manipulated the number of exposures to the familiar object presented on Test day (Figure 4.1). For rats in condition High Familiarity (HF), the familiar object was presented on all three previous days, for rats in condition Moderate Familiarity (MF), the familiar object had been presented on the two previous days and for rats in condition Low Familiarity (LF) the familiar object was presented only once, on the day prior to Test day (Figure 4.1). This ensured that all rats in these conditions had the same number of exposures to novel and familiar items, and also had the same expectation: each day a novel and familiar item would be presented. Rats in the control condition were presented with the same two objects on all days.

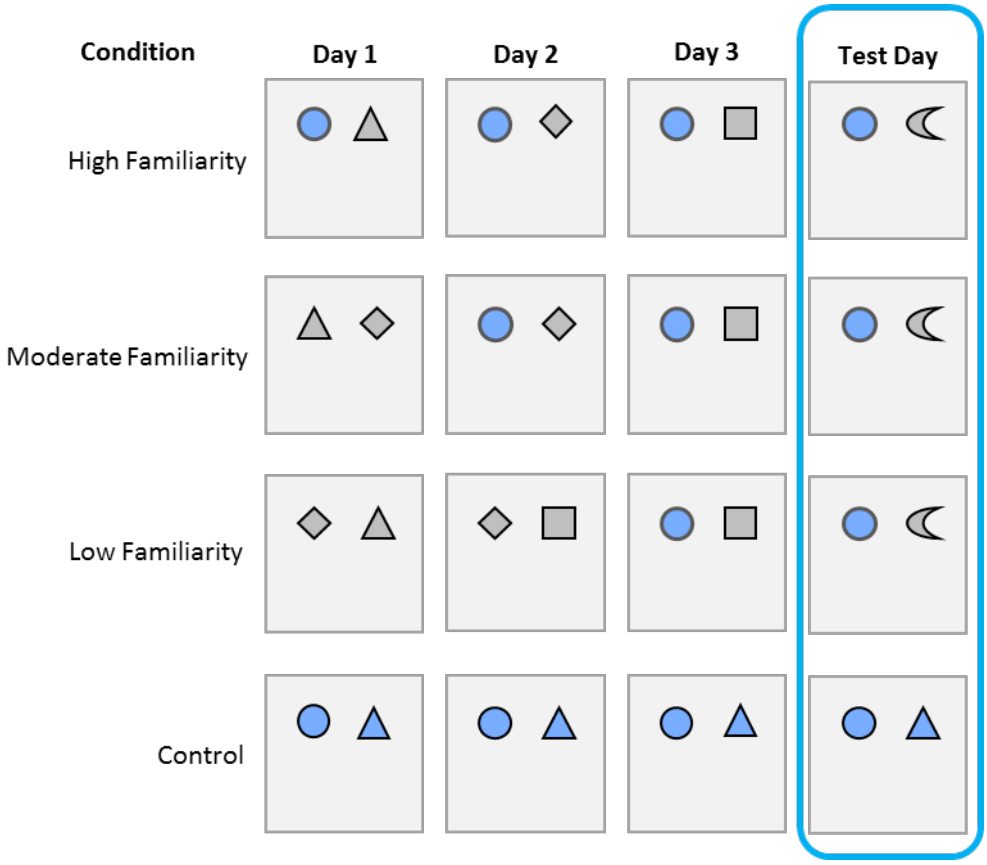

Figure 4.1: Schematic of the experimental design for Experiment 4. Rats in all experimental conditions were presented with a novel and a familiar object each day. Objects in blue represent the familiar item presented on test day, and depict the experimental manipulation whereby the relative familiarity of this item was varied between experimental groups by repeating rats' exposure to it across days. Control rats saw the same objects every day.

To control for any object-place confounds, for each rat, a given object (e.g. object A) was always presented in the same location (left/right) of the testing box. The novel/familiar status of objects, along with the location of presentation (left/right) of familiar objects was counterbalanced between rats. The same two objects (e.g. A and Z) were presented on test day for all experimental condition rats, with half of the rats experiencing a particular object (e.g. A) as familiar while the other half experienced it as novel, where the opposite was true of the other object (e.g. Z).

4.2.4 Perfusions and Histology

An hour after completion of behavioural testing, animals were given an overdose of sodium pentobarbitone. They were then perfused transcardially with 50ml phosphate-buffer saline, followed by at least 250ml of 4% paraformaldehyde solution made up with 0.1% phosphate buffer. Brains were removed and refrigerated in 20% sucrose solution (made up in 0.1% phosphate buffer) until sectioning.

Series of 50µm coronal sections were cut on a freezing microtome within 6 days of the end of testing, with an equal number of brains from animals in each group being cut on any given day. One in four sections were used for subsequent staining and quantification. The sections were stored in antifreeze (Appendix E) in a freezer pending *c-fos* activation immunohistochemistry.

Sections were processed for *c-fos* activation immunohistochemistry as described previously (Ainge, Jenkins, & Winn, 2004; Wilson, Langston, et al., 2013). After being washed in phosphate buffer, sections were placed in blocking solution (20% normal goat serum) for 60 minutes. These sections were then incubated in anti-*c-fos* primary antibody at a concentration of 1: 8 000 (Oncogene Research Products, Calbiochem) overnight. Sections were then removed, washed in phosphate buffer and placed in biotinylated IgG (anti-rabbit, Vectastain Elite ABC kit) in a concentration of 1:200 for 60 minutes before finally being incubated in avidin-biotin complex (Vectastain Elite ABC kit) at a concentration of 1:50 for a further 60 minutes. Sections were then reacted with nickel enhanced 3,3-diaminobenzidine teetrahydrochloride (Sigma) before being mounted, dehydrated, and cover slipped with DPX.

4.2.5 Calculation and Statistical Data Analysis

4.2.5.1 Behavioural Analysis

A behavioural measure of object recognition in the form of an exploration-based Discrimination Index (DI) was obtained from the task. The DI is a measure reflecting the preferential exploration allocated to a novel object as compared to a familiar one, as a proportion of total exploration time (to control for intrinsic variability in rats' levels of exploration). To calculate this, exploration timing and duration for each object during the first three minutes of each session were collected. Object exploration was only scored when the rat was facing the object with its nose less than 2cm away from the object. Moments when the rat was touching the object with another part of the body, or when leaning or rearing against it in order to investigate the area above it were not scored as object exploration. The DI was then calculated as follows, based on the exploration duration for the new (T_{new}) and familiar ($T_{familiar}$) objects, as well as the sum of these providing a total exploration duration for the trial (T_{total}):

$$\mathrm{DI} = \frac{(T_{new}) - (T_{familiar})}{(T_{total})} \quad (8)$$

For control rats, the DI was calculated based on object identity rather than mnemonic status as follows:

$$\mathrm{DI} = \frac{(T_{objectA}) - (T_{objectB})}{(T_{total})} \quad (9)$$

4.2.5.2 Histological analysis

Regions of Interest. Regions of interest were identified using a combination of a Digital atlas of the rat hippocampal region (Kjonigsen, Leergaard, Witter & Bjaalie,

2011) and a stereotaxic atlas of the whole rat brain (Paxinos & Watson, 2006). Examples of all regions of interest from which *c-fos* positive cell counts were obtained are depicted in Figure 4.2a. Regions of interest paralleled those identified by Albasser et al., (2010). The perirhinal cortex (PrH) was sub-divided into three sub regions: rostral (from AP -2.76 to -4.68 relative to bregma), mid (from AP -4.68 to -6.12 relative to bregma) and caudal (from AP -6.12 to -7.56 relative to bregma). These were further sub-divided into perirhinal areas 35 and 36 (Figure 4.2b; Burwell, 2001). Being a large input area into the perirhinal cotex and having been previously implicated in novelty detection (Wan et al., 1999), counts were also obtained from area Te2 (from AP -2.76 to AP -7.56 relative to bregma). Anatomically and functionally placed between the perirhinal cortex and the hippocampus, the lateral-entorhinal cortex (LEnt) was also examined. This was sub-divided into three regions: rostral (from AP -3.12 to -4.68 relative to bregma), mid (from AP -4.68 to -6.12 relative to bregma) and caudal (from AP -6.12 to -7.56 relative to bregma). Hippocampal sub-regions examined were the CA1, CA3 and dentate gyrus (DG). While DG counts were only obtained rostrally (from AP -2.76 to -4.68 relative to bregma), CA1 and CA3 were sub-divided into two regions: rostral (from AP -2.76 to -4.68 relative to bregma) and mid (from AP -4.68 to -6.12 relative to bregma).

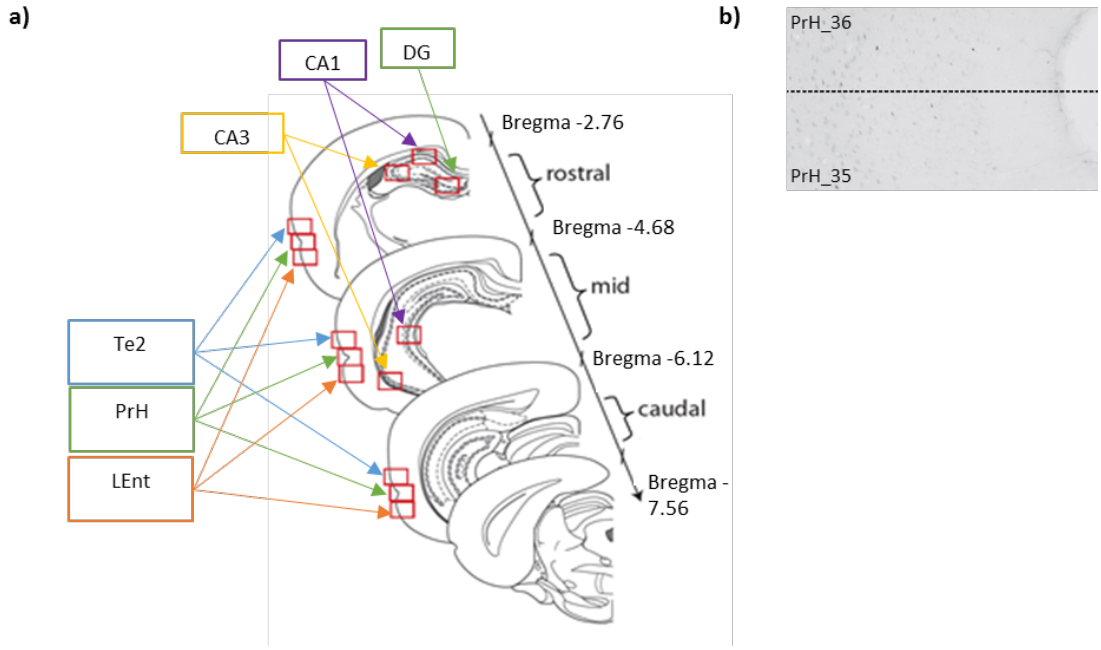

Figure 4.2: Regions of interest for Fos quantification. a) Coronal sections from Paxinos & Watson (2009) showing regions of interest from which images for *c-fos* quantification were taken. b) Perirhinal cortex image from a coronal section showing sub-regions 35 (bottom) and 36 (top).

Fos Quantification. Fos quantification was as carried out as by Wilson et al., (2013). Fos quantification was carried out blind to the experimental condition. Photographs of the regions of interest were taken at 10x magnification with a consistent light level. While the aim was for Fos expression to be quantified for four sections bilaterally for each region of interest, some sections were damaged in processing, meaning that this was not always possible. However, Fos expression was quantified bilaterally for a minimum of three (and maximum of four) sections per region of interest, with a mean of 3.87 (SD = 0.2) sections being counted bilaterally for each region of interest across all animals. Images were processed using Scion Image software (v4.0.3.2) as follows: *c-fos* expression was identified by taking a mean grayscale for each image and identifying pixels that were 2 standard deviations darker (or more saturated) than the mean. *c-fos* positive neurons were classified as groups of more than 50 and less than 1000 adjacent pixels whose saturation was greater than 2 standard deviations from the mean for that image, and their count recorded. Density of *c-fos* positive neurons was calculated by outlining a region of

interest on the section image, measuring the area of that region and dividing the total *c-fos* positive cell count within this region by the area. Thus, cell count densities were available as neurons per mm^2. To allow comparisons of activities across different brain regions with differing cell densities, cell counts were scaled by dividing them by the mean count for that area across groups and multiplying by 100.

4.2.5.3 Statistical Analysis

Rats' behaviour was compared to chance by submitting each experimental group's DI (preferential exploration of objects) to a single sample t-test, with zero as the value of comparison.

Rats discrimination behaviour was compared across experimental groups by submitting DIs to a one-way (groups: HF, MF, LF) ANOVA. Furthermore, to ascertain if the different experimental groups treated novel and familiar items differently, the duration of time spent exploring novel (T_{new}) and familiar ($T_{familiar}$) items were also submitted to separate one-way (group: HF, MF, LF) repeated-measures ANOVAs. Where one-way ANOVAs were significant, follow-up Bonferroni corrected pairwise comparisons were carried out.

To reduce Type 1 error, scaled *c-fos* expressing cell densities were analysed in three mixed-factorial ANOVAS based on regional groupings. These were: i) the parahippocampal cortex, including perirhinal areas 35 and 36 for three (rostral, mid, caudal) sub-regions and area Te2, ii) the hippocampus, including the CA1 (rostral and mid), the CA3 (rostral and mid) and the DG (rostral), and iii) the lateral entorhinal cortex (rostral, mid and caudal). Scaled cell densities were analysed across groups and regions using mixed-factorial ANOAVs, with experimental group (HF, MF, LF, C) as the between-subjects factor and sub-region as the within-subjects factor. Following any significant group x region interaction, simple effects were examined using Bonferroni corrected pairwise comparisons to assess how the *c-fos* expressing cell densities within each sub-region differed between groups. The same analyses were then repeated using raw cell densities as opposed to scaled cell densities.

Perirhinal *c-fos* expressing cell densities were collapsed across all perirhinal sub-regions (rostral, mid and caudal for both area 35 and 36) and correlated to DI using Pearsons' correlation to investigate potential relationships between recognition behavior and activity within the perirhianl cortex.

4.3 Results

4.3.1 Behavioural Results

Figure 4.3a displays the mean discrimination indices (DIs) for all groups. The positive DIs for all experimental groups demonstrate preferential exploration of the novel object compared to the familiar object as a proportion of total exploration time. Single sample t-tests confirmed that this preferential exploration was above chance for all experimental groups: High Familiarity (M =0.44, SD = 0.20), $t_{(5)} = 5.32$, p = 0.003, d = 2.17; Moderate Familiarity (M =0.31, SD = 0.16), $t_{(5)} = 4.95$, p =0.004, d = 2.02; Low Familiarity (M = 0.35, SD = 0.12), $t_{(5)} = 7.13$, p = 0.001, d = 2.91. The control group showed an object preference as depicted by DI being significantly below chance (M = -0.12, SD = 0.08), $t_{(4)} = 3.29$, p = 0.030, d = 1.47.

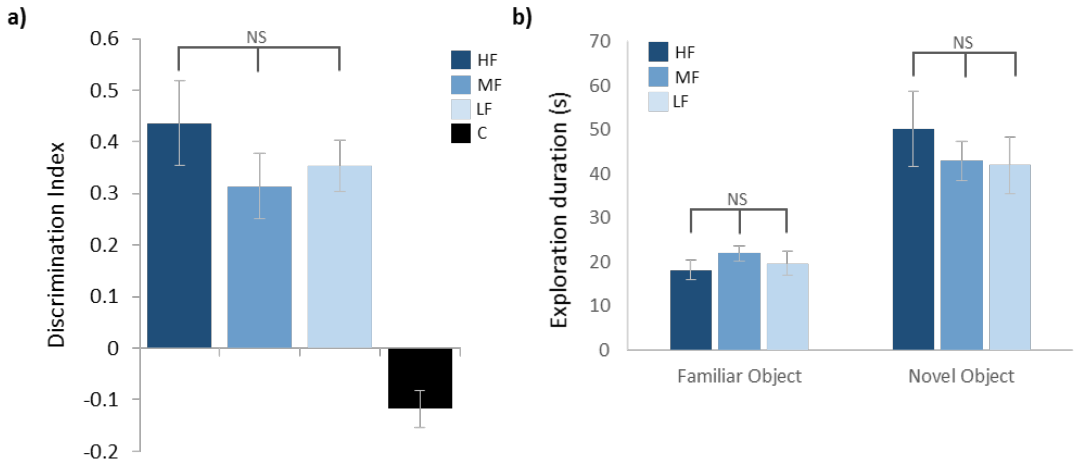

Figure 4.3: Mean discrimination indices for all groups a) (HF = high familiarity, MF = moderate familiarity, LF = low familiarity and C = control) and b) exploration durations for experimental groups, with error bars representing standard error.

Rats in the three experimental groups discriminated between the novel and familiar objects to the same extent (Figure 4.3a), as confirmed by the non-significant one-way ANOVA, $F_{(2,25)} = 0.883$, $p = 0.434$, $\eta_p^2 = 0.11$.

Mean exploration durations for novel and familiar objects for each experimental condition are displayed in Figure 4.3b. There were no differences between exploration times for novel and familiar objects between experimental groups as confirmed by two one-way ANOVAs, $F_{(2,15)} = 0.456$, $p = 0.642$, $\eta_p^2 = 0.06$, and $F_{(2,15)} = 0.685$, $p = 0.519$, $\eta_p^2 = 0.08$, respectively.

4.3.2 Immediate-Early Gene Results

As a measure of active cells, *c-fos* expression was measured in the perirhinal cortex, the hippocampus and the lateral enthorhinal cortex. Cell densities were scaled (see Section 4.2.5.2) such that comparisons across regions could be undertaken. Mean scaled active cell densities are presented in Figure 4.4.

Perirhinal cortex and area Te2. Figure 4.4a illustrates that there was no difference in the *c-fos* immunoreactivity across groups in any of the Perirhinal cortex and area Te2 sub-regions, as confirmed by a mixed-factorial ANOVA revealing no main effect of

group, $F_{(3,19)}$ = 0.291, p = 0.832, η_p^2 = 0.044, and no Group x Sub-region interaction, $F_{(18,114)}$ = 0.991, p = 0.475, η_p^2 = 0.135. As the cell densities were scaled for each sub-region, a main effect of sub-region was not possible (here $F_{(6,114)}$ = 0.007, p = 1.000, η_p^2 = 0.000). The same pattern of results was observed when the raw cell densities were submitted to the same analysis.

Hippocampus subfields. Figure 4.4b illustrates the mean scaled cell densities for the different groups across hippocampal sub-regions. Differences in the *c-fos* immunoreactivity within hippocampal sub-regions depending on group were confirmed by a greenhouse-Geisser corrected mixed-factorial ANOVA of the hippocampal sub-regions, following a significant Mauchly's test showing violation of the assumption of sphericity, $\chi^2_{(9)}$ = 0.158, p < 0.001, demonstrating a significant group x sub-region interaction, $F_{(5.95, 37.73)}$ = 2.604, p = 0.033, partial η^2 = 0.291, in the absence of a main effect of group, $F_{(3,19)}$ = 0.540, p = 0.661. As the cell densities were scaled for each sub-region, a main effect of sub-region was not possible (here $F_{(1.98, 37.73)}$ = 0.006, p = 0.994, η_p^2 = 0.000). Looking at Figure 4.4b, it would appear that the main differences occurred between the Low familiarity and the Control group in CA1_R, CA3_R and DG_R. However, following Bonferroni correction, none of the pairwise comparisons reached significance. The same pattern of results was observed when the raw cell densities were submitted to the same analysis.

Lateral entorhinal cortex. Figure 4.4c illustrates that there was no difference in the *c-fos* immunoreactivity across groups in any of the lateral entorhinal cortex sub-regions, as confirmed by a mixed-factorial ANOVA, revealing no main effect of group, $F_{(3,19)}$ = 0.425, p = 0.737, η_p^2 = 0.062, and no Group x Sub-region interaction, $F_{(6,38)}$ = 0.686, p = 0.662, η_p^2 = 0.098. As the cell densities were scaled for each sub-region, a main effect of sub-region was not possible (here $F_{(2,38)}$ = 0.004, p = 0.100, η_p^2 = 0.000). The same pattern of results was observed when the raw cell densities were submitted to the same analysis.

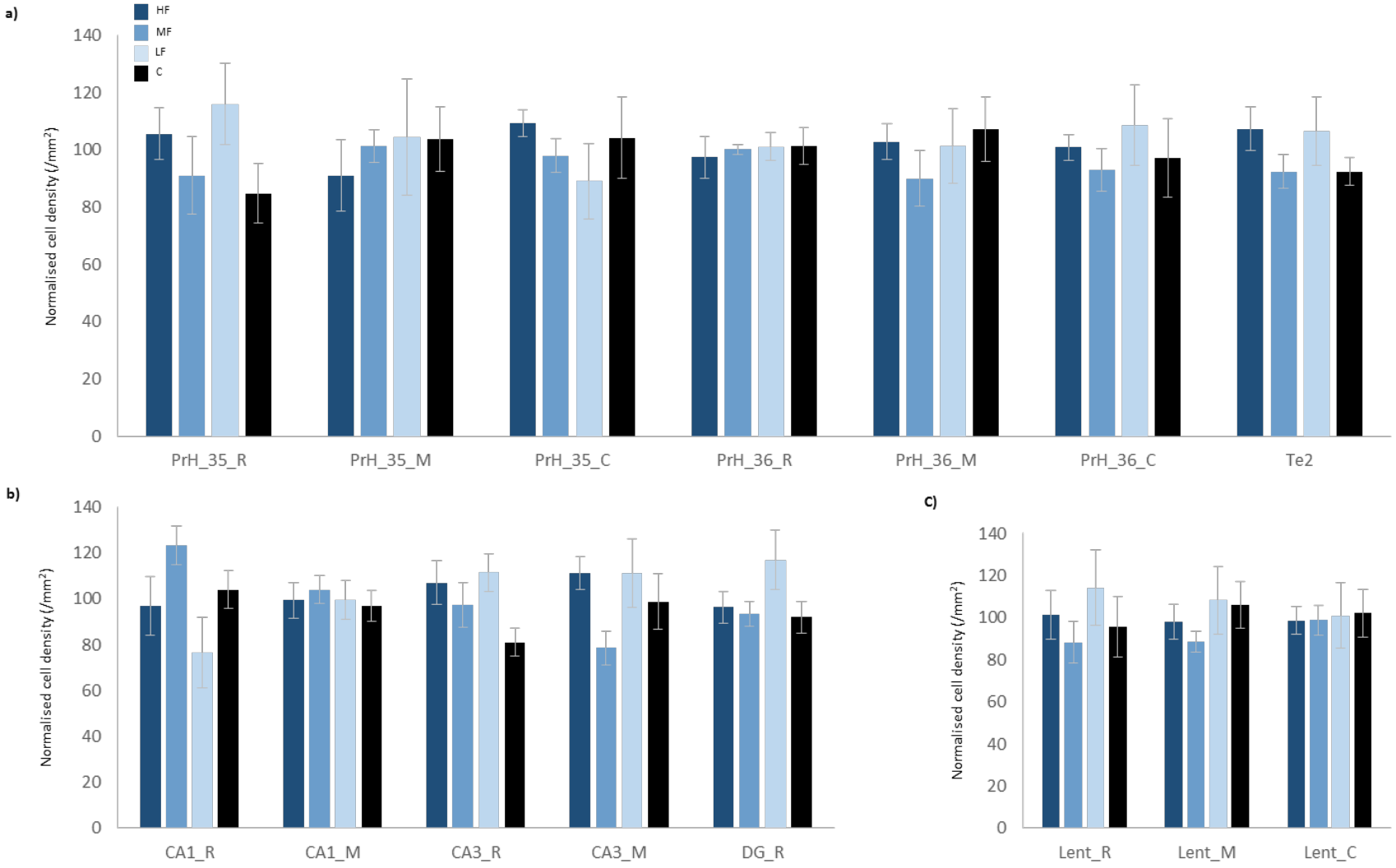

Figure 4.4: Mean and standard errors for scaled cell density counts for a) the perirhinal cortex and area Te2, b) the hippocampus and c) the lateral entorhinal cortex, where "_R" denotes rostral, "_M" denotes mid and "_C" denotes caudal sub-regions.

These results show that there was no effect of the relative familiarity of the object presented to rats, nor was there an effect of the presence of a novel object (as depicted by the lack of differences between experimental and control groups were found) on cell activity in the perirhinal cortex, and the lateral entorhinal cortex. The hippocampal subfields did show differential cell activity dependent on group, but no pattern consistent with an increasing familiarity response or decreasing novelty response was seen and specific comparisons between region counts from each group failed to reach significance after controlling for multiple comparisons.

4.3.3 Behaviour and Immediate Early Gene Correlation

The relationship between DI and perirhinal cortex *c-fos* expressing cell density is represented in Figure 4.5. Pearsons' correlation confirmed that there is no significant relationship between DI and perirhinal cortex *c-fos* expressing cell density, $r_{(21)} = -0.104$, $p = 0.682$, suggesting no relationship between overt recognition behavior and perirhinal cortex activity as measured by *c-fos*.

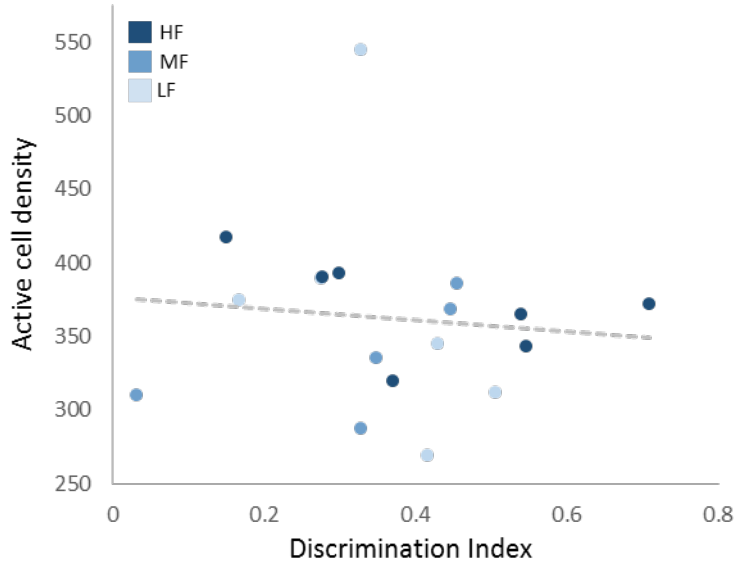

Figure 4.5: Scatter plot demonstrating the lack of a relationship between DI, a behavioural measure of recognition memory , and the perirhinal cortex *c-fos* expression for rats presented with a novel and a familiar item on test day.

4.4 Discussion

This aim of this experiment was to establish if presenting a familiar item alongside a novel item would disrupt the perirhinal cortex's neural response to the novel item. This was investigated within the context of questioning the assumption that novelty and familiarity are terms underlying a single neural process. Based on single unit recordings and *c-fos* expression in the perirhinal cortex demonstrating greater levels of neural activity for novel as compared to familiar items (see Section 1.4.2), it was hypothesised that rats presented with two items of which one was novel, would have greater *c-fos* expression in the perirhinal cortex than rats presented with two familiar items. Furthermore, as it has been suggested that the firing rate of perirhinal neurons codes for both novelty and familiarity (where these are inverses of each other), it was hypothesised that concurrent familiar items would disrupt the increased perirhinal activity seen for novel objects, such that the greater the level of concurrent familiarity, the greater the decrease in the perirhinal neuronal activity. The results from the current experiment do no provide support for either of these hypotheses. No differences in the *c-fos* expression between control rats (presented with two familiar items) and experimental group rats (presented with a novel and a familiar item) was demonstrated. Furthermore, the familiarity of the concurrent item did not have the hypothesised graded effect on the perirhinal cortex *c-fos* expression, where no differences in perirhinal cortex *c-fos* expression were observed between experimental groups.

Similarly to the findings by Gaskin and colleagues (2010), the relative familiarity of the item presented alongside the novel item in the experimental groups failed to affect single item recognition behaviour. Two interpretations of these data are possible. Firstly, this may reflect that in the current experiment a familiarity ceiling was reached, such that exposure to an item for 10 minutes on the previous day was sufficient to make this item as familiar as these will ever be, and hence no difference was observed in the exploration between experimental groups. However, Gaskin and colleagues (2010) found similar results with object presentations lasting 5 minutes. Hence, it is proposed that the more plausible interpretation is that these data support the notion that recognition behaviour in the SOR task is driven by novelty. Specifically, the exploration of familiar objects is a baseline, and a

novel object leads to increases in exploration from this baseline, as opposed to familiar items leading to decreases in exploration from a novel item exploration baseline. This parallels the findings from Experiments 2 where participants' recognition memory was not differentially affected by concurrent low or high familiar items.

As in Kinnavane, Amin, Horne & Aggleton (2014), the current findings failed to replicate the increase seen in the perirhinal cortex's *c-fos* expression following the presentation of a novel object (Wan, Aggleton & Brown, 1999; Xiang & Brown, 1998; Zhu, et al., 1995; 1996; Albasser, Poirier & Aggleton, 2010), despite rats demonstrating clear single object recognition behaviour. Indeed, the perirhinal *c-fos* expression was shown to be uncorrelated to novel object recognition performance. It is important to note here that the suggestion is not that the perirhinal cortex activity is unrelated to recognition behavior, rather, the number of *c-fos* expressing neurons in the perirhinal cortex is not related to behavioural expressions of recognition memory.

The lack of replication of previous findings showing greater perirhinal *c-fos* expression for rats (or visual fields) presented with a novel object compared to a familiar object is considered to result from the simultaneous presentation of both a novel and familiar object in the current experiment. Indeed, previous studies have presented either single items to rats (or visual fields; Wan, Aggleton & Brown, 1999; Xiang & Brown, 1998; Zhu, et al., 1995; 1996) or two items of the same mnemonic status (two novel or two familiar; Albasser, Poirier & Aggleton, 2010). Indeed, Kinnavane and colleagues (2014) who also failed to replicate greater *c-fos* expression in the perihrinal cortex as a response to novelty also presented a novel and familiar item concurrently. While it may be argued that the lack of an effect of a novel item on *c-fos* expression in the perirhinal cortex of animals in the experimental groups of the current experiment is a result of the low number of trials resulting in a lack of driving the novelty response (Roloff, Muller & Brown, 2016), this lack of an effect was also demonstrated by Kinnavane and colleagues (2014) despite the use of a bow-tie maze and twenty SOR trials. Hence, when taken into the context of these other studies, the current results may therefore be tentatively considered to demonstrate that a concurrent familiar item provides interference large enough to abolish the novelty-related

perirhinal signal. This appears to be occurring despite objects not being attended to simultaneously (see Section 3.4, page 77).

However, a potential limitation of the current study limiting this interpretation was that the objects presented to control rats on test day were different to those presented to rats in the experimental groups. Considering the perirhinal cortex has been implicated in the processing of visual objects (Buckley et al., 2001; Murray & Bussey, 1999; see Section 1.4.3), presenting differing objects to the control and the experimental groups makes these difficult to compare: given that different objects lead to different levels of perirhinal cortex recruitment (Bussey, Saksida & Murray, 2002; 2003), differences, or lack thereof, between experimental groups and the control group are confounded by object identity.

While no differences were observed in the density of *c-fos* expressing cells between groups for any individual sub-region, it is important to consider that neural structures do not work in isolation. Previous research using *c-fos* has robustly depicted two diverging pathways engaged by the presentation of novel and familiar items (Albasser, Poirier & Aggleton, 2010; Kinnavane, et al., 2016, see Section 1.5). The presentation of purely novel items leads to the engagement of the performant pathway between the parrahippocampus and hippocampus (LEnt **->** DG **->** CA3 **->** CA1), while presentation of purely familiar items leads to the engagement of the temporo-ammonic pathway (LEnt **->** CA1; Albasser, Poirier & Aggleton, 2010; Kinnavane, et al., 2016). These differing networks have been established even in the absence of differences in the densities of *c-fos* expressing cells within the perirhinal cortex (Kinnavane, et al., 2016). The differences in the engagement of the DG and CA3 for novel items may underlie the differences seen in *c-fos* expression in the CA3 and DG between the Low Familiarity group, exposed to the most novelty (a novel item and a least familiar one), and the control group, although these differences were not robust enough to sustain control for multiple comparisons.

To investigate this, the same methodology used in Experiment 4 was used in Experiment 5 to assess how concurrently presenting items of differing familiarity levels affects these "novelty" and "familiarity" neural networks. To allow this, larger sample sizes adequate for

structural equation modelling were used. Experiment 5 also rectified the object identity confound between the experimental and control groups such that comparisons between these groups could be interpreted with greater confidence.

4.5 Introduction Experiment 5

Experiment 5 was designed under the same theoretical premise as Experiment 4, such that the effect of concurrently presenting items of differing familiarity levels on novelty processing at a neural level was investigated. While this was investigated by looking at the active cell densities within different medial temporal lobe sub-regions in Experiment 4, the current experiment will further this analysis to consider the effects of a concurrent familiar item on novelty processing at a neural network level. Thus, following the investigation of the dissociation between novelty and familiarity at a cognitive and individual neural structure level, here this is also investigated at a neural network level. Previous research in rats has identified two neural networks within the MTL, differentially engaged by the presentation of novel and familiar items (Albasser, Poirier & Aggleton, 2010; Kinnavane, et al., 2016). These networks differ in their parrahippocampal-hippocampal effective connectivity. Familiar items engaged the more direct temporo-ammonic pathway from lateral entorhinal cortex to CA1 (Figure 4.6a), while novel items engaged connectivity along the performant pathway from lateral entorhinal cortex to dentate gyrus, and then to CA1 through CA3 (Figure 4.6b).

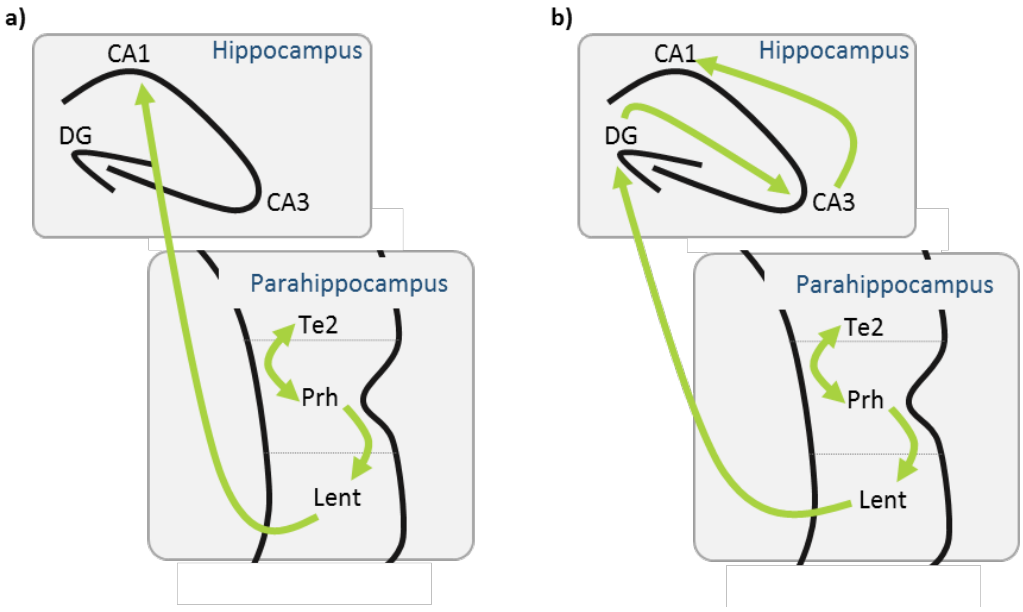

Figure 4.6: Anatomical representation of the hippocampus and parahippocampus with effective connectivity networks for a) familiar and b) novel items as suggested by Albasser et al., 2010.

These networks were demonstrated in rats presented with either purely novel or purely familiar items. Thus these networks have been identified under conditions devoid of competing mnemonic statuses for items. Here the effect of concurrently presenting items of differing mnemonic statuses on these novelty and familiarity networks will be investigated following analysis of the *c-fos* expressing cell densities in MTL sub-regions. It is hypothesised that for the control group presented with only familiar objects, only the familiarity network will be engaged, while for the experimental groups presented with both a novel and a familiar object, both the novelty and familiarity networks will be engaged. Furthermore, it is hypothesised that for the experimental groups, the greater the level of familiarity of the concurrent item, the greater the engagement of the familiarity network, as depicted by stronger effective connectivity within the familiarity network.

4.6 Materials and Methods

The experiment was run as two repetitions of the same protocol. Behavioural testing and Fos quantification was undertaken with the assistance of Karina Vitanova and Veronika Ambrozova.

4.6.1 Subjects

Cohort A. Cohort A consisted of 16 naïve male Lister-Hooded rats (Harlan Olac Ltd, Bicester, UK) weighing between 315 and 395g at experiment commencement who were house in pairs. Four rats were assigned to each of the following testing conditions: High Familiarity (HF), Moderately Familiarity (MF), Low Familiarity (LF) and Control (C; see below for condition details).

Cohort B. Cohort B consisted of 20 naïve male Lister-Hooded rats (Harlan Olac Ltd, Bicester, UK) weighing between 315 and 380g at experiment commencement who were also house in pairs. Due to health complications, one rat was omitted from testing, such that final testing

and data pertained to 19 rats: four rats assigned to condition HF and five rats assigned to all other conditions (MF, LF, C).
A total of 36 rats were subjects in this experiment, with data from 35 rats available for analysis: eight from the HF condition, nine from the MF condition, nine from the LF condition and nine from the C condition.

All rats were kept on a 12-hour light/dark cycle, with behavioural testing taking place during the light phase. To allow for greater motivation for the rats on the tasks, their food access was controlled such that their weights were maintained at approximately 90% of their free-feeding weight. Rats had *ad libitum* access to water in their home cages. Each behavioural repetition (i.e. per cohort) was undertaken over a period of two weeks. All procedures were carried out under the Project License numbers 70/8306 and 60/4069, and Personal License number 60/13883. All procedures were approved by the Animal Welfare Ethics Committee of the University of St Andrews, and complied with national (Animal [Scientific Procedures] Act, 1986) and international (European Communities Council Directive of 24 November 1986 [86/609/EEC]) legislation governing the maintenance of laboratory animals and their use in scientific research.

4.6.2 Apparatus

All apparatus was as used in Experiment 4 (Section 4.2.2).

4.6.3 Behavioural Testing

All behavioural testing was as in Experiment 4 (Section 4.2.3) with the exception of the objects used for the control group. Objects presented each day to the control group were selected to be the same as those that the rats from experimental groups were exposed to on test day (Figure 4.7).

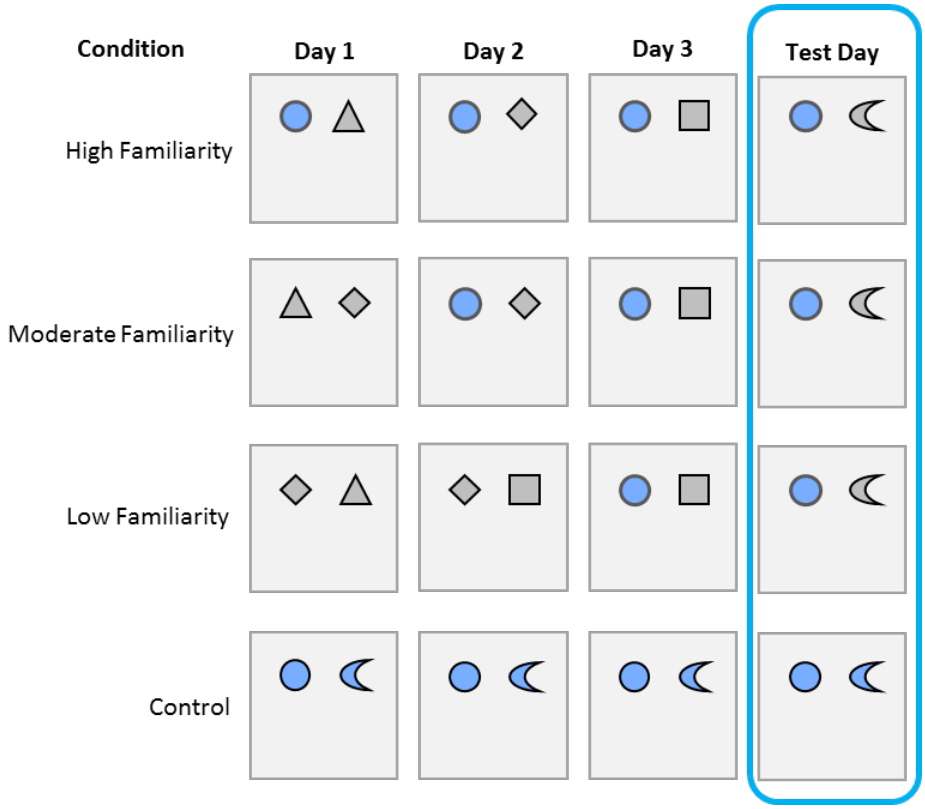

Figure 4.7: Schematic of the experimental design for Experiment 5. Rats in all experimental conditions were presented with a novel and a familiar object each day. Objects in blue represent the familiar item presented on test day, and depict the experimental manipulation whereby the relative familiarity of this item was varied between experimental groups by repeating rats' exposure to it across days. Control rats saw the same objects every day.

4.6.4 Perfusions and Histology

Perfusions and histology was run as in Experiment 4 (Section 4.2.4) with the following changes: *c-fos* activation immunohistochemistry was initiated one day after sections were cut and the *c-fos* primary antibody used was from a different source (Synaptic Systems, Germany), at a concentration of 1: 8000.

4.6.5 Calculation and Statistical Data Analysis

4.6.5.1 Behavioural Analysis

Behavioural analysis was identical to that in Experiment 4 (Section 4.6.5.1).

4.6.5.2 Histological analysis

Regions of interest and Fos quantification was as in Experiment 4 (Section 4.6.5.2), with the exception that Fos expression was quantified bilaterally for a minimum of two (and maximum of four) sections per region of interest, with a mean of 3.67 (SD = 0.8) sections being counted bilaterally for each region of interest across all animals.

4.6.5.3 Statistical Analysis

To allow comparison of behaviour between cohorts, a paired-samples t-test was performed on cohorts' DIs. Furthermore, any interaction between cohort and cell densities for different regions of interest were investigated using a 2 (cohort: A, B) x 6 (Sub-region: PrH, Te2, CA1, CA3, DG, LEnt) mixed-factorial ANOVA, with sub-region as the within subjects variable. Subsequent statistical analysis was as in Experiment 4 (Section 4.2.5.3).

4.6.5.4 Structural Equation Modelling

Structural equation modelling (SEM) enables the inter-relationships between variables, in this case neural activation in different regions, to be assessed. SEM extends simple and multiple regression by allowing more complex relationships between multiple independent and multiple dependent variables to be mapped (Schumacker & Lomax, 2010). Models of the relationships between variables are hypothesised, and SEM is subsequently used to test whether these hypothesised models are supported by the sample data (Schumacker & Lomax, 2010).

SEM is used here as it allows a finer grained analysis of the *c-fos* data. It is possible for activity in given regions to not significantly differ between conditions when tested using cruder measures of activity (i.e. cell count densities), while the relationships among these regions do significantly differ between the same conditions (e.g. Albasser, Poirier & Aggleton, 2010). Given that SEMs depict the level of the repercussion on a variable of changing another (Schumacker & Lomax, 2010), when applied to *c-fos* quantification this allows neural activity to be considered not simply in terms of the number or density of neurons active, but also with regards to the amount of influence this activity has on downstream structures. Hence, when used for neuroanatomical network model, SEM

models depict the effective connectivity between various regions (Protzner & McIntosh, 2006). SEM is therefore a fitting tool to investigate the effect of experimental manipulations on neural networks, and indeed SEM has previously been employed to investigate neural networks in the MTL based on *c-fos* expression (Albasser, Poirier & Aggleton, 2010; Orlate-Sanchez et al., 2014; Kinnavane et al., 2016).

An overview of SEM path analysis, the manner in which it is applied to *c-fos* data, and its interpretation will now be outlined. The purpose of SEM, as stated previously, is to test whether hypothesised models are supported by observed data. Hence, models to be tested are first specified, typically using path diagrams such as that in Figure 4.8. Observed variables are depicted using rectangles (e.g. A in Figure 4.8) and the residual error associated with that variable depicted as circles (e.g. e1 in Figure 4.8). The residual error represents the unspecified influences (not accounted for by the model) on the variable they point to, including any measurement error (Hoyle, 2012). The hypothesised relationships between the observed variables are depicted using arrows, known as paths, and specify the direction of the effects of the relationships between these variables (Schumacker & Lomax, 2010).

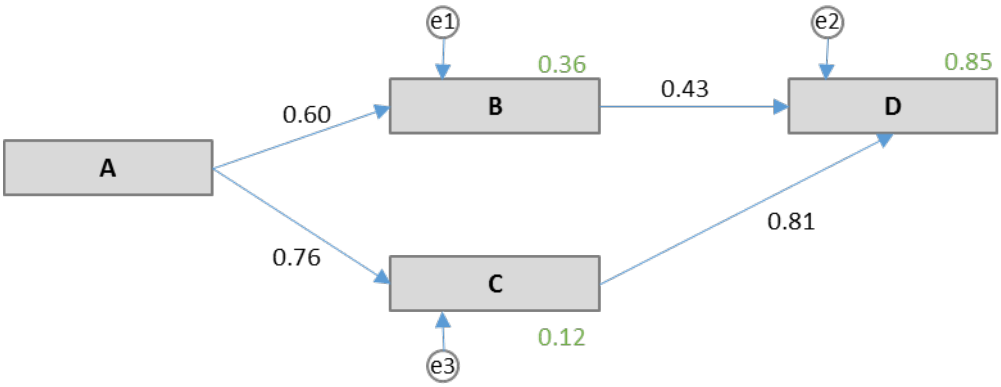

Figure 4.8: Example path diagram with observed variables (rectangles), residual errors (circles), paths (blue) and their coefficients, and coefficients of determination (R^2; green).

Observed relationships in the data in the form of correlations and covariances between variables are then supplied for the model. Using multiple-regression analysis, SEM software attempts to reproduce the covariance matrix from the implied set of covariances based on the specified model. The parameter estimates for the relationships between variables are path coefficients. A path coefficient is a standardized regression coefficient (equivalent to standardized regression coefficients in multiple regressions [Hoyle, 2012]), and is expressed numerically alongside the paths representing them (see Figure 4.8). These path coefficients specify the extent to which a change in the variable at the start of a path (tail of the arrow) is transmitted to the variable at the end of that path (head of the arrow; Loehlin, 2004). For example, in Figure 4.8, the path coefficient of 0.60 between variable A and B outlines that if the mean of variable A was raised by 1 standard deviation, the mean of variable B would increase by 0.60 (Loehlin, 2004). SEM path analysis models also calculate a coefficient of determination (R^2) for all variables to which paths are drawn (variables at the head of a path/arrow; known as endogenous variables). These represent the portion of the variance for that variable accounted for by its inputs (variables at the tail of the path/arrow, from which the paths are hypothesised) in the model and are depicted as numbers next to observed variables (numbers in green in Figure 4.8).

It is important to ensure that the generated SEM path analysis models accurately represent or “fit” the data. This is determined based on how well the covariance matrix predicted from the estimated SEM model replicates the covariance matrix observed for the raw data. Various fit indices exist, each with a unique set of advantages and disadvantages (Hooper, Coughlan, & Mullen, 2008). Therefore, reporting several indices is favoured. Indices of fit, along with the use of the SEM path analysis for this experiment are outlined below.

When using SEM path analysis for *c-fos* data, the specified models should be constrained by neuroanatomy. Neuroanatomical regions of interest are entered in the model as observed variables, with the paths (or relationships) and their directions based on known neural connections between regions. As novelty and familiarity networks differently engaging the perforant and temporo-ammonic pathways between parahippocampal and hippocampal structures respectively have been repeatedly identified (Albasser, Poirier & Aggleton, 2010;

Kinnavane, et al., 2016; Olarte-Sanchez, Kinnavane, Amin & Aggleton, 2014), these networks, depicted diagrammatically in Figure 4.9a&b (omitting residual errors for clarity), are used as the specified models for the SEM analysis in this chapter. Furthermore, given the significant input from the perirhinal cortex into the lateral entorhinal cortex (Sugar, Witter, van Strien, & Cappaert, 2011; van Strien, Cappaert, & Witter, 2009), these same models but with input to the lateral entorhinal cortex arising from the perirhinal cortex rather than area Te2 were also tested (Figure 4.9c&d). Parameter estimates were calculated based on raw cell densities (rather than scaled cell densities) as the absolute differences between regions was not being compared.

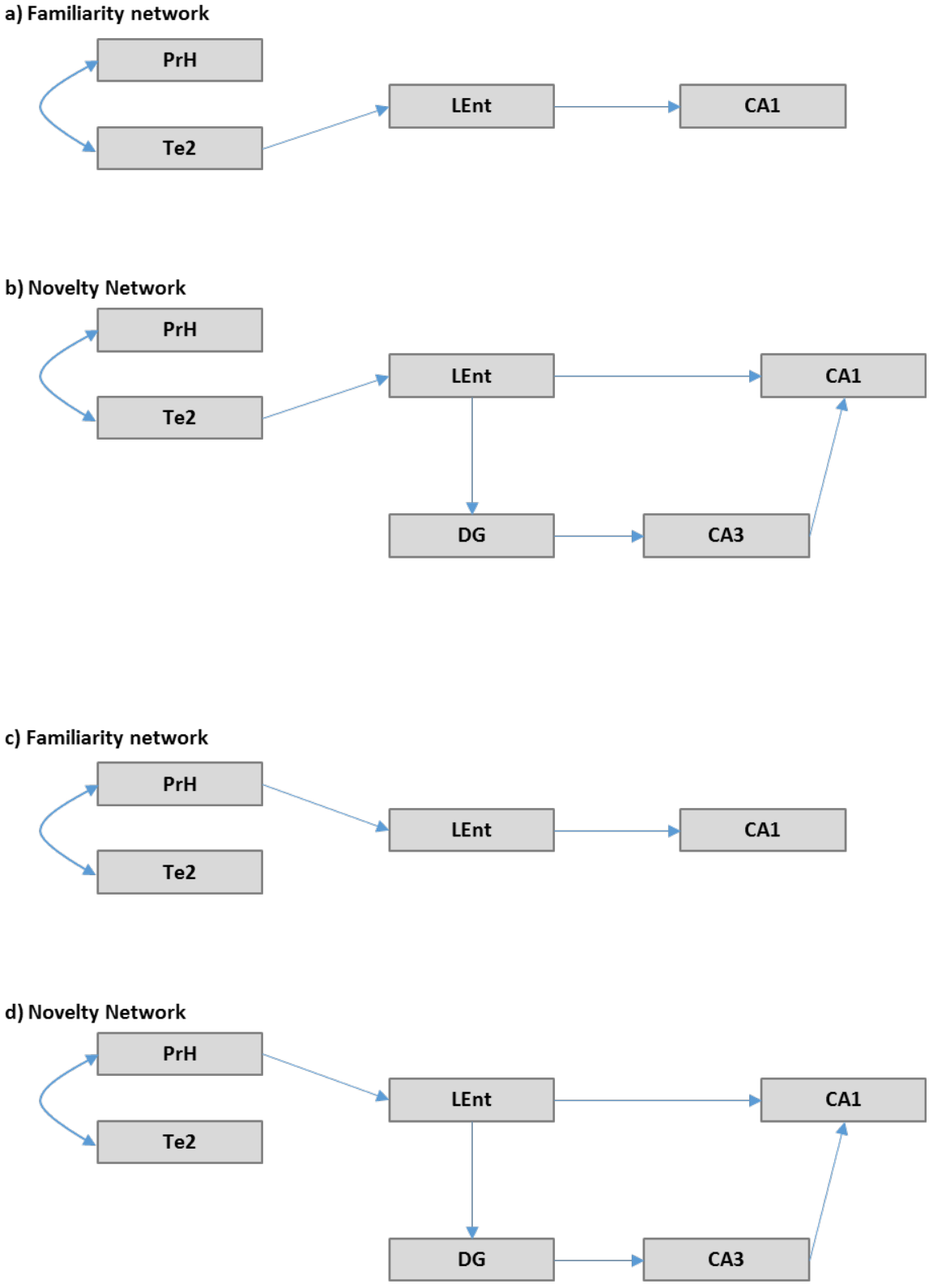

Figure 4.9: Path diagrams of the a) familiarity and b) novelty neural networks in the MTL outlined by Albasser et al., (2010), and the modified c) familiarity and d) novelty networks, with input into the lateral entorhinal cortex (Lent) arising from the PrH rather than area Te2, tested in this chapter. These show the hypothesised relationships between the Perirhinal cortex (PrH), area Te2, LEnt, and the hippocampal subfields, the dentate gyrus (DG), CA1 and CA3, investigated in this chapter.

Paralleling the work of Albasser and colleagues (2010) that this analysis is based on, three indices of model fit are reported here: Chi-square (χ^2), comparative fit index (CFI) and root mean square error approximation (RMSEA). A χ^2 analysis assesses whether the covariance matrix generated by the estimated model significantly differs from that observed for the sample data. Thus, a good fit is indicated by a non-significant χ^2, such that the estimated model produces a covariance matrix not significantly different from that obtained from the sample data. To minimize the effect of a small sample size on the χ^2 analysis, a model with a non-significant χ^2 was only accepted if the ratio of the χ^2 value to the degrees of freedom was less than two (Hooper, Coughlan & Mullan, 2008). In contrast to the χ^2 fit index, the CFI and RMSEA provide an indication of the *extent* of the model's fit to the data. These are used as they are most robust to small sample sizes (Albasser et al., 2010; Hooper et al., 2008; Hu & Bentler, 1998). According to convention, models were considered to have a good fit if the CFI was between 0.90 and 0.95 or the RMSEA was less than 0.07 (Hooper, Coughlan & Mullan, 2008; Albasser et al., 2010).

The aim of this experiment was to question whether the different experimental conditions, exposing rats to various levels of familiar objects along with a consistent novel object, lead to the engagement of differing neural networks, or the same networks in differing ways. To question this, the SEM models generated for each condition need to be compared. The novelty network model specified here is one which includes the familiarity network model specified (i.e. the familiarity network is "nested" in the novelty network) while having additional observed variables and paths (i.e. additional "free" parameters to be estimated). As such, the novelty network is the more restrictive of the two specified networks. As the specified familiarity model can be nested within the specified novelty model, comparison between these models between groups can thus be undertaken using a χ^2 difference test (Schermelleh-Engel, Moosbrugger, & Müller, 2003; Steiger, Shapiro, & Browne, 1985), using the following reasoning. Models are a method of summarizing data. Ideally, the aim of a model is to represent the data well in as few parameters or components as possible. Assuming the models generated are a well-fitting, the greater the number of parameters, the better the data are accounted for. When using nested model, a χ^2 difference test questions whether the model with the greater number of parameters does indeed fit the

data better than its nested "smaller" model with fewer parameters. Hence, a non-significant χ^2 difference result demonstrates that both models fit equally well, and thus the additional parameters did not significantly improve the model fit. Here the more constrained model with fewer parameters better represents the data. Contrarily, a significant χ^2 difference allows the interpretation that the more restrictive model (in this case the specified model for the familiarity network which is nested within the specified novelty network model) has a significantly wrose fit than the less restrictive network (in this case the specified familiarity network), and thus the less restrictive model better represents the data (Schermelleh-Engel, Moosbrugger, & Müller, 2003).

The SEM software package SPSS AMOS version 20.0 (IBM Corp, Armonk, NY, USA) was used to compute the path analyses reported in this chapter.

4.7 Results

To allow collapsing across cohorts, the lack of cohort effects on behavior and active cell densities must first be verified. Cohorts did not differ in their behavioural performance of the task as outlined by an independent samples t-test on DI collapsed across all conditions, $t_{(33)} = -0.704$, $p = 0.487$, $d = 0.236$. Comparing the active cell densities between cohorts would be a misleading way of testing possible cohort effects. The immunohistochemistry for the different cohorts was run separately, where this processing and staining was a significant source of variability in active cell densities. Therefore, the same number of animals from each condition was processed in the same batch for each cohort, such that any variability due to staining in a given cohort is spread evenly between conditions. Possible interactions between the cohort and the cell densities for different regions of interest were investigated using a mixed-factorial ANOVA. A greenhouse-Geisser corrected mixed-factorial ANOVA demonstrated no significant interaction between cohort and active cell densities for the different areas of interest, $F_{(2.34, 77.16)} = 1.541$, $p = 0.218$, $\eta_p^2 = 0.045$.

Given the lack of evidence that rats behaved differently across cohorts, the methodological design, and the lack of an interaction between cohorts and active cell densities, the data were collapsed across the cohorts.

4.7.1 Behavioural Results

Figure 4.10a displays the mean discrimination indices (DIs) for all groups. The positive DIs for all experimental groups demonstrate preferential exploration of the novel object compared to the familiar object as a proportion of total exploration time. Single sample t-tests confirmed that this preferential exploration was above chance for all experimental groups High Familiarity (M =0.31, SD = 0.21), $t_{(7)} = 4.21$, p = 0.004, d = 1.49 ; Moderate Familiarity (M =0.31, SD = 0.23), $t_{(8)} = 3.97$, p =0.004, d = 1.32; Low Familiarity (M = 0.28, SD = 0.20), $t_{(8)} = 4.14$, p = 0.003, d = 1.38. Contrastingly, the Control group's DI (M = 0.04, SD = 0.20) shows that there was no preference for either of the control objects presented on test day, as confirmed by a single sample t-test, $t_{(8)} = 0.501$, p = 0.630, d = 0.167 (Figure 4.10a).

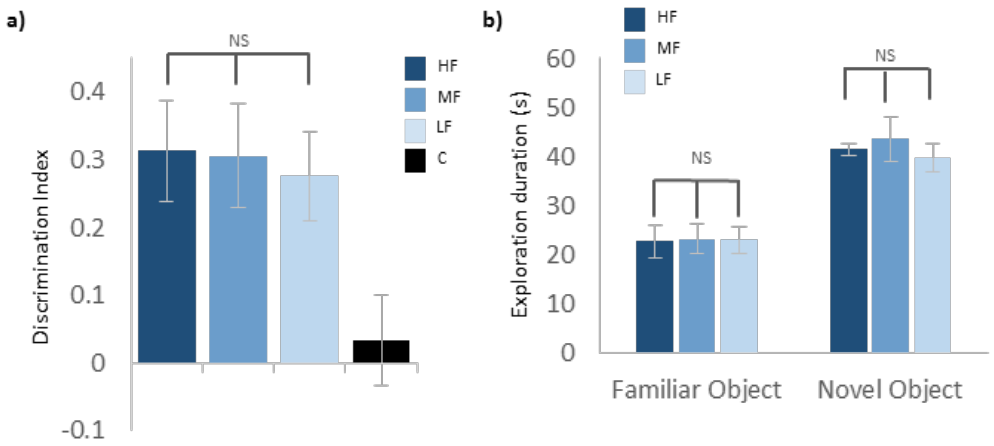

Figure 4.10: Mean discrimination indices for all groups a) (HF = high familiarity, MF = moderate familiarity, LF = low familiarity and C = control) and b) exploration durations for experimental groups, with error bars representing standard error.

Rats in the three experimental groups discriminated between the novel and familiar objects to the same extent (Figure 4.10a), as confirmed by the non-significant one-way ANOVA, $F_{(2,23)} = 0.075$, $p = 0.928$, $\eta_p^2 = 0.007$.

Mean exploration durations for novel and familiar objects for each experimental condition are displayed in Figure 4.10b. There were no differences between exploration times for novel and familiar objects between experimental groups as confirmed by two separate one-way ANOVAs, $F_{(2,23)} = 0.371$, $p = 0.694$, $\eta_p^2 = 0.000$, and $F_{(2,23)} = 0.006$, $p = 0.994$, $\eta_p^2 = 0.001$, respectively. Thus, the experimental groups' exploration behaviour towards the novel and familiar objects was not modulated by the degree of familiarity of the familiar object.

4.7.2 Immediate Early Gene Results

As a measure of active cells, *c-fos* expression was measured in the perirhinal cortex, the hippocampus and the lateral entorhinal cortex. Cell densities were scaled (see section 4.2.5.2) such that comparisons across regions could be undertaken. Mean scaled active cell densities are presented in Figure 4.11.

Perirhinal cortex and area Te2. Figure 4.11a illustrates that there was no difference in the active cell density across groups in any of the perirhinal cortex and area Te2 sub-regions, as confirmed by a mixed-factorial ANOVA revealing no main effect of Group, $F_{(3,31)} = 0.481$, $p = 0.698$, $\eta_p^2 = 0.044$, and no Group x Sub-region interaction, $F_{(18,186)} = 0.415$, $p = 0.984$, $\eta_p^2 = 0.039$. As the cell densities were scaled for each sub-region, a main effect of sub-region was not possible (here $F_{(6,186)} = 0.001$, $p = 1.000$, $\eta_p^2 = 0.000$). The same pattern of results was observed when the raw cell densities were submitted to the same analysis.

Hippocampus subfields. Figure 4.11b illustrates that there was no difference in the active cell density across groups in any of the hippocampal sub-regions, as confirmed by a mixed-factorial ANOVA revealing no main effect of Group, $F_{(3,31)} = 0.274$, $p = 0.844$, $\eta_p^2 = 0.026$, and no Group x Sub-region interaction, $F_{(12,124)} = 0.600$, $p = 0.838$, $\eta_p^2 = 0.055$. As the cell densities were scaled for each sub-region, a main effect of sub-region was not possible

(here $F_{(4,124)}$ = 0.003, p = 1.000, η_p^2 = 0.000). The same pattern of results was observed when the raw cell densities were submitted to the same analysis.

Lateral entorhinal cortex. Figure 4.11c illustrates that there was no difference in the active cell density across groups in any of the hippocampal sub-regions, as confirmed by a mixed-factorial ANOVA revealing no main effect of Group, $F_{(3,31)}$ = 1.047, p = 0.386, η_p^2 = 0.092, and no Group x Sub-region interaction, $F_{(6,24)}$ = 0.364, p = 0.899, η_p^2 = 0.034. As the cell densities were scaled for each sub-region, a main effect of sub-region was not possible (here $F_{(2,62)}$ = 0.001, p = 0.999, η_p^2 = 0.000). The same pattern of results was observed when the raw cell densities were submitted to the same analysis.

These results, consistent with those from Experiment 4, show that there was no effect of the relative familiarity of the object presented to rats, nor was there an effect of the presence of a novel object (as depicted by the lack of differences between experimental and control groups were found) on cell activity in the perirhinal cortex, the hippocampus and the lateral entorhinal cortex.

The relationship between DI and perirhinal cortex *c-fos* expressing cell density is represented in Figure 4.12. Pearsons' correlation confirmed that there is no significant relationship between DI and perirhinal cortex *c-fos* expressing cell density, $r_{(24)}$ = 0.118, p = 0.567.

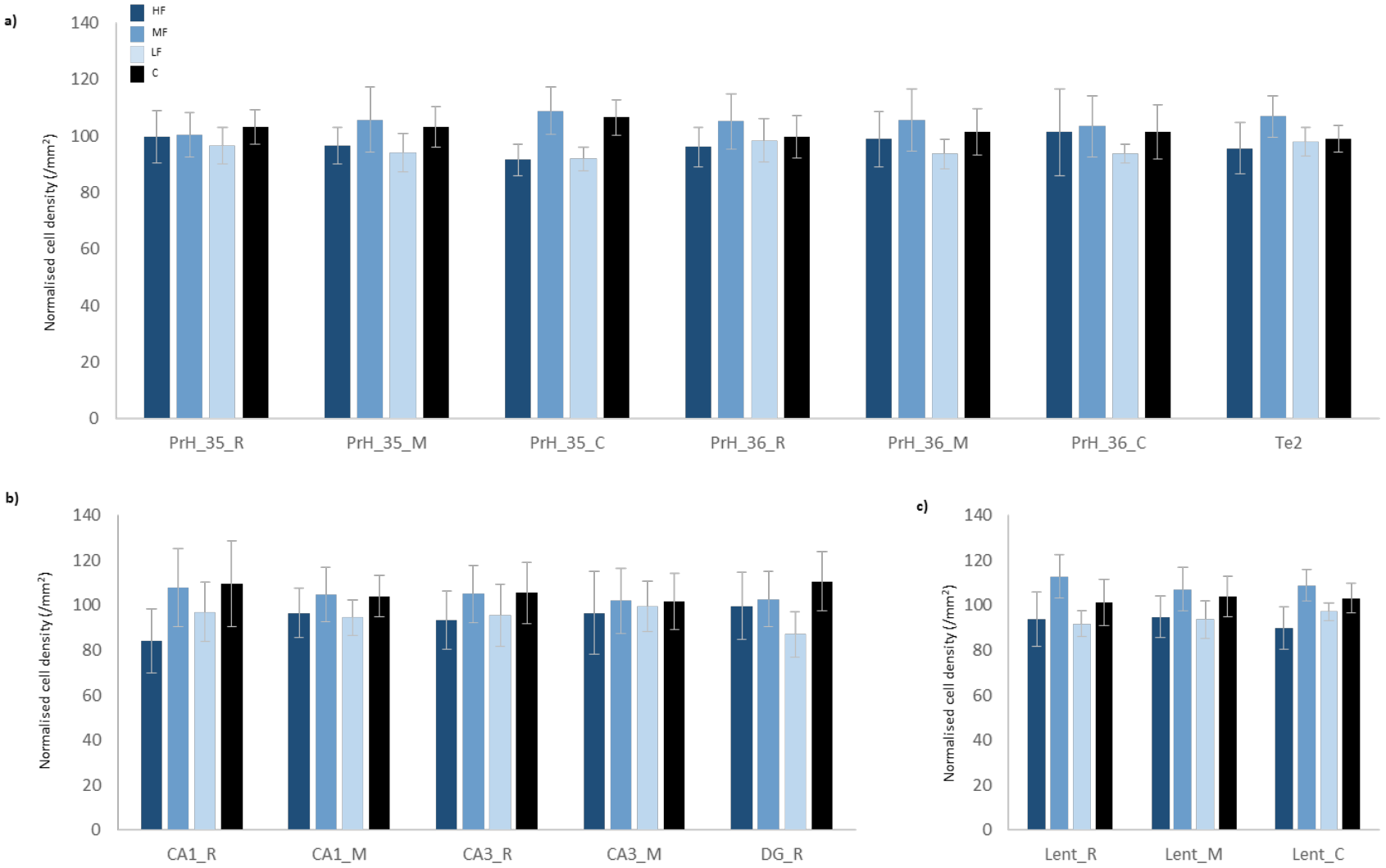

Figure 4.11: Displaying mean and standard errors for scaled cell density counts for a) the perirhinal cortex and area Te2, b) the hippocampus and c) the lateral entorhinal cortex, where "_R" denotes rostral, "_M" denotes mid and "_C" denotes caudal sub-regions.

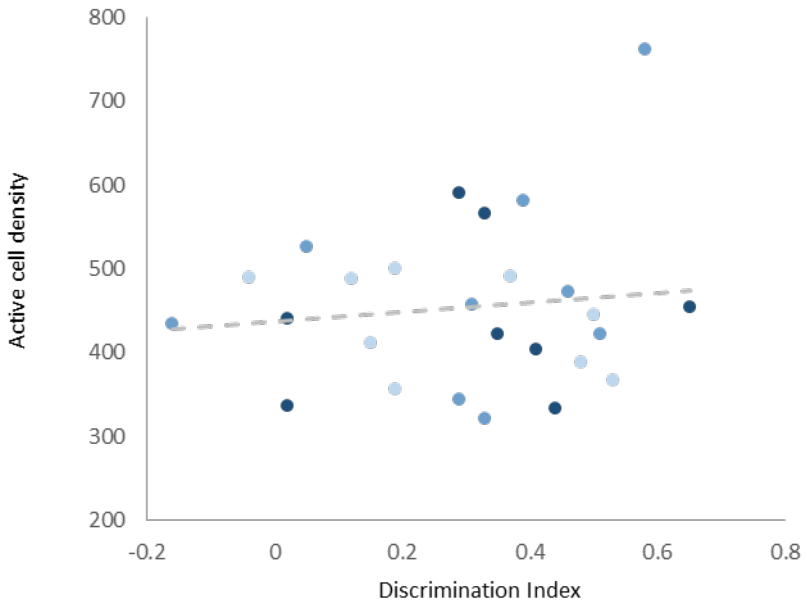

Figure 4.12: Scatter plot demonstrating the lack of a relationship between DI and active cell density in the perirhinal cortex.

4.7.3 Structural Equation Modelling Results

As raw cell densities were used as the basis for the SEM modeling, rather than the scaled densities used for the analyses above, mean raw cell densities are presented in Figure 4.13.

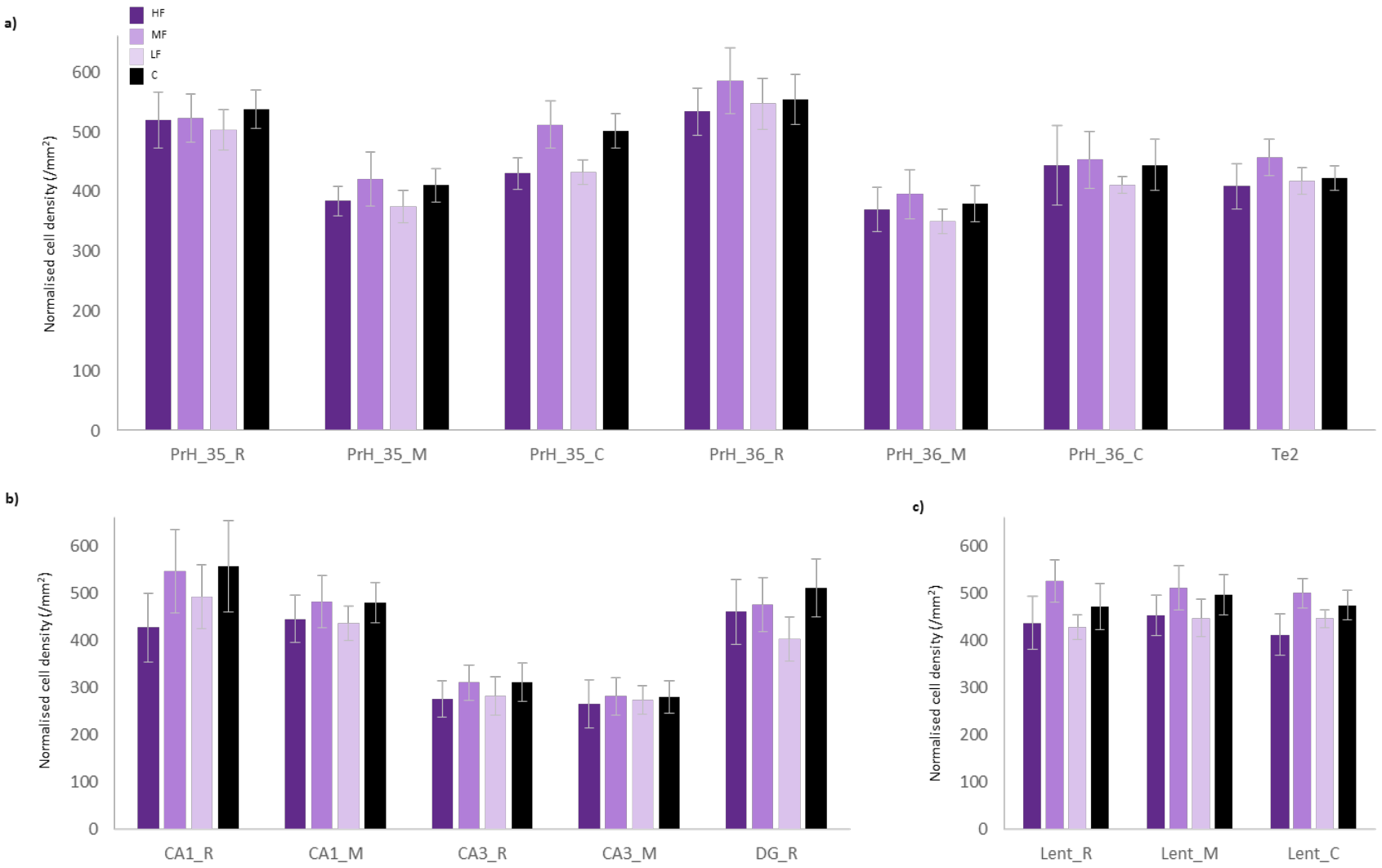

Figure 4.13: Displaying mean and standard errors for raw cell density counts for a) the perirhinal cortex and area Te2, b) the hippocampus and c) the lateral entorhinal cortex, where "_R" denotes rostral, "_M" denotes mid and "_C" denotes caudal sub-regions

C-fos expressing cell densities were used to estimate the effective connectivity between MTL sub-regions as in Albasser et al., (2010). The novelty and familiarity networks previously identified in the literature (Albasser, Poirier & Aggleton, 2010; Kinnavane, Amin, Olarte-Sanchez & Aggleton, 2016), are depicted in Figure 4.9a&b (see section 4.6.5.4). These were used as the specified models tested in this experiment using the *c-fos* expressing cell densities, along with two similar models where input into the lateral entorhinal cortex from the perirhinal cortex rather than area Te2 (Figure 4.9c&d) was considered.

As no differences in *c-fos* expressing cell densities were observed within subfields of the perihinal cortex, the hippocampus or the lateral entorhinal cortex, these densities were collapsed across sub-regions for each rat such that SEM models were estimated using overall perirhinal cortex, lateral entorhinal cortex, CA1, CA3 and DG *c-fos* expressing densities. It is important to note here that this input for the models differs from that in Albasser and colleagues (2010), who found models best fitting when only caudal perirhinal cortex *c-fos* expressing cell densities were entered into the model. However, contrary to Albasser and colleages' (2010) findings, we failed to demonstrate a differences in *c-fos* expressing cell densities between the caudal perirhinal cortex and other sub-regions of the perirhinal cortex for any group, and as such exclusively entering caudal perirhinal cortex *c-fos* expressing cell densities is unsupported. Thus *c-fos* expressing densities for all perirhinal cortex sub-regions were collapsed and this overall perirhinal cortex *c-fos* expressing density was entered into the model. SEM analyses are reported for each group below.

4.7.3.1 Control Group

Of the four models tested, only the two familiarity based models depicting direct effective connectivity between the lateral entorhinal cortex and the CA1 "fit" based on the chi-square results (i.e. non-significant). Of these, the optimal model (as per RMSEA and CFI measures of fit) was that depicted in Figure 4.14, with input into lateral entorhinal cortex from the perirhinal cortex rather than area Te2, where all

the pathways were significant ($p < 0.001$) and the model had good fit ($\chi^2_{(3)} = 3.436$, $p = 0.329$, RMSEA = 0.135, CFI = 0.991; Table 4.1)

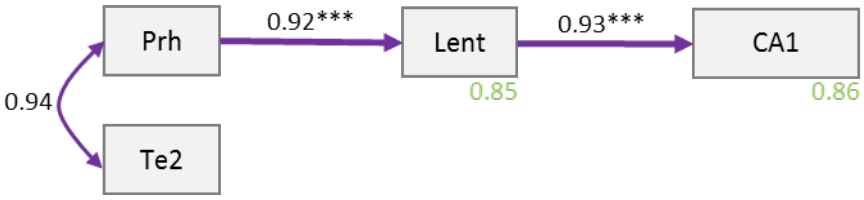

Figure 4.14: Optimal fitting SEM model of the four tested models for the Control group. * denotes significance at p<0.001**

4.7.3.2 High Familiarity

Of the four models tested, none "fit" based on the chi-square results as all models returned a significant chi-square statistics (Table 4.1).

4.7.3.3 Moderate Familiarity

As for the Control group, only the two familiarity based models depicting direct effective connectivity between the lateral entorhinal cortex and the CA1 "fit" based on the chi-square results (i.e. non-significant). Of these, the optimal model (as per RMSEA and CFI measures of fit) was that depicted in Figure 4.15, with input into lateral entorhinal cortex from the perirhinal cortex ($\chi^2_{(3)} = 2.405$, $p = 0.493$, RMSEA < 0.001, CFI = 1.000). However, the pathway between the lateral entorhinal cortex and the CA1 was non-significant, and thus this model only accounts for variance in *c-fos* expressing cell density in the perirhinal cortex, area Te2 and the lateral entorhinal cortex, but not the CA1 (Table 4.1).

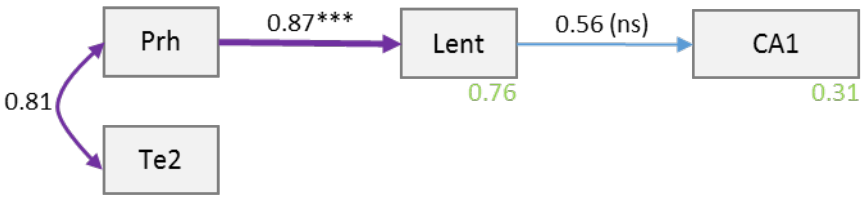

Figure 4.15: Optimal fitting SEM model of the four tested models for the Moderate Familiarity group. Purple arrows and * denote significance at p<0.001, blue arrows and ns denotes non-significance.**

4.7.3.4 Low Familiarity

Of the four models tested, only those where the lateral entorhinal cortex input was modeled from the perirhinal cortex "fit" the data. Both the familiar_Prh and novel_Prh models "fit" based on the chi-square results (i.e. non-significant), and these models were equally fitting (as per RMSEA and CFI measures of fit; familiar_PrH: $\chi^2_{(3)} = 0.832$, p = 0.842, RMSEA < 0.001, CFI = 1.000; novel_PrH: $\chi^2_{(9)} = 7.854$, p = 0.549, RMSEA < 0.001, CFI = 1.000). Thus, both of these "optimal" models are depicted below. These models were compared using the chi-squared differences test for comparing nested models (see section 4.6.5.4). This revealed a non-significant chi-square, suggesting the larger model with more free parameters did not account for the data significantly better than the more constrained model (Table 4.1).

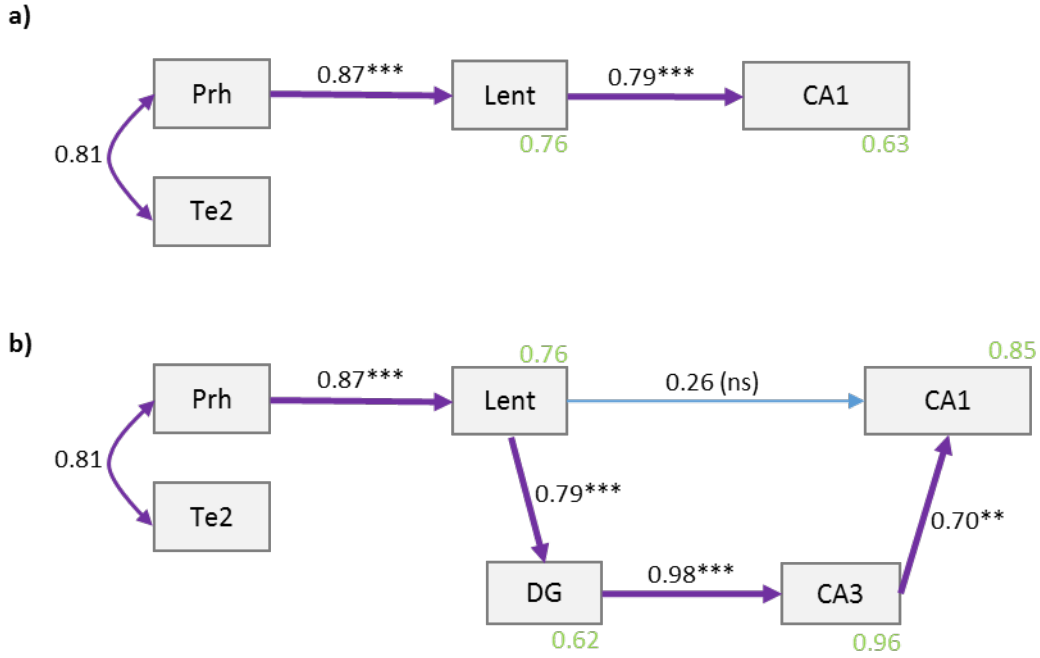

Figure 4.16: Optimal fitting a) familiarity and b) novelty SEM models of the four tested models for the Low Familiarity group. Purple arrows denote significance, with * denoting p<0.001 and ** denoting p = 0.001, blue arrows and ns denotes non-significance.**

4.7.3.5 All Experimental Groups

The lack of differences observed in the recognition behaviour of the three experimental groups suggests the behavioural manipulation had no effect. Furthermore, contrarily to controls, all experimental groups were presented with a novel and a familiar object on test day. As such, the four models were also tested with the three experimental groups collapsed. Of the four models tested, only the familiarity based model, depicting direct effective connectivity between the lateral entorhinal cortex and the CA1, in which input into the lateral entorhinal cortex was from the perirhinal cortex "fit" based on the chi-square results (i.e. non-significant; $\chi^2_{(3)} = 6.005$, p = 0.111, RMSEA = 0.200, CFI = 0.957; Table 4.1), and is depicted in Figure 4.17.

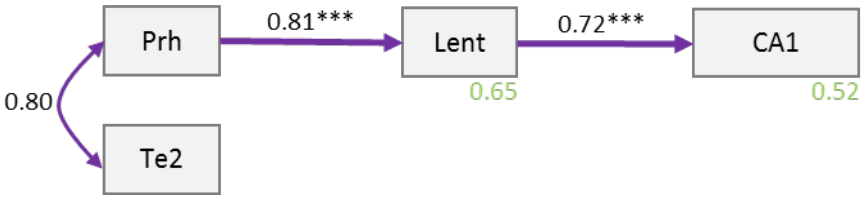

Figure 4.17: The only fitting SEM model of the four tested for all of the experimental groups collapsed together. Purple arrows and * denote significance at p<0.001, blue arrows and ns denotes non-significance.**

Table 4.1: Table showing SEM model fit results for each model tested for each group.

Group	Model	χ^2	df	p	RMSEA	CFI
Control	**Familiarity, Te2->Lent**	**4.113**	**3**	**0.250**	**0.215**	**0.976**
	Novelty, Te2->LEnt	18.621	9	0.029	0.360	0.879
	Familiarity, Prh->LEnt	**3.436**	**3**	**0.329**	**0.135**	**0.991**
	Novelty, Prh->Lent	17.944	9	0.036	0.352	0.887
High Familiarity	Familiarity, Te2->Lent	15.710	3	0.001	0.778	0.756
	Novelty, Te2->LEnt	32.316	9	0.000	0.608	0.702
	Familiarity, Prh->LEnt	13.496	3	0.004	0.707	0.799
	Novelty, Prh->Lent	30.102	9	0.000	0.579	0.731
Moderate Familiarity	**Familiarity, Te2->Lent**	**2.405**	**3**	**0.493**	**0.000**	**1.000**
	Novelty, Te2->LEnt	28.584	9	0.001	0.522	0.512
	Familiarity, Prh->LEnt	**4.000**	**3**	**0.261**	**0.204**	**0.948**
	Novelty, Prh->Lent	19.280	9	0.023	0.378	0.744
Low Familiarity	Familiarity, Te2->Lent	10.636	3	0.014	0.564	0.519
	Novelty, Te2->LEnt	17.658	9	0.039	0.347	0.839
	Familiarity, Prh->LEnt	**0.832**	**3**	**0.842**	**0.000**	**1.000**
	Novelty, Prh->Lent	**7.854**	**9**	**0.549**	**0.000**	**1.000**
Experimental groups collapsed	**Familiarity, Te2->Lent**	**6.005**	**3**	**0.111**	**0.200**	**0.957**
	Novelty, Te2->LEnt	24.344	9	0.004	0.261	0.885
	Familiarity, Prh->LEnt	10.837	3	0.013	0.888	0.323
	Novelty, Prh->Lent	29.176	9	0.001	0.849	0.299

Note: models displayed in bold have a non-significant $\chi 2$ and are therefore considered to fit the data

4.8 Discussion

This aim of this experiment was to investigate whether concurrently presenting items of different familiarity levels concurrently to a novel item would affect the neural processing of novelty and familiarity, both within the perirhinal cortex and across MTL neural networks. The results from the current experiment, detailed below, do not support the hypothesis that the presence of a novel object will lead to

engagement of the novelty network, and that greater levels of familiarity will lead to greater engagement of the familiarity network.

Similarly to Experiment 4, *c-fos* expressing cell densities in the perirhinal cortex, the hippocampal sub-regions and the lateral entorhinal cortex were not affected by the presentation of novel items, or items of varying levels of familiarity. As no differences were observed in the *c-fos* expressing cell densities between the Control group and all other experimental groups, this data also failed to replicate the frequently referred to increase in *c-fos* expression within the perirhinal cortex after exposure to a novel item (Wan, Aggleton & Brown, 1999; Xiang & Brown, 1998; Zhu, et al., 1995; 1996; Albasser, Poirier & Aggleton, 2010; see Kinnacane et al., 2014). This finding was maintained from Experiment 4 despite the identity of items presented at test being controlled for between the experimental and control groups. This suggests the result from Experiment 4 are not confounded by the visual processing of differing objects between the experimentl and control groups. It is suggested here that, as for Experiment 4 (see Section 4.3.2), the lack of replication of greater *c-fos* expression in the perirhinal cortex of rats after presentation of a novel item results from presenting both a novel and a familiar item simultaneously (see Section 1.4.3 for more details). As discussed in the introduction to Experiment 4 (see Section 4.1), lesions of the perirhinal cortex disrupt rats' SOR for novel and familiar objects presented concurrently, but leaves behavioural responses to novelty and familiarity intact when these are presented separately (Orlate-Sanchez et al., 2015; Albasser, et al., 2011). Orlate-Sanchez and colleagues (2015) argue that under conditions of simultaneous novelty and familiarty presence, the perirhinal cortex allows binding of the mnemonic status of an item to that item. Thus the perirhinal cortex is not involved in the amount of novelty or familiarty per se but rather is involved specifically in object recognition through the identification of the mnemonic status of a specific object. Within this capacity, the task demands are equal for experimental and control groups – both require identification of the mnemonic status of two items, and hence the lack of a difference between these groups is understandable.

In accordance with the results of Experiment 4, the lack of any differences in the perirhinal cortex *c-fos* expressing cell densities between experimental and control groups was seen in conjunction with behavioral demonstration of recognition memory, where these two measurements were uncorrelated. Thus, this finding supports the understanding that the perirhinal cortex activity as measured by *c-fos* expression is not driving the behavioural expression of recognition memory (see the discussion of Experiment 4 - Section 4.4 - for a further discussion of this). Similarly, to Experiment 4, the lack of an effect of the relative familiarity of the familiar item presented on behaviour suggests that novelty drives SOR behaviour (see Section 4.4 for more details).

The neural network analyses suggested that the input from the perirhinal cortex into the lateral entorhinal cortex was more significant than that from area Te2, in contrast to the findings of Albasser and colleagues, (2010). The differences in the suitability of the hypothesised neural networks in explaining the pattern of *c-fos* expressing cell densities within the MTL structures identified between conditions clearly indicates an effect of the mnemonic status of the presented item on these networks. However the results from the analyses, as outlined below, do not depict a cohesive picture.

For the Control condition in which rats were only presented with highly familiar items, the familiarity network outlined by Albasser and colleagues (2010) was engaged, in which effective connectivity is along the more direct temporo-ammonic pathway from the lateral entorhinal cortex to CA1 (PrH -> Lent -> CA1). In contrast, for the Low Familiarity condition in which rats were presented with the highest level of relative novelty, the novelty network outlined by Albasser and colleagues (2010) was engaged, in which effective connectivity is along the performant pathway from lateral entorhinal cortex to dentate gyrus, and then to CA1 through CA3. However, the pattern of *c-fos* expressing cell densities for the Low Familiarity condition was also explained by the familiarity network. While this familiarity network was considered to better account for the data statistically, Protzner and McIntosh (2006)

demonstrate that when SEM models are based on anatomical connections, inferences about models can reliably be made regardless of absolute model fit, and thus both models are considered to valuably depict the patterns of effective connection in this condition. While it is tempting to view the engagement of both of these networks as a consequence of presenting both a familiar and a novel item, this conclusion is not supported by the lack of adequate fit for either of these models in the High Familiarity and Moderate Familiarity condition, where rats were also presented with a novel and a familiar item. Indeed, the familiar item presented to the High and Moderate Familiarity groups was relatively more familiar and thus should engage the familiar network to a greater extent if this conclusion was to be supported. It should be noted here that the familiarity network was considered an adequate fit for the data in the Moderate Familiarity condition. However, only the components of the familiarity neural network upstream from the hippocampus were supported by the patterns of activity within the sub-regions measured, and based on the lack of effective connectivity downstream from the lateral entorhinal cortex, this model as a whole was considered to inadequately explain the data despite statistical significance (see Protzner & McIntosh, 2006).

One possible interpretation of the data is that the presence of items of concurrent mnemonic status disrupt the novelty and familiarity networks of the MTL. This interference appears not to be systematic. The presence of a novel item did not simply result in engagement of the performant pathway, while incremental changes in the familiarity of an item presented alongside a novel item did not lead to stronger engagement of the temporo-ammonic pathway. However, the single trial methodology used in this experiment may not have driven the engagement of these pathways significantly enough to allow the changes in *c-fos* expression to the extent necessary to question this. Indeed, these networks were identified and further corroborated from experiments using twenty-one novel or familiar objects presented sequentially in a bow-ties maze (Albasser, Poirier & Aggleton, 2010; Kinnavane, Amin, Olarte-Sanchez & Aggleton, 2016). As cells in the perirhinal cortex have been shown to respond to the presence (Burke et al., 2012) as well as the

familiarity (Brown & Xiang, 1998) of objects, the variety of objects presented in the twenty trials in the previous literature are likely to have caused more neurons to fire within the network, allowing differences in the networks to be substantial enough for analysis through *c-fos* expression.

Moreover, recent findings have suggested that novelty/familiarity processing in the perirhinal cortex is dependent upon firing frequency, where stimulation of the perirhinal cortex with frequencies of 30-40Hz caused rats to treat familiar images as novel, and frequencies of 10-15Hz caused rats to treat novel images as familiar (Ho et al., 2015). As such, more sensitive quantification of activity, such as measures of firing rate and synchronicity obtained through single unit recordings, may be more suited to interrogating the differences in the neural processing of novelty and familiarity when these are investigated using methodologies which do not markedly drive the engagement of these processes.

Finally, while it is important to acknowledge that single regions do not work in isolation, and to therefore consider the neural network involved, it is also important to remember that these networks are also not closed loops, such that various networks interact with each other. Indeed, Lisman and Grace (2005) propose a model in which networks within the MTL interact with dopamine networks to enable the processing of novel information such that it is then entered into long-term memory. This model incorporating other neural regions of interest, such as the Ventral-Tegmentum Area (VTA), which should also be considered within the context of the questioning of the processing of novelty and familiarity.

In conclusion, the results obtained from this experiment suggest that despite normal behavioural expression of recognition memory, there is some disruption of the neural processing of novelty and familiarity by presenting concurrent items of differing mnemonic statuses, but the quality and extent of this disruption remains unclear. Extended anatomical networks should be considered, paralleled with the use of techniques allowing for finer measurements of neural activity, to further

investigate the processing of novelty and familiarity at a neural level. This should take into consideration both the networks within the MTL and those interacting with MTL structures such as the dopamine based networks.

5. CHAPTER FIVE: RECONCILING THE RECOGNITION MEMORY MEASURES OBTAINED FROM HUMAN AND RODENT TASKS

5.1 General Introduction

When considering the integration of the empirical investigations outlined in the previous experimental chapters 2 – 4 which question the dissociation of novelty and familiarity as separate processes that support recognition memory across differing levels of analysis (cognitive and neural) and differing species (humans and rodents), an important consideration became apparent: are the animal and human literature testing same components of recognition memory? Thus, the experiments within this chapter aim to investigate and bridge the methodologies used to test recognition memory across humans and rodents. More specifically, does the SOR capture both the components of sensitivity (*d'*) and bias (*c*) known to contribute to human recognition? These are important considerations as, since its development in 1988 by Ennaceur and Delacour, the use of the (spontaneous) novel object recognition (NOR/SOR) task has become mainstream in animal recognition memory research. Indeed, the above mentioned paper in which this task was introduced has more than 1,400 citations to date (ISI, Web of Science, 10th October 2016). Furthermore, using a web of science search for "novel object recognition" or "spontaneous object recognition" paired with either "rat" or "mouse", Ameen-Ali, Eacott & Easton (2012) estimate that approximately 43,000 rats or mice have been used between the years 2007 - 2012 in the 534 peer-review papers listed in the result. Despite this heavy use of the SOR task in rodents, we have little understanding, if any, of how the indirect recognition memory measure obtained from the SOR tasks (the Discrimination Index - DI) relates to the memory parameters of sensitivity (*d'*) and bias (*c*) typically extracted from tests of human recognition memory.

The manner in which the SOR task and its behavioural results are discussed in the literature strongly imply that the DI is a measure of recognition memory sensitivity. Animals who fail to present a novelty preference in the SOR task are considered to lack the ability to discriminate between novel and familiar items in memory, where this is the precise definition of sensitivity. However, the SOR task is also discussed as being based on an "innate preference for", or predisposition to, explore novelty. As response bias reflects an individuals' predisposition towards identifying novelty, the DI and recognition memory bias may be related such that both reflect an individual's general preference or predisposition towards novelty. Within the framework of the novelty-encoding hypothesis, novelty is considered to elicit additional cognitive processing, which in turn leads to better subsequent memory (Tulving & Kroll, 1995). Additional cognitive processing is often considered to come at the cost of time – the more considerable the amount of processing, the longer required to undertake it. Hence, within the novelty-encoding hypothesis framework, a causal relationship is suggested between DI and memory sensitivity: a greater novelty preference, as indicated by a larger DI, will lead to greater processing, and thus presumably encoding, which will result in greater memory sensitivity. However, this does not preclude an effect of, or relationship between, bias on/and DI.

Data from lesion and neurophysiological studies using the SOR paradigm is also not conclusive as to whether sensitivity and/or bias are reflected in the DI. Memory sensitivity is considered to depend upon memory processing structures such as the MTL and surrounding rhinal cortex, while bias is a cognitive control/decision-making process which has been shown to be dependent upon the prefrontal cortex (PFC; Fuster, 1980; Goldman-Rakic, 1987; Duncan & Desimone, 1995). While Perirhinal cortex lesions reliably lead to an SOR deficit (e.g. Ennaceur et al., 1996; Barker et al., 2007; Aggleton et al., 2010; see Section 1.4.1 introduction for more details), PFC lesions have also been demonstrated to cause SOR deficits (Dias & Honey, 2002), although this is not reliable (see Morici et al., 2015 for a review; see Section 1.4.4 of this thesis for more details). Thus, these data would suggest that the DI is

predominantly related to memory sensitivity while also being related to bias in certain circumstances.

Thus, empirical evidence is required to ascertain how the DI obtained in the animal literature relates to the components of recognition memory obtained from human research. This will further our understanding of whether research in these two species is testing the same recognition memory components and processes. Consequently, the aim of Experiments 6 and 7 was to investigate the relationship between individual people's (Experiment 6) and rats' (Experiment 7) DI and measures of sensitivity and bias.

5.2 Introduction Experiment 6

Investigating the relationship between the discrimination index (DI) and sensitivity (*d'*) and bias (*c*) in humans requires the use of both a standard old/new single item recognition test, from which to obtain measures of *d'* and *c*, and an analogue to the Spontaneous Object Recognition task from which to obtain a DI. For the current experiment, the Visual-Paired Comparison (VPC) task was used as this analogue to the SOR. The VPC is run similarly to the SOR. In a sample trial, participants are presented with two identical images (e.g. AA) side by side for a specific duration. After a specified delay, in a test trial, participants are presented with one of the same image (A) and a novel image (e.g. B). Fixation durations for the novel and familiar objects presented are recorded and compared. Similarly to the SOR, novel objects are found to be preferentially fixated upon (Crutcher et al., 2009; Manns, Stark, & Squire, 2000; Richmond, Sowerby, Colombo, & Hayne, 2004). Furthermore, as for the SOR, the novelty preference exhibited in the VPC is innate (see Rose, Feldman, & Jankowski, 2004 for a review), the task requires no rule learning, and is unrewarded (see Section 1.2.1 of this thesis). As such, the VPC has been used in developmental research with infants (see Rose, Feldman & Jankowski, 2004 for a review), in older adults with cognitive impairments (e.g. Crutcher, et al., 2009) and in

non-human primates (e.g. Zeamer, Meunier, & Bachevalier, 2011). For the reasons outlines above, the VPC is considered to correspond well to the SOR used in the rodent literature.

Interestingly, while selective hippocampal lesions have been demonstrated not to affect the novelty preference seen in the SOR task (e.g. Langston & Wood, 2010; Mumby et al., 2002 see Section 1.4.1), these same lesions in monkeys (Zeamer et al., 2011; S M Zola et al., 2000) and humans (Pascalis, Hunkin, Holdstock, Isaac, & Mayes, 2004) significantly disrupt the novelty preference seen in the VPC. As the hippocampus is considered to support recollection rather than familiarity processing, and the SOR is considered to be dependent upon familiarity rather than recollection (outlined in detail in Section 1.4 of the introduction; Brown & Aggleton, 2001), the lesion data presented above would suggest that the VPC and SOR tasks differ in the memory processes which underlie them. Specifically, the hippocampal lesion data suggest that the VPC is dependent upon recollection, while the SOR is not.

Pascalis and colleagues (2004) propose a different interpretation. Their patient YR with selective hippocampal damage demonstrated significantly lower novelty preference than controls when the delay between the sample phase in which two identical images were presented, and the test phase in which one of the items seen during the sample phase and a new item was greater than zero seconds. However, YR was not impaired on an item-recognition task requiring her to point to a previously presented familiar item presented alongside a novel item. These apparent contradictory results, which are paralleled in experiments using monkeys with hippocampal lesions (Nemanic et al., 2004), lead Pascalis and colleagues (2004) to propose that longer fixation times allocated to familiar items are a result of an inability to recollect, because the individual is unable to ascertain why the item appears familiar. As such, reduced novelty fixations are a consequence of a recollection deficit without this reflecting an item recognition deficit. Following this explanation, the VPC is not considered to be directly dependent upon the hippocampus or recollection per se, rather, lesions of this leads to deficits which interfere with the VPC. Consequently, following the proposition by Pascalis and

colleagues (2004), the use of the VPC in intact animals and participants without recollection impairments should be an appropriate analogue to the SOR.
Stimulus similarity has also been considered to explain the discrepancy in the performance of hippocampal lesioned monkeys and patients between tasks of conscious response-based item recognition and the VPC (Zeamer, Meunier, Bachevalier, 2011). When stimuli are perceptually dissimilar, and thus presumably can be suitably differentiated based on familiarity (see Section 1.4.3), monkeys with hippocampal lesions perform normally on the VPC. Contrastingly, when the presented stimuli are visually similar, and hence recollection would aid their differentiation, these same individuals are impaired on the VPC. Although this suggests important consideration is required in the choice of stimuli for the VPC (see also Winters, Dubuc & Higham, 2015), this finding does not explain the pattern of results for patient YR, as black and white images of objects were used in both the VPC and the item recognition task in which she overtly pointed to familiar items (Pascalis, et al., 2004). Thus, on the basis of the evidence currently available, for the purpose of this experiment, given the use of intact participants, and the considered selection of stimuli (outlined below), the VPC is considered an appropriate method from which a DI measurement can be obtained.

To the author's knowledge, novelty preference in the VPC has not previously been compared to measures of participants' recognition memory sensitivity and bias. However, Manns and colleagues (2000) have examined the VPC for consideration as a measure of declarative memory more generally. In their procedure, participants performed a VPC task in which two identical images (e.g. AA) were presented for 5 seconds, and, after a 5-minute delay, one of these old images and a novel image (e.g. AB) were presented together. Following a 24-hour delay, participants were tested using a yes/no recognition paradigm for the old images (i.e. A, but never B) seen in the VPC task and novel images not previously seen in the experiment (e.g. C). Unexpectedly, the percentage of time spent looking at the novel item in the VPC was uncorrelated to the percentage of items correctly identified during the yes/no recognition task. Seemingly, this provides evidence against a relationship between

novelty preference in the VPC and single item recognition memory. However, this experiment is a prime example of the importance of considering recognition responses to both old (Hits and Misses) and new (Correct Rejections and False Alarms) items, and the combined effect of these (sensitivity and bias). Indeed, by using the blunt summary measure of percentage of correctly identified items for the single item recognition task, the data are difficult to interpret. For example, as suggested by the authors, the lack of a correlation may result from a trade-off between correctly identifying new and old items as a result of the novelty preference in the VPC. Specifically, greater fixation durations for the novel item in the VPC by definition results in lower fixation durations for the old item. As such, based on the understanding that greater fixation durations reflect deeper encoding, more important novelty preferences may result in a corresponding decrease in the correct identification of old items, which once identified as familiar are given little further processing in the test phase of the VPC. Interestingly, if the novelty preference observed in the VPC positively correlate to correct rejection rates, such that participants spending more time fixating on novelty are also better able to identify this same novelty, when these enhanced correct rejection rates are collapsed with the reduce hit rates to obtain a measure of overall accuracy, the novelty preference in the VPC will be uncorrelated to this measure of accuracy. This is supported by the findings in the same experiment that DI correlated to both reaction times and participants' confidence, which are commonly reported to be highly correlated to recognition memory ability (e.g. Cave & Squire, 1992; Juslin, Olsson & Winman, 1996). Furthermore, differences in bias are left unconsidered by this measure of declarative memory.

However, in a similar manner to a forced choice task in which participants are asked to identify the old (or new) item of two presented items, the VPC is considered to be a "criterion-free", or bias free, task (Macmillan & Creelman, 2005). Indeed, participants are spending more time looking at the *relatively* newest item presented, rather that identifying the items presented in terms of absolute "new" or "old". As bias reflects the level of evidence required for a participant to differentially make an

old or new judgment, this is not required when simply comparing the strength of evidence between two items to identify which is least/most novel. As such, the VPC is not considered to be impacted by bias.

Thus, the current experiment aimed to determine whether the participants' novelty preference in a VPC task, as measured by a fixation duration based discrimination index (DI), relates to the recognition memory components of sensitivity (*d'*) and bias (*c*). Drawing on the assumptions in the rodent literature, together with the lesion data, as outlined above in Section 5.1, it was hypothesised that the DI would be positively related to *d'*, but unrelated to *c*. Furthermore, as lesions to the hippocampus do not disrupt SOR behaviour while perirhinal lesions do (Brown & Aggleton, 2001), and that the VPC is not considered to be dependent upon the hippocampus and recollection in intact individuals, it is additionally hypothesised that the positive relationship between DI and *d'* would be strongest under conditions where participants were more singularly reliant upon familiarity (i.e. when information is shallowly encoded at study) rather than also having access to a significant recollection component (i.e. when information is deeply encoded at study).

To test these hypothesis, the following experiment used eye-tracking during a VPC task to obtain an accurate measurements of novelty preference (the DI), along with a standard old/new single item recognition judgement task. Importantly, the VPC was always administered before the single item recognition task. This ensured that participants did not carry over the recognition instructions from the single item recognition task to the VPC, as these instructions have been demonstrated to lead to greater fixation times for the old compared to the new item presented (Ryan, Hannula, & Cohen, 2007). Furthermore, the stimuli of choice were carefully considered. As differing stimuli lead to differing levels of *d'* and *c* (e.g. see Experiment 2 and 3), for assessment of the relationship between DI and *d'* and *c,* it was established that the same class of stimuli should be used for both experimental components. However, to avoid the issues resulting from testing single item

recognition on the same items as those presented during the VPC (see the discussion of Manns, Stark & Squire, 2000 above), differing sets of stimuli from this same class were used for the VPC and the single item recognition task. Thus, visual stimuli were required, which, unlike the fractals used in Experiment 2, were suitably memorable. Consequently, the stimuli of choice for Experiment 6 were images of Digimon and Pokémon characters[6] as in Experiment 3. The variety in these and use of large numbers of trials was considered to counteract any stimuli specific effects on the VPC (Zeamer, Meunier, Bachevalier, 2011; Winters, Dubuc & Higham, 2015).

5.3 Materials and Methods

Behavioural Testing was undertaken with the assistance of Imogen Callan.

5.3.1 Participants

Data was collected for a total of 37 participants with self-reported normal or corrected-to-normal vision, compensated £7 for their time. Nine participants were excluded from the analyses for the following reasons: failure to follow task instructions (n = 1); self-reported estimate that > 35% of stimuli were familiar or recognized (n = 3); failure to calibrate the eye tracker (n = 1); failure to fixate on both stimuli presented on screen during the Visual Paired Comparison task on more than 50% of trials (n = 3); failure to reach a minimum overall *d*' of 0.1 in the image judgment task (n = 1). Hence, the final sample consisted of 28 participants (75.68% of the original sample; 20 females, mean age = 23.86 years, age range = 18 - 32 years). Informed consent was obtained in accordance with the University Teaching and Research Ethics Committee of St Andrews (Appendix F).

[6] The release of the game Pokemon Go (Niantic, Inc., 2016), which re-introduced Pokémon characters into popular culture, occurred on the 13th of July 2016, during the course of this experiment (which ran from 28th June 2016 – 10th August 2016). However, 67.74% of participants were tested before its release, and the remaining 32.26% declared not having played the game.

5.3.2 Stimuli

The stimuli used were from the same set as those in Experiment 3. A set of 425 Pokémon generation II-VI (© 1995-2016 Nintendo/Creatures Inc./GAME FREAK inc. Pokémon) and Digimon (© 1997-2008 Bandai) characters was selected from online databases ("Pokemon Wiki", n.d.; "Wikimon", 2005)[7]. Images measured 200 x 200 pixels (5.29 cm^2). For each participant, a set of 320 items was randomly sampled from this pool, 80 of which were used for the Visual Paired Comparison task, and the remaining 240 for the image judgment task.

5.3.3 Apparatus

5.3.3.1 Visual Paired Comparison Task

Eye movements were recorded using an SR Eyelink 1000 eye tracker (SR Research Ltd., Mississauga, Ontario, Canada) with tower mount apparatus, sampling at 250 Hz. Fixations were determined using a spatial resolution of 0.1 deg (SR Research Ltd, 2013). Participants were seated approximately 40 cm from a CRT computer screen, resolution 1280 x 1024, used to display the stimuli. A chin rest reduced participants' head movements and increased participants' comfort. Calibrations of gaze direction were nine-point calibrations, and ensured that recordings had a mean spatial error < 0.5 deg for each participant. During calibration, participants were asked to fixate on the fixation crosses presented individually on screen. The spatial error between the measured eye-tracked location and the known spatial location of the fixation point on screen was computed by the software. Drift corrections were also implemented. These are used throughout testing to ensure calibrations are still valid, and consist of a single fixation cross on which participants fixate. Again, a mean spatial error < 0.5 deg is required for validation, where a mean spatial error > 0.5 deg triggers a new nine-point calibration.

[7] These stimuli do not infringe any copyright regulations as they are used under the "Fair dealing" law, originating in Section 29 and 30 of the Copyright, Designs and Patent Act 1988. This states that "fair dealing with a literary, dramatic, musical or artistic work for the purpose of research for a non-commercial purpose does not infringe any copyright in the work provided that it is accompanied by sufficient acknowledgement".

5.3.3.2 Image Judgement Task

The image judgement task was run on a Lenovo T410i laptop, screen resolution 1024 x 768 pixels, using Matlab (The Mathworks Inc., Natick, MA, R2011b) and Psychophysics Toolbox (Brainard, 1997).

5.3.4 Procedure

All participants undertook the visual paired comparison task immediately followed by the image judgment task.

5.3.4.1 Visual Paired Comparison Task.

After an initial calibration, participants were presented with a series of 80 trials consisting of two items displayed side-by-side (separated by a 158 pixel/4.18 cm margin) on a white screen. Participants were instructed to "simply look at the screen as if you are watching TV". No overt recognition responses were required of participants. These 80 trials consisted of a sequence of 40 sample-test pairs. In a Sample trial, two copies of the same (target) item were presented. In the corresponding Test trial, one copy of this same (target) item and a new (lure), not previously seen item, were presented onscreen (Figure 5.1a). New items were presented on the left and right hand sides of the screen equally frequently, in a pseudorandom order.

Each trial was presented for two seconds with a 0.5 second inter-trial interval (ITI), during which a fixation-cross appeared centrally on the screen. Breaks were available to the participant every 20 trials, during which participants could remove their head from the chin-rest. Eye tracking calibrations were undertaken (as outlined above) after every break, regardless of whether or not the participant removed their head from the chin rest. Drift corrections were also implemented every 10 trials to ensure adequate eye tracking accuracy. After completion of the 80 trials, participants were seated at a separate table in the same testing room and undertook the image judgement task.

5.3.4.2 Image Judgement Task.

Participants completed two self-paced study-test blocks. Each study phase consisted of a series of 60 characters presented individually at the center of the screen. Character presentation was spaced by a 0.5 second interval, consisting of a centrally presented fixation cross. Participants completed an incidental encoding task with different levels of processing (LOP) for each block. A question at the top of the screen indicated whether participants were being asked to rate the amount of yellow on a character ("YELLOW?", from "0 = none to "3+" = lots; shallow processing) or rate the character's friendliness ("FRIENDLY?", from "0" = very unfriendly – "3" = extremely friendly; deep processing) for the given block (Figure 5.1b). Encoding questions remained on screen throughout the study phase and were presented in different colours ("YELLOW?" in yellow, "FRIENDLY?" in blue) to further differentiate these.

During each test phase participants were presented with a series of 120 individual cartoon characters displayed centrally on the screen, 60 of which were targets (had been seen during the preceding study phase) and 60 of which were lures (had not previously been seen at study). Again, a 0.5 second interval consisting of a central fixation cross spaced the character presentation. Using a single-item recognition procedure, participants made "OLD" or "NEW" judgements, responding by key press (1 = "OLD", 0 = "NEW"). The order of the two study-test blocks, and therefore the level of processing used at study, was counterbalanced between participants. Participants' judgments and reaction times (RT) were recorded.

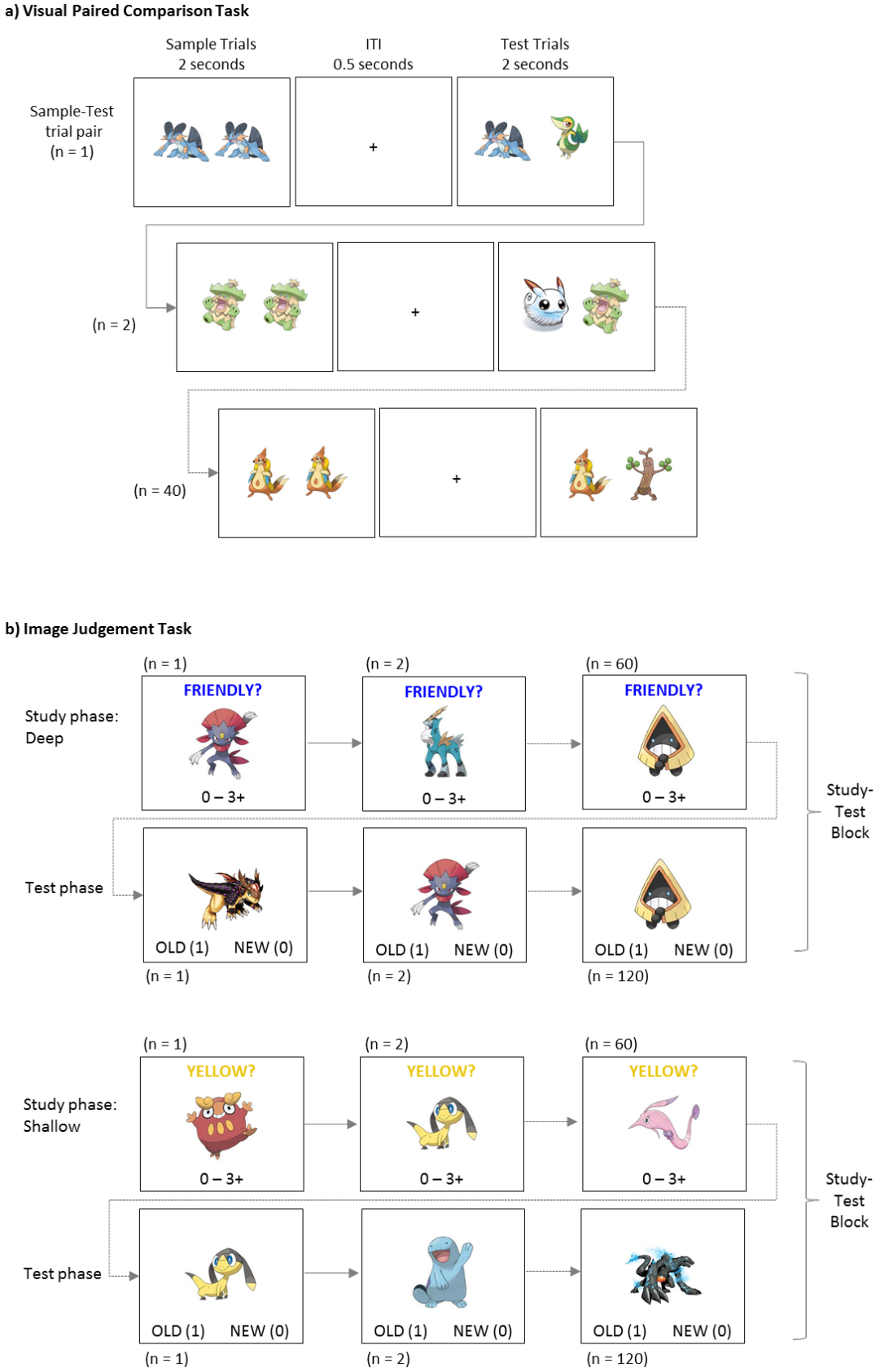

Figure 5.1: Experimental Design for Experiment 6.a) The Visual Paired Comparison task and b) the Image Judgment Task n denotes the trial number.

5.3.5 Calculations

Mirroring the measures used in the animal Novel Object Recognition literature, a fixation duration-based Discrimination Index (DI) was the principal measure obtained from the visual paired comparison task. The DI is a measure reflecting the preferential exploration allocated to a novel object as compared to a familiar one, as a proportion of total exploration time (to control for intrinsic variability in participants' levels of exploration). Here, fixations were used as a proxy for exploration time. For a given Test trial, fixation durations for the lure (new) item were summed (T_{new}) as were those for the target (previously encountered) item ($T_{familiar}$). The sum of all fixation durations for both items was used as the total trial exploration time. Hence the DI was calculated as follows:

$$DI = \frac{(T_{new}) - (T_{familiar})}{(T_{new} + T_{familiar})} \quad (10)$$

For sample trials, as participants saw two identical items on screen side by side, a left/right preference *DI* was calculated. Here fixation times for novel and familiar items are substituted by fixations times for the left and right character as follows:

$$DI_{left/right} = \frac{(T_{left}) - (T_{right})}{(T_{left} + T_{right})} \quad (11)$$

As in previous experiments depicted in this thesis, sensitivity (*d*') and bias (*c*) parameter estimates from the equal variance signal-detection model (Macmillan & Creelman, 2005) were the principal measures obtained from the Image Judgement Task. These were calculated for each participant as outlined in Section 2.2.4 (Chapter 2).

5.3.6 Data Analysis

Participants' preference for fixating on old and new images during the VPC was compared by submitting DIs to a single sample t-test, with zero as the value of comparison (where zero shows no preference for old or new items).

The effect of the LOP on *H'* and *CR'* rates in the Image judgment task was investigated. *H'* rates for both the shallow and deep LOP were submitted to a paired-samples t-test to reveal any differences between these. The same analysis was run separately on *CR'* rates.

The effect of LOP on *d'* and *c* were also investigated. *d'* for both the shallow and deep LOPs were submitted to a paired-samples t-test to reveal any differences between these. The same analysis was run separately on *c*.

To investigate the effect of LOP and the identification of old and new items on RTs in the Image Judgement Task, a 2 (LOP: shallow vs deep) x 2 (response: *H* vs *CR*) repeated-measures ANOVA was conducted.

Of most interest for this experiment, to investigate the relationship between the DI and measures of *d'* and *c*, these data were submitted to Pearsons' correlations separately for each LOP. Following significant correlations between DI and *d'* or *c* for both LOPs, these correlations were compared across LOPs using Steiger's Z-test for correlated correlations (Lee & Preacher, 2013). In essence this test converts the correlations to a z-score and then compares them in a similar manner to a t-test to establish if the correlations are significantly different from each other.

Finally, to help establish whether the processing of novelty and/or familiarity were related to participants *d'* and/or *c*, Pearson's correlations were also performed between participants RTs in the Image Judgement Task and their *d'* and *c*.

5.4 Results

Components entered into the correlations of interest are first examined individually.

5.4.1 Visual Paired Comparison Task: Analysis of Discrimination Index (DI)

To control for object-place novelty, data interpretation from the Test trials is dependent upon the assumption that the participant has seen the target in the previous Sample phase on *both* the left and right hand side of the screen. Therefore, only Test trials for which participant had fixated at least once on each item presented in the previous Sample Trial *and* during that Test trial, were included in the analyses (M = 84.91% of Test trials, SD = 13.90). Subsequent Sample trial analyses only included the Sample trials corresponding to the included Test trials. A paired samples t-test confirmed that there was no significant difference in the number of test trials included in the analysis in which the new character was presented on the left (M = 16.68, SD = 3.22) or right (M = 17.29, SD = 2.77) hand side of the screen, $t_{(27)} = 1.41$, $p = 0.171$, $d = 0.266$. This ensured that all further results were not confounded with location (left/right) of presentation.

Participants spent equivalent amounts of time fixating on the two identical characters presented during study trials, as confirmed by a one-sample t-test ($DI_{left/right}$: M = -0.017, SD = 0.105), $t_{(27)} = - 0.836$, $p = 0.410$, $d = 0.158$. In contrast, for test trials, participants had a DI significantly above chance (M = 0.321, SD = 0.151), indicating that they preferred looking at the novel compared to the familiar character presented on screen, $t_{(27)} = 11.257$, $p < 0.001$, $d = 2.13$ (Figure 5.2)

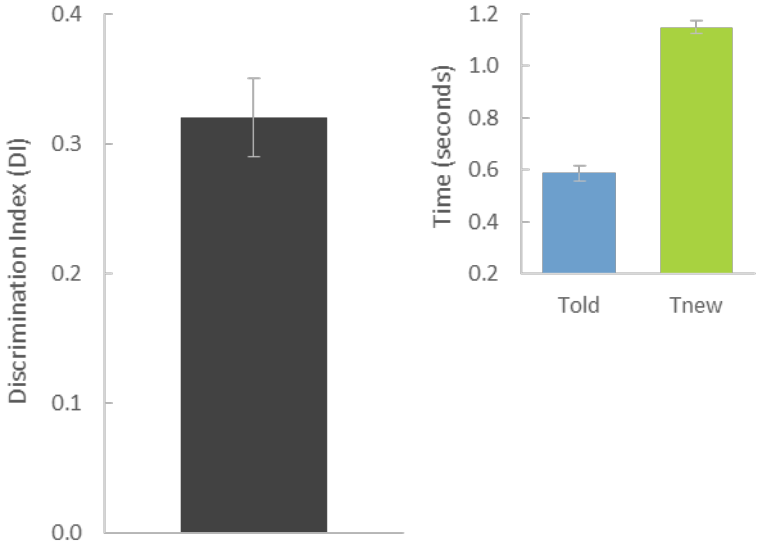

Figure 5.2: Mean Discrimination Index for items presented during the Visual Paired Comparison task, with mean exploration time for old and new items (inset). Error bars represent standard errors.

5.4.2 Image Judgment Task: Analysis of Hits (*H'*) and Correct Rejections (*CR'*)

Although hit and correct rejection rates are not directly used in the correlations of interest within this experiment, these are outlined here as they are the basis for calculations of sensitivity and bias, which are of primary interest within these correlations. Figure 5.3 depicts the mean *H'* and *CR'* rates for items in both the shallow and deep LOPs. Paired-samples t-tests demonstrated that *CR'* rates were unaffected by the LOP (Shallow: M = 0.87, SD = 0.09; Deep: M = 0.86, SD = 0.11), $t_{(27)}$ = 0.904, p = 0.374, d = 0.071, while *H'* rates were higher for items deeply encoded (M = 0.72, SD = 0.17) than those shallowly encoded (M = 0.54, SD = 0.19), $t_{(27)}$ = -5.57, p < 0.001, d = 1.05.

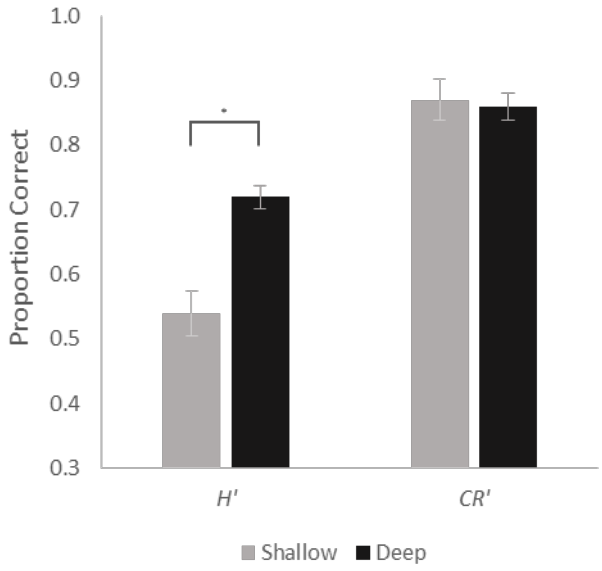

Figure 5.3: Mean adjusted hit (*H'*) and correct rejection (*CR'*) rates for both shallow and deep level of processing. Error bars represent standard errors.

5.4.3 Image Judgment Task: Analysis of Sensitivity (*d'*) and Bias (*c*)

The parameters of *d'* and *c* are of primary interest with respect to their correlations with DI. Mean *d'* for each LOP are presented in Figure 5.4a. As expected, participants' had a greater *d'* for items in the deep (M = 1.84, SD = 0.76) compared to the shallow (M = 1.34, SD = 0.55) LOP block as confirmed by a paired-samples t-test, $t_{(27)} = -4.284$, $p < 0.001$, d = 0.810. Due to the differences in *d'* across LOP, to allow comparisons of *c* across these, *c* was scaled to *d'* on a participant by participant basis, as in Experiment 1 to obtain adjusted *c* (*c'*; see Section 2.2.4). Mean *c'* for each LOP are presented in Figure 5.4b. Participants had a significantly more conservative *c'* in the shallow (M = 0.60, SD = 0.73) as compared to the deep (M = 0.14, SD = 0.54) LOP block, as confirmed by a paired samples t-test, $t_{(27)} = -3.418$, $p = 0.002$, d = 0.646.

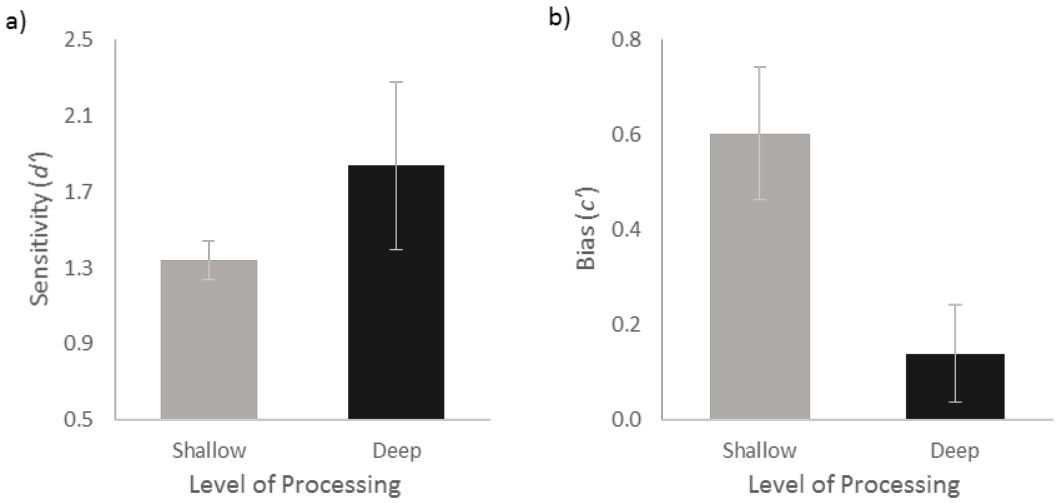

Figure 5.4: Mean a) sensitivity and b) bias estimates for both shallow and deep level of processing. Error bars represent standard errors.

5.4.4 Image Judgment Task: Analysis of Reaction Times (RTs)

Reaction times (RTs) for hits (*H'*) and correct rejections (*CR'*) are presented in Figure 5.5. A 2 (LOP: shallow vs deep) x 2 (response: *H'* vs *CR'*) repeated measures ANOVA revealed that participants were faster at making correct old (*H'*) compared to correct new (*CR'*) judgements, $F_{(1,27)} = 10.26$, $p = 0.003$, $\eta_p^2 = 0.275$, while LOP had no effect on RT, $F_{(1,27)} = 1.07$, $p = 0.311$, $\eta_p^2 = 0.038$, and the LOP x judgement interaction was also non-significant, $F_{(1,27)} = 1.86$, $p = 0.184$, $\eta_p^2 = 0.064$.

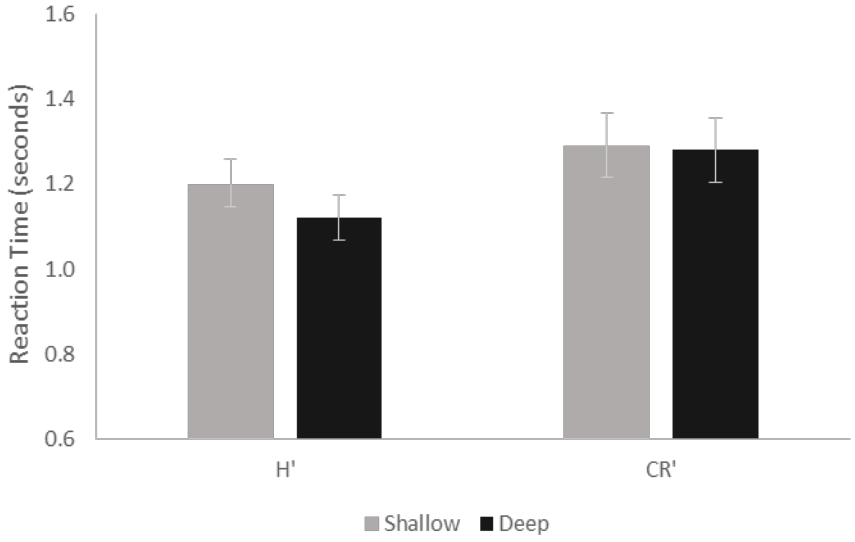

Figure 5.5: Mean reaction times for Hits and Correct Rejections under shallow and deep levels of encoding. Error bars represent standard errors.

5.4.5 Combining the Visual Paired Comparison Task and the Image Judgement Task results: Analysis of Relationships between Recognition Memory Measures

The correlations between DI and *d'* and *c* are of greatest interest for the purposes of this experiment. Pearsons' correlations run on DI and shallow and deep *d'* and *c'* are presented in Table 5.1. Statistically significant positive correlations were seen between DI and *d'* for both the shallow, $r_{(26)} = 0.528$, $p = 0.004$, and deep, $r_{(26)} = 0.576$, $p = 0.001$, encoding conditions (Figure 5.6). These correlations are not significantly different from each other as tested using Steiger's Z-test for correlated correlations, $Z = -0.338$, $p = 0.074$, (Lee & Preacher, 2013).

No correlation was observed between DI and *c'*. *c'* measures for the shallow and deep LOP blocks were positively correlated, $r_{(26)} = 0.403$, $p = 0.034$. Although participants significantly shifted their bias in the different LOP blocks (see Section 5.4.3), they did so in a similar manner. To investigate the relationship between the time taken for processing of novelty and familiarity and participants sensitivity and

bias within the Image Judgment Task, Pearson's correlations were also run on *d'*, *c'* and RTs for hits and correct rejections (Table 5.1).

Table 5.1: Correlation matrix for measures obtained from the Image Judgement Task and the Visual Paired Comparison Task. Correlations between reaction times (RT), the parameter estimates of sensitivity (*d'*) and bias (*c'*) obtained from the Image Judgement Task, along with the discrimination index (DI) calculated from the Visual Paired Comparison Task (n = 28).

		Shallow				Deep				
		d'	***c'***	**RT *H'***	**RT *CR'***	***d'***	***c'***	**RT *H'***	**RT *CR'***	**DI**
Shallow	***d'***	-								
	c'	**-0.618***	-							
	RT *H'*	0.317	-0.352	-						
	RT *CR'*	**0.386***	**-0.458***	**0.808*****	-					
Deep	***d'***	**0.603***	-0.181	0.118	0.106	-				
	c'	0.094	**0.403***	0.022	-0.007	0.037	-			
	RT *H'*	0.252	-0.278	**0.762*****	**0.563***	0.087	-0.053	-		
	RT *CR'*	0.263	-0.267	**0.606****	**0.592****	0.216	-0.040	**0.754*****	-	
	DI	**0.528***	-0.346	-0.008	0.152	**0.576***	-0.003	0.028	0.047	-

*Note: * denotes significance at p < 0.05, ** denotes significance at p = 0.001, *** denotes significance at p < 0.001.*

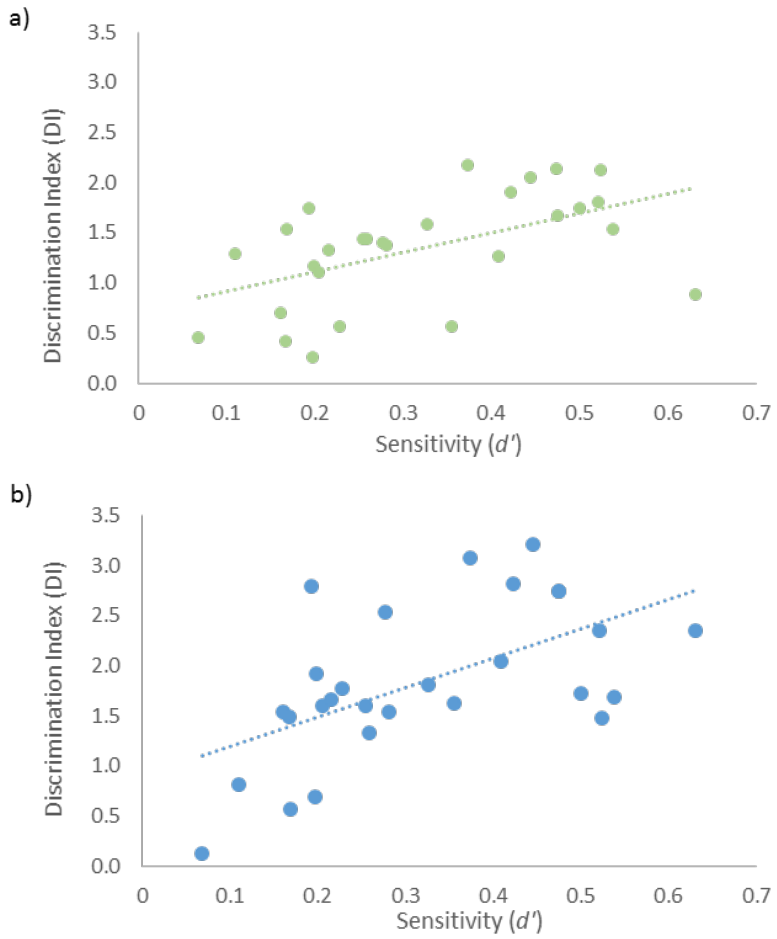

Figure 5.6: Correlations between DI and *d'* for a) shallowly encoded stimuli ($r_{(26)}$ = 0.528, p = 0.004) and b) deeply encoded stimuli ($r_{(26)}$ = 0.576, p = 0.001) (n = 28).

Relationships between participants' *d'*, *c'* and RTs for the image judgement task are only apparent in the shallow encoding condition. For items presented in the shallow encoding condition, RTs to correctly identified old items (*H'*; M = 1.20, SD = 0.31) did not correlate to either *d'* or *c'*, while RTs to correctly identified new items (*CR'*; M = 1.29, SD = 0.41) were positively correlated to *d'*, $r_{(26)}$ = 0.386, p = 0.042, and negatively correlated to *c'*, $r_{(26)}$ = -458, p = 0.014. Thus, the longer participants took to make *CR*s, the better their sensitivity, and the less conservative (i.e. less likely to make a "new"/more likely to make an "old" decisions under conditions of uncertainty) they were. For the deep encoding condition mean RT for *H*s was 1.12 (SD = 0.28) and for *CR* was 1.28 (SD = 0.40).

5.5 Discussion

Supporting the hypothesis, the result from Experiment 6 suggest that the DI is correlated to memory sensitivity, as individuals who attributed/directed a larger proportion of their looking time towards novel objects were also better able to differentiate old from new items in memory. However, contrarily to what was hypothesised, the correlations between DI and *d'* were not stronger under conditions of lower levels of encoding. As hypothesised, and explained by the VPC being a bias free task (see Section 5.2), the DI was not correlated to *c'*.

This serves not only to validate the manner in which the DI is interpreted and discussed in the numerous studies using the SOR task in rodents, but also supports our understanding of the role of novelty processing on memory, such as that outline by the novelty-encoding hypothesis (Tulving, Markowitsch, Craik, Habib & Houle, 1996). While obtained from differing tasks, the greater the preferential attribution of processing time to the novel item in the VPC by participants, the greater their memory performance in the Image Judgement Task. Although correlational rather than causational, and due to the between-task design, it is not possible to ascertain whether greater novelty preference in the VPC leads to better memory for those same stimuli subsequently. However, taken with the results from Manns and colleagues (2000) who demonstrated that novelty preference in the VPC was predictive of subsequent memory performance indicators for the same stimuli, these data suggests a relationship between novelty processing and subsequent memory performance.

Further supporting this influential contribution of novelty processing to recognition memory, participants sensitivity and bias appeared to be exclusively correlated to the reaction times for correct rejections, and not those for hits. As reaction times are considered to provide an indication of cognitive processing, this suggests that recognition memory sensitivity and bias are more closely associated to the cognitive processing of novelty rather than familiarity. Participants who spent longer

processing correctly identified novel items were better able to differentiate these from familiar items, and were less likely to endorse a "new" response under conditions of uncertainty. Although again correlational and thus not causational, these results suggest that the strength of memory evidence underlying recognition judgements, and the manner in which this is assessed, are driven by similar processes supporting novelty processing. Taken together with the positive correlation between DI and *d'* and the results from the experiments by Manns and colleagues (2000), these results suggest an important connection between novelty processing and recognition memory.

As expected due to the forced-choice nature of the task, the DI measure does not appear to capture the response bias component of recognition memory. Sensitivity alone is not fully representative of the recognition memory process (see Figure 1.1, page 8, in Section 1.2.2 for a representation of this). Indeed, response bias can be affected without a corresponding effect on sensitivity (e.g. Mill & O'Connor, 2014), such that participants adopt different strategies (e.g. preferring to mistakenly call new items olds than old items new) in the face of consistent memory evidence (see Section 1.2.2 for a discussion of sensitivity and bias). Hence, these result suggest that the rodent SOR only allows investigations into memory sensitivity rather than bias, which has significant implications for rodent studies investigating the neural correlates of recognition memory. Indeed, in using the SOR to determine the neural structures which support recognition memory, the current results suggest only memory sensitivity can be considered. To verify this claim, the correlations observed between DI and *d'* and the absence of these between DI and *c'* should be replicated using rats as subjects. Hence, Experiment 7 aimed to replicate the findings of Experiment 6 in rats. Here the use of a task allowing measures of sensitivity and bias in rats required. Such a task has been developed by Fortin and colleagues (2004), and will be outlined in detail in the Introduction to Experiment 7. However, it is important to note that as the SOR does not appear to capture the bias component of recognition, the use of this task should also be considered for its suitability in

allowing investigations of recognition memory bias in rats to further enable the translational research between the animal and human literatures.

Finally, while investigations of methodology for testing recognition memory were not the central aim of this study, the strong correlation between DI and sensitivity suggests the need for consideration of the VPC task as a tool for assessing recognition memory. The aspects of the VPC which make it a suitable analogue to the SOR, i.e. the innate nature of the response, the lack of a requirement for specific instructions and its relatively short duration (≈ 15 mins), are the principal reasons for its common use in infants (see Rose, Feldman & Jankowski, 2004s for a review). Indeed, these same traits sanction it as an appropriate method for testing recognition memory sensitivity for participants who are unable to provide keypress responses, or who have difficulty understanding or following instructions, such as patients or elderly participants. Indeed, previous research has demonstrated that individuals with mild cognitive impairments (MCI) at risk of developing dementia spent significantly less time than control participants fixating on the novel item in a VPC once a delay of 2 minutes was introduced between sample and test trials (Crutcher et al., 2009). Importantly, in a longitudinal study, the novelty preference in the VPC was shown predict a) the progression of MCI participants to more severe dementia, and b) the progression of normal controls to MCI up to three years before changes in clinical diagnosis were made using standard assessments (Zola, Manzanares, Clopton, Lah, & Levey, 2013). This suggests an important role for the VPC in the future of cognitive memory testing, where, as far as the author is aware, the relationship between the novelty preference in the VPC and the specific component of recognition memory sensitivity never having previously been established.

5.6 Introduction Experiment 7

Experiment 7 was devised to corroborate the finding in Experiment 6 using rats. Thus the aim was to investigate into the relationships between the discrimination index (DI) and sensitivity (*d'*) and bias (*c*) in rats. While the SOR is widely use in the animal literature and can be used to obtain a measure of DI, tasks paralleling single item recognition in humans allowing measures of sensitivity and bias to be calculated are less common in rats. Such a task was however developed by Fortin, Wright and Eichenbaum (2004). In this task, over the course of extensive training, rats are taught to dig in odours not previously seen that day for rewards, and to approach an empty cup for rewards for odours previously seen that day. This task enables the recording of correct recognition of old (*H*) and new (*CR*) odours, as well as errors in the recognition of both old (*M*) and new (*FA*) odours to be obtained. This task is thus used here to obtained measures of sensitivity (*d'*) and bias (*c*), calculated based on rats of *H* and *FA*s.

To closely parallel the widely used SOR task in the literature, for the current experiment, the SOR was run with 3D visual objects. However, given the challenging nature of the judgement task, this was undertaken using odour stimuli as rats are better able to discriminate these than visual stimuli (Nigrosh, Slotnick, & Nevin, 1975).

Under the same theoretical premise as Experiment 6 (see Section 5.2), it was hypothesised that DI would be positively correlated to *d'*, while showing no relationship to *c*.

5.7 Materials and Methods

5.7.1 Subjects

Six naïve male Lister-Hooded rats (Charles River, UK) weighing between 300 and 350g at the experiment commencement were housed in groups of three and kept on a 12-hour light/dark cycle. Behavioural training and testing took place during the light portion of the cycle. To allow for greater motivation for the rats on the tasks, their food access was controlled to maintain their weights at approximately 90% of their free-feeding weight. Rats had *ad libidum* access to water in their home cages. All procedures were carried out under the Project License numbers 70/8306 and 60/4069, and Personal License number 60/13883. All procedures were approved by the Animal Welfare Ethics Committee of the University of St Andrews, and complied with national (Animal [Scientific Procedures] Act, 1986) and international (European Communities Council Directive of 24 November 1986 [86/609/EEC]) legislation governing the maintenance of laboratory animals and their use in scientific research was ensured.

5.7.2 Apparatus

Odour judgement Task. All behavioural testing took place in a two-compartment plastic arena with 45cm opaque black walls and dark grey floors. The testing compartment measured 47cm by 48cm, with the holding compartment annexed to one end, measuring 47cm by 21cm (see Figure 5.7a). Small ceramic bowls with an internal diameter of 7cm and a depth of 4cm were used for the rats to dig in. Dry play sand was mixed with 40 ground household spices, herbs, flowers or berries to create odour stimuli (Table 5.2). The 40 odour stimuli were subdivided into two sets of 20 odours (Table 5.2), where rats were exposed to each set on alternative testing days to reduce possible interference. Chocolate Wheetos (Weetabix, Kettering, UK) were used as rewards throughout the experiment.

Novel Object Recognition Task. All behavioural testing took place in a wooden 67cm square arena with 40cm high grey patterned walls and a dark blue floor (see Figure

5.7b). Behaviour was monitored live and recorded using an HP HD 4310 webcam attached to a fixed wooden pole located centrally on the wall opposite object placement. All objects used were 3D easily cleanable household objects and toys of approximately the same size as a rat in one dimension. Objects were fixed to the floor using Dual Lock Velcro (3M2, St. Paul, MN).

Table 5.2: Table of the concentrations used to create odour stimuli, using household spices, herbs, flowers and berries.

Set	Odour	Concentration (g/100ml)	Set	Odour	Concentration (g/100ml)
A	Sumac	0.8	B	Cardamom	0.3
	Juniper	0.7		Cinnamon	0.4
	Coffee	0.4		Clove	0.2
	Passion Flower	1.0		Cocoa	1.2
	Mint	1.7		Coconut	4.8
	Dill	0.8		Coriander	1.2
	Onion Powder	0.4		Cumin	0.3
	Chamomile Flower	0.8		Eucalyptus	2.1
	Fenugreek	1.2		Fennel	1.5
	Oats	1.2		Garlic	0.5
	Parsley	1.4		Ginger	0.5
	Aniseed	0.8		Asafoetida	0.4
	Thyme	0.8		Hops	2.4
	Curry Leaf	1.8		Lovage	0.3
	Lemongrass	0.8		Mango	1.6
	Savory (summer)	3.6		Nutmeg	0.3
	Lavender	1.2		Orange	3.4
	Allspice	0.5		Paprika	0.6
	Celery	0.8		Rosebuds	1.3
	Schisandra Berries	1.0		Sage	1.8

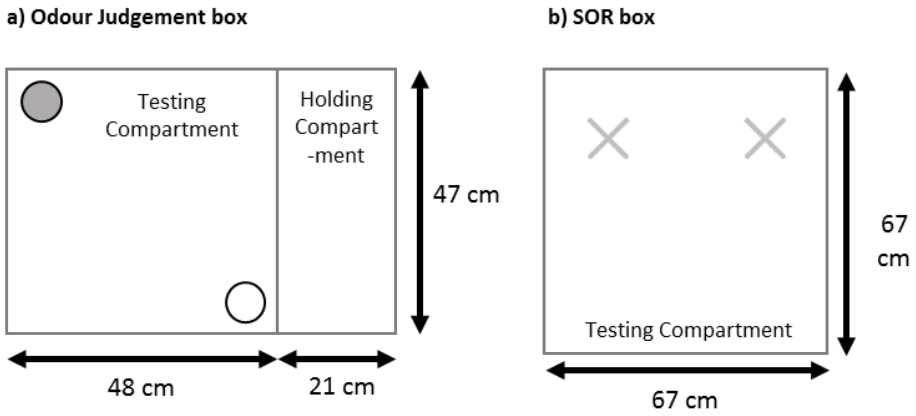

Figure 5.7: Schematics of the Testing Arenas used in Experiment 7, a) Odour Judgement Task and b) SOR Task, with walls omitted for clarity. Circles in a) represent ceramic bowls: shaded = sand/odour, unshaded = empty. Crosses in b) represent object locations.

5.7.3 Behavioural Testing Procedure

Rats were handled by the experimenter daily for five days prior to any behavioural testing or habituation. Behavioural testing for both the Odour Judgement Task and the Novel Objet Recognition task was undertaken in the same room. An overview of the sequence of behavioural testing procedures described in detail below is presented in Figure 5.8.

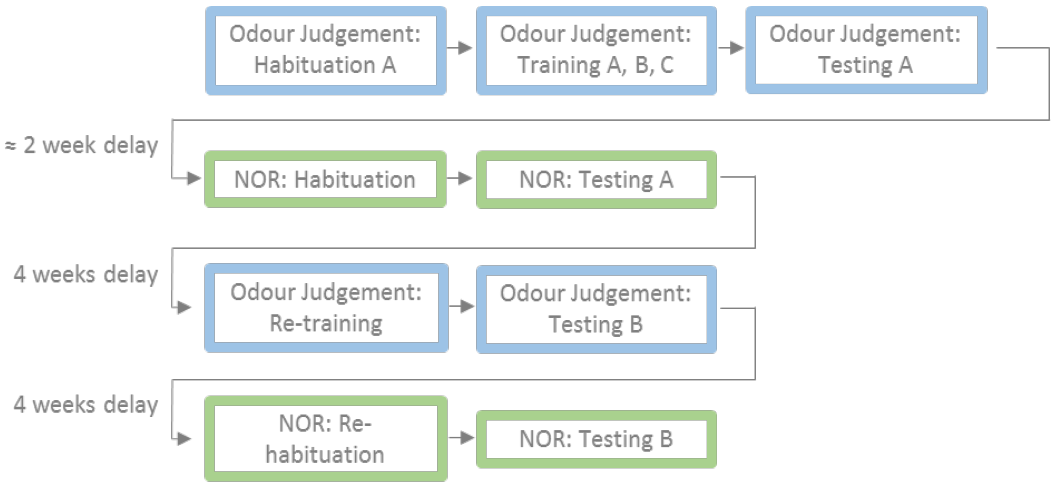

Figure 5.8: Schematic of the entire behavioural testing sequence for Experiment 7. All rats started SOR habituation on the same day, but as rats reached criterion for the different phases of the Odour Judgement Task on different days, the delay between the Odour Judgement Task and the SOR was different for each rat. The mean delay was 2 weeks, with the minimum being 1 week and the maximum 3.17 weeks.

5.7.3.1 Odour judgment Task

Animals were trained in successive stages, as outlined below.

Habituation to Digging

Rats had access to two bowls of odourless sand in their home cages for 2 days prior to habituation, and throughout the first phase of habituation (Habituation A). These bowls had Wheetos in them and were used to encourage rats to learn to dig in the sand bowls.

Habituation A

Habituation A served to habituate the rats to the testing and holding compartments of the apparatus. Rats were placed in home-cage groups in the testing compartment for 10 minutes, and subsequently in the holding compartment for 5 minutes for two sessions. This was repeated for a further two sessions where rats were placed in these individually. A single session was undertaken each day.

Habituation B

Habituation B served to habituate rats to retrieving rewards from an (odourless) sand-filled and an empty bowl presented in the testing environment (Figure 5.7). Both the sand bowl and the empty bowl contained a Wheeto. If rats retrieved both Wheetos this was considered a "trial". Rats were given 3 minutes to finish a trial. If both Wheetos had not been collected within 3 minutes, the rats were placed in the holding compartment for a brief delay and then placed back into the testing compartment for another opportunity to finish a trial. Rats were given either a maximum of 5 trials or 5 opportunities for trials per session. Sessions were repeated daily until rats performed 5 trials in less than 10 minutes.

Training A

Training A served to teach the rats an odour recognition rule. Sessions were now divided into blocks, each consisting of a study and a test phase (see Figure 5.9a). During all phases two bowls were always present in the testing environment: one

empty and one sand-filled. In a given study phase rats were presented with an odour bowl (scented sand, e.g. sage). This odour bowl was baited and rats were given 1.5 minutes to dig in the odour and retrieve the reward. When rats retrieved the reward or after 1.5 minutes, the rat was removed from the testing compartments and was placed in the holding compartment. If the rat had not retrieved the reward, this study phase was repeated. If the rat still did not retrieve the reward, then the trial was abandoned and the rat moved on to the study phase for the subsequent block.

Each test phase consisted of two components: one Target test trial where rats were presented with the same odour (old) as they had seen during the study phase (e.g. sage), and one Lure test trial where rats were presented with an odour they had not seen that day (new; e.g. cumin). The order of these two test trials was pseudorandom across blocks such that either old or new trials occurred first for no more than three consecutive blocks. New odours were baited and required the rat to dig to retrieve the reward, while old odours were not baited and required the rat to approach the empty bowl to be rewarded with two Wheetos delivered by the experimenter into the bowl. Rats' responses were coded as follows: Correct Rejections (*CR*; correctly digging in a new odour trial) Hits (*H*; correctly approaching the empty bowl in an old odour trial), False Alarams (*FA*; incorrectly approaching the empty bowl in a new odour trial), and finally Misses (*M*; incorrectly digging in an old odour). Apart from the first training A session where rats were allowed to self-correct after a *M*, rats were removed from the testing compartment and placed in the holding compartment after incorrect responses (i.e. a *FA* or *M*). Rats were given 2 minutes to make a response, after which if no response was made the rat was placed in the holding compartment and the next trial (study or test) was initiated.

Rats undertook 10 blocks in each daily session, with odours never repeating across blocks within a session (i.e. 20 odours were used for each session). This was repeated until rats reached a criterion of 80% correct on 15 out of 21 consecutive blocks (i.e. 80% on 5 out of 7 consecutive sessions). This criterion was chosen as trials were numerous and rats' motivation across all blocks in a given session was not

always maintained. As such, this criterion allowed rats to demonstrate having learnt the rule while allowing for days with lower motivation.

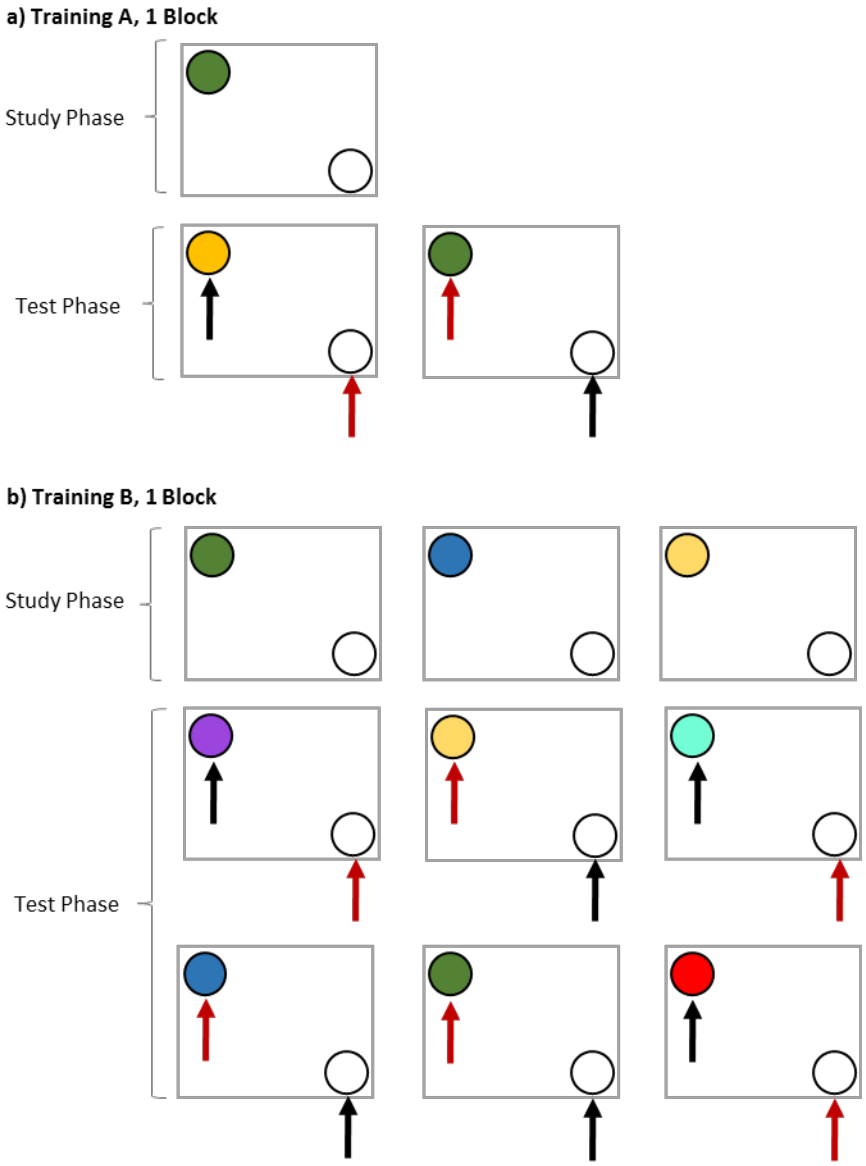

Figure 5.9: Schematic of the Odour Judgement Task. Example Study-Test blocks as used in a) Training Phase A, and b) Training Phase B. Circles represent ceramic bowls within the testing environment. White bowls are empty, and coloured bowl represent different odours. Red arrows represent incorrect responses/digging, and black arrows represent correct responses/digging. Training C, Testing A and Testing B were run in a similar manner as Training B, but the number of odours presented at study and test was increased.

Training B

Training B served to increase the difficulty of the task by increasing the number of odours presented at study and test. Again sessions were divided into blocks, each consisting of a study and a test phase (see Figure 5.9b). Two bowls were always present in the testing environment: one empty and one sand-filled. Form here onwards, to ensure rats were not simply digging in new odours because they could smell the Wheeto in it, at least 20% of new odours were un-baited and rats were rewarded with a Wheeto placed in the sand once they had already started digging. Furthermore, at least 20% of old odours were also baited, where if a rat started to dig the trial was terminated, i.e. the rat was placed in the holding compartment, before the rat could retrieve the reward.

Similar to Training Phase A, rats were presented with a baited odour bowl (e.g. sage), and rats were given 1.5 minutes to dig in the odour and retrieve the reward. However, when rats retrieved the reward or after 1.5 minutes, the odour bowl was removed and replaced with a new odour bowl (e.g. mint). Again rats were given 1.5 minutes to dig in the odour and retrieve the reward. This was repeated such that three different odours were presented during the study phase, after which the rat was placed in the holding compartment.

Test phases were run identically to Training Phase A test phases but were comprised of 6 test trials: 3 Target and 3 Lure trials. These were pseudorandomly ordered such that rats encountered no more than two consecutive Target or Lure test trials. Rats undertook 3 blocks per daily session with odours never repeating across blocks within a session (i.e. 18 odours were used for each session). If rats failed to respond for three consecutive trials, the block was terminated, responses for the remained of the block were recorded as incorrect, and the subsequent block was initiated. Daily sessions were undertaken until rats reached a criterion of 85% correct on 14 out of the last 15 blocks to ensure rats had learnt the rule.

Training C

Training C served to further increase the difficulty of the task. This was designed and run identically to Training B except that 5 odours were presented during a study phase and 10 (5 targets and 5 lures) were presented during a test phase. Test trials were pseudorandomly ordered such that rats encountered no more than 3 consecutive old or new test trials. Rats undertook 2 blocks per session (i.e. 20 odours were used for each session). This was repeated until rats reached a criterion of 80% correct on 10 consecutive blocks.

Testing A

Testing A served as critical days to test rats' memory performance on the final level of difficulty for the task. Measurements of errors in the form of Misses and False Alarms are required to obtain estimates of sensitivity (*d'*) and bias (*c*). Hence, following demonstration that the "dig in a new odour, don't dig in an old odour" rule was learnt following Training C, task difficulty was increased such that rats were more likely to make mistakes. Testing A was designed and run identically to Training B/Training C except that 10 odours were presented during a study phase and 20 (10 targets and 10 lures) were presented during a test phase. Test trials were pseudorandomly ordered such that rats encountered no more than 3 consecutive target or lure test trials. Rats undertook 1 block per session (i.e. 20 odours were used for each session). Rats memory performance was assessed across 6 sessions run on consecutive days.

Re- Training

After being undertaking to the SORtask, rats were re trained on the odour judgment task by undertaking identical training sessions as in Training B until they reached the pre-determined criterion of 85% correct across six block. Rats then completed Testing B.

Testing B

This was identical to Testing A but controlled for "noise" in the sand scent. As rats were digging in multiple odours sequentially, contamination from other odours was likely to occur through transfer, introducing "noise" to the odours. As substantial volumes of sand were required for the experiment, and hence new sand could not be used each day, this was controlled for by having two receptacles for a given odour. For each animal, bowls were filled from container 1 and emptied into container 2 after testing. At the end of the day, scented sand from container 1 and 2 was mixed and placed back into container 1. This ensured a consistent level of noise in the sand scent for each rat.

5.7.3.2 Novel Object Recognition Task

All SORsessions were run on consecutive days. Rats completed the first SOR task (SOR A) after the odour judgement task testing A.

Habituation

Rats were habituated to the testing arena during four sessions. During the first two sessions, rats were placed in the testing arena in home-cage groups for 10 minutes. The subsequent two sessions were identical except that rats were placed in the testing arena individually. Rats were then given four sessions of object habituation, where they were again placed in the testing arena for 10 minutes individually but this now contained two 3D objects. All objects used during habituation were not subsequently used for testing. Objects were always placed in the same location in the arena.

Testing A

Testing occurred over 4 sessions, one daily, with each session comprising a study and a test phase. Novel objects were used for each session. During the study phase two identical novel objects were present in the arena. Rats were placed into the arena facing the back wall. The study phase ended after 3 minutes or after the rat had

explored each object for a minimum of 15 seconds, whichever occurred first. Rats were removed from the testing arena and placed in a holding cage (≈ 1 minute). The testing arena was cleaned and a third identical copy of the object seen during the study phase (old) and a new object were placed in the testing arena. Rats were then returned to the testing arena for a further 3 minutes. The location of the novel object (left or right) was counterbalanced across animals.

After undertaking the second testing session of the odour judgement task, rats completed the second SOR task (SOR B).

Re-Habituation

Firstly, rats were given two repeat habituation sessions: during the first rats were placed individually in the empty testing arena for 10 minutes, and this was repeated during the second but with two novel objects also placed in the arena.

Testing B

The second round of SOR testing was undertaken in an identical manner to Testing A, and using the same object pairs, except that for each animal the memory status of the objects in a given pair were reversed: what was the old object during Testing A became the new object for the same object pair during Testing B. As rats have been shown not to show novel object preference for objects after delays of 24 hours, and SOR B was undertaken more than 9 weeks after SOR A, the assumption is that all objects appear novel to the rats even though these have been seen during SOR A. Thus, this was designed to reduce any object preference noise unintentionally introduced to the task.

Table 5.3 depicts the mean, minimum and maximum number of sessions (i.e. days) and trials taken to reach each training sessions' criterion performance.

Table 5.3: Behavioural Training sessions and trials required for rats to reach criterion for each training/test phase of the protocol.

	Sessions			Trials Attempted		
Phase	**Mean**	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**
Habituation A	4	NA	NA	NA	NA	NA
Habituation B	2.5	2	3	13.2	10	15
Training A	10.17	7	15	264.3	180	414
Training B	8.33	5	14	208.7	129	283
Training C	5.33	5	6	157	150	180
Test A	6	NA	NA	30	NA	NA
Re-training	2	2	2	18	18	18
Test B	6	NA	NA	30	NA	NA

5.7.4 Data Analysis

5.7.4.1 Behavioural data coding

All calculations were identical to those used in Experiment 6. For all data pertaining to the SOR, exploration durations for each rat for SOR A and SOR B for each day (i.e. same objects but different mnemonic status) were averaged. This combined data was then used in all further analyses. For the Discrimination Index (DI, Equation (8), page 104) exploration time rather than fixation time of the familiar ($T_{familiar}$) and the new (T_{new}) objects was used. Only behavioural data from Testing B of the odour judgement task was used in subsequent analyses.

Exploration behavior was coded using recorded videos and Observe software via keypress. All behavior was coded while blind to the experimental condition of the rats. To check for reliability, a subset of 10% of the videos coded by the experimenter were re-coded by a third party observer, and these scores were found to be consistently within 10% of the experimenter's. Rats were deemed to be exploring an object when the rat was facing the object with its nose less than 2cm away from the object. Moments in which the rat was touching the object with another part of the body, leaning on or rearing against the object in order to investigate the area above it was not scored as object exploration.

5.7.4.2 Statistical Analysis

As in Experiment 6, rats' object preference was compared to chance by submitting the DIs to a single sample t-test, with zero as the value of comparison.

Similarly to Experiment 6, of most interest in this experiment is the investigation of relationships between DI and *d'* and/or *c*. As such, Pearson's correlations were separately run between DI and *d'* and DI and *c*.

5.8 Results and Discussion

5.8.1 SOR: Analysis of Discrimination Index

As presented in Figure 5.10a, rats did not spend significantly longer exploring the novel (M = 38.34 secs, SD = 4.01) compared to the familiar (M = 42.76 secs, SD = 7.48) objects during the three minute test trial, as reflected by the DI not being significantly different to zero (M = 0.13; SD =0.13), as confirmed by a single-sample t-test, $t_{(5)} = 2.485$, $p = 0.055$, $d = 1.11$. As preferential exploration of the novel object decreases with time (Clark, Zola, Squire, 2000), rats exploration of objects for the first minute of the test trials were also analysed. In the first minute of the test trial, rats spent significantly longer exploring novel (M = 35.83 secs, SD = 6.11) compared to familiar (M = 16.33 secs, SD = 2.43) objects (Figure 5.10b), as reflected by the positive DI (M = 0.348, SD = 0.067), $t_{(5)} = 12.666$, $p < 0.001$, $d = 5.66$.

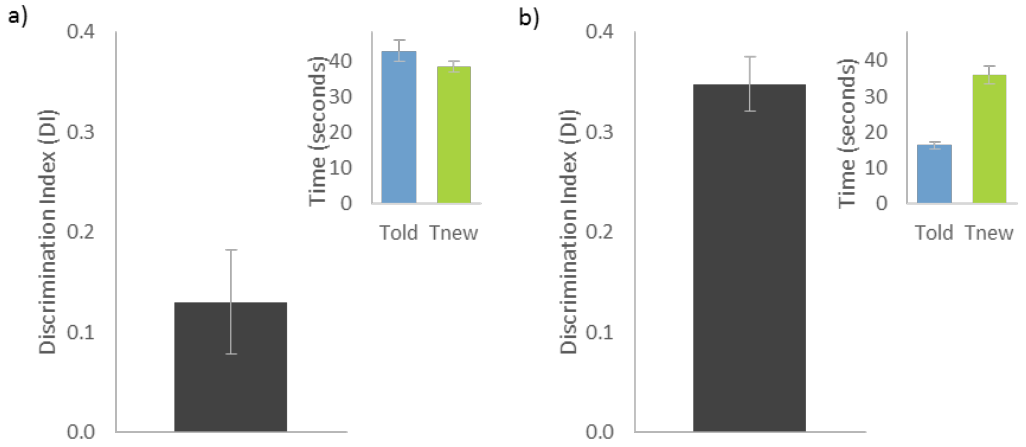

Figure 5.10: Mean Discrimination Index and exploration times for novel and familiar objects for a) the whole 3 minutes of a test trial and for b) the first minute of a test trial.

5.8.2 Odour Judgement Task: Analysis of Hits (*H'*) and Correct Rejections (*CR'*)

Although hit and correct rejection rates are not directly used in the correlations of interest within this experiment, these are outlined here as they form the basis for the calculations of sensitivity and bias, which are of primary interest within these correlations. Mean *H'* and *CR'* rates for odours tested in Testing B are presented in Table 5.4. Rats' performance on the task was high, as outlined by both *H'* and *CR'* rates of approximately 80% (*H'*: M = 0.80, SD = 0.06; *CR'*: M = 0.78, SD = 0.10).

5.8.3 Odour Judgement Task: Analysis of Sensitivity (*d'*) and bias (*c*)

The parameters of *d'* and *c* are of primary interest with respect to their correlations with DI for the first minute of the test trial. Mean *d'* and *c* are also presented in Table 5.4. As in Experiment 6, *c* was scaled to *d'* on a participant by participant basis, with adjusted *c* (*c'*) also presented in Table 5.4.

Table 5.4: Descriptive statistics for performance on the odour judgement task: adjusted Hits, Correct Rejections and estimates of sensitivity (d'), bias (c) and adjusted bias (c').

	H'	*CR'*	*d'*	*c*	*c'*
Mean	0.80	0.78	1.7	0.71	0.47
Standard Deviation	0.06	0.1	0.28	0.58	0.48

5.8.4 Combining the Odour Judgement Task and the Image Judgement Task results: Analysis of Relationships between Recognition Memory Measures

As in Experiment 6, the correlations between DI and *d'* and *c'* are of greatest interest for the purpose of this experiment. As the interest lies in rats' discrimination of novel and familiar object, the DI for the first minute of the test trial was used as an indication of this and therefore used in all subsequent correlation analysis. Scatter plots showing the relationship between DI and *d'* and DI and *c'* are presented below (Figure 5.11). With six data points (see Discussion below for the reasons for this), interpretation and analysis of relationships between these measures is highly tentative. Nevertheless, in the interest of completeness, this data was analysed in a way to mirror the data obtained in Experiment 6.

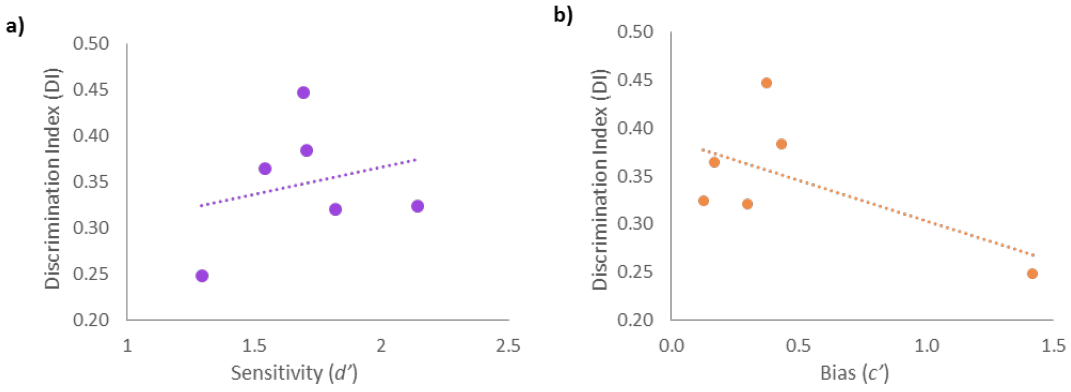

Figure 5.11: Scatterplot of the correlations between DI and measures of sensitivity and bias. a) DI and *d'* ($r_{(4)}$ = 0.245, p = 0.639) and between b) DI and *c'* ($r_{(4)}$ = - 0.602, p = 0.206) (n = 6).

Pearsons' correlations run on DI and *c'* and *d'* are presented in Figure 5.11. Unlike for Experiment 6, no significant correlation was seen between DI and *d'*, $r_{(4)} = 0.245$, $p = 0.639$ (Figure 5.11a). Although the scatter plot for DI and *c'* suggests that the data contains an outlier, this is difficult to ascertain given the sample size (Figure 5.11b). Pearson's correlations between DI and *c'* were also non-significant, whether or not this data point was included; $r_{(4)} = - 0.602$, $p = 0.206$; and $r_{(4)} = 0.597$, $p = 0.288$, respectively.

5.9 Discussion

The behavioural odour judgement task developed by Fortin and colleagues (2004) was found to elicit adequate hit and correct rejection rates for investigating the recognition memory parameters of sensitivity and bias in rats. Rats were able to learn the rule but task demands ensured rats were not performing at ceiling. Furthermore, rats level of sensitivity and bias in Experiment 7 were similar to those obtained in human participants in Experiment 6, suggesting that this task was well matched to, and thus provides a good analogue for, the human Image Judgement Task. However, the amount of training rats required to learn the rule was substantial. Indeed, while the intention was to run multiple cohorts of rats on this task to increase the sample size, the time requirements for training prohibited this. The advantages of the SOR in terms of the lack of a requirement for rewards, the lack of rule learning, and the relative rapidity and simplicity of running the task, are lost in the odour judgment task. As such, while this task is of significant merit in enabling a measure of recognition bias to be obtained from rats, and potential manipulations of, or neural structures supporting this to be investigated, its regular use in testing recognition memory in rats is limited.

Due to the small sample size in the current experiment, the correlations outlined between DI and sensitivity and DI and bias are inconclusive. It is not possible to discern relationships between the recognition memory measures of interest. Based

on the finding from Experiment 6 in humans, and on the understanding that the SOR is a bias-free forced-choice task, the DI is expected to correlate to sensitivity but not to bias, however this cannot be ascertained here: the data can be used neither to support nor reject this hypothesis. While further repetitions of the current experiment are encouraged to allow Experiment 6 to be replicated in the rodent literature, assuming the VPC is accepted as a satisfactory analogue to the SOR, it is proposed here that the findings from Experiment 6 alone suitably demonstrate the relationship between the DI and sensitivity and bias.

While the human and rodent tasks were well paralleled, one difference between these is worth considering. The stimuli used for the VPC and the Image Judgement Task in the human experiment were all sampled from a set of similar stimuli: a mixture of Pokémon and Digimon characters. In comparison, the rats were presented with 3D visual objects during the SOR task and odours in the Odour Judgement Task. Given that different stimuli are differently memorable, and therefore lend themselves to differing levels of sensitivity (see Experiments 2 & 3, Chapter 3), a hypothetical absence of a relationship between sensitivity derived from an odour recognition task and novelty preference from a visual task may be falsely representative of differences generated by the stimuli rather than a genuine lack of relationship between these. However, assuming no floor or ceiling effects, an individual's recognition memory sensitivity/ability is considered to be stable and hence should be scaled by the memorability of stimuli rather than being completely dependent upon it. If this were the case, individual's recognition memory sensitivity for differing stimuli would be highly correlated and these should therefore not impact further correlations between sensitivity and other factors. These suggestions should be investigated empirically, where Experiment 6 suggests the use of the VPC and single item recognition tasks in humans are an effective way to achieve this.

5.10 Conclusion

The aim of the presented experimental chapter was to evaluate the relationship between novelty preference as a recognition memory measure obtained in the rodent literature, and measures of recognition memory sensitivity and bias typically obtained in the human literature. Novelty preference obtained from eye-tracking was found to be positively correlated to memory sensitivity in humans, while not being related to bias. This was attempted to be replicated in rats in Experiment 7. An Odour Judgement Task was found to elicit behaviour which enabled adequate measurements of sensitivity and bias in rats to be obtained. However, the training demands to establish this behaviour were such that sample size was limited and the results from Experiment 6 could neither be corroborated nor refuted.

6. CHAPTER 6: GENERAL DISCUSSION

6.1 Summary of Thesis

The aim of the current thesis was to integrate experimental methodologies across human and rodents to further investigate novelty processing at both a cognitive and neural level, and assess whether it is dissociable from familiarity processing. This aim was driven by the conflict between the assumption in the literature that novelty and familiarity are words referring to the same recognition memory process (present both in the methodologies used to test recognition memory (see Section 1.2.1), and in the theoretical understanding of recognition memory (see Section 1.2.2)), and empirical findings which have suggested differently (e.g. Albasser et al., 2010; Kumaran & Maguire, 2007; Xiang & Brown, 1998), see Section 1.5). Thus, the experimental approach outlined in chapters 2 – 4 was based upon the theoretically driven hypothesis that if novelty and familiarity are simply words ascribed to different directionalities of the same, single, memory strength continuum, then novelty and familiarity processing should be equally and oppositely affected by experimental manipulations. Furthermore, to better assimilate the findings from these experimental chapters, and the human and animal recognition memory literature more broadly, the relationship between measures of recognition memory typically obtained from animal and human research was investigated empirically in Chapter 5. The key findings from these experiments are presented below, and their implications for the methodological and theoretical investigations of recognition memory are discussed in sections 6.2 and 6.3.

A simple initial investigation into whether novelty and familiarity are dissociable processes is to ascertain whether participants can/do differentially assess these. Mill & O'Connor (2014) demonstrate that participants assessed their memory differently as a result of test question, such that a "new?" test question lead to a more liberal bias (more likely to endorse an old response) whereas an "old?" test question lead to a more conservative bias (less likely to

endorse an old response). Assuming a single process contributes to memory strength evidence, amendments in bias should occur uniformly across the spectrum of memory strength (see Section 2.1 for a detailed discussion of this). However, if differing sources contribute to memory strength then they may be differentially interrogated to enable differential assessments of memory strength based on these (see Section 2.1 for a detailed discussion of this). Thus, whether shifts in bias for high, medium and low old and new confidence level judgements as a consequence of test question were uniform was investigated in Experiment 1. Participants rated either the level of familiarity or the level of novelty of word stimuli in a single item recognition task, and their recognition memory performance was compared across these two conditions. With recollection accounted for, an interaction was observed between participants' bias for making high, medium and low confidence judgments and whether they were rating familiarity or novelty. This interaction suggested that participants were differentially assessing their memory when novelty or familiarity was emphasised, where this appeared to be most prominent for high confidence new judgements. It is argued in Chapter 2 that this supports the notion that memory strength evidence is gained by more than one process, where these are considered to reflect familiarity and novelty.

Experiments 2 and 3 followed on from this by investigating whether concurrent, and thus conflicting, novelty and familiarity processing caused interference, and whether this interference was quantitatively equal and opposite. This design was established as it was considered to parallel well with the SOR recognition memory paradigm used in rodents, in which two items of conflicting mnemonic statuses are presented at test (Ennaceur & Delacour, 1988). When taken together the results from Experiment 2 and 3 were unclear. In Experiment 2, participants' identification of novel items as such was interfered with by the mnemonic status of a concurrent item, leading to changes in memory sensitivity, while no such effect was observed for their identification of familiar items. Similarly to Experiment 1, this suggests a dissociation between novelty and familiarity processing. However, this was not replicated in Experiment 3 when the memorability of stimuli was improved. Nevertheless, while the effect of the mnemonic status of a concurrent item did not affect correct identification of either old or new items in Experiment 3, the presence of a

concurrent item did differentially affect correct identification of novel and familiar items. Identification of old items was not impacted by the presence of a concurrent item, while identification of a novel item was aided by the presence of a concurrent highly familiar item compared to when this was presented alone. Thus, while the different stimuli used in these two experiment significantly affected participant's recognition performance and the patterns in the data, both experiments demonstrate differential interference for novelty and familiarity detection. Thus, in line with Experiment 1, the results from Experiment 2 and 3 provide evidence suggesting that novelty and familiarity are dissociable. The differing patterns of result however did not allow insight into the characterisation of the differences between these processes.

Having established evidence for the dissociation of novelty and familiarity processing at a cognitive level, this dissociation was subsequently investigated at a neural level in Chapter 4. This investigation was based upon the previously established significant role that the perirhinal cortex plays in item recognition (see Section 1.4 for a review). Indeed, high activity in the perirhinal cortex has been shown for novel items, with this activity decreasing as items become more familiar over time or due to the number of exposures (e.g. Roloff et al., 2016; Xiang & Brown, 1998). If the activity of the perirhinal cortex reflects familiarity processing, and familiarity and novelty are words ascribed to a same neural process, concurrently presenting a familiar item alongside a novel item should disrupt the increased perirhinal activity seen for a novel item. Hence, this was tested using the same theoretical premise as, and a similar methodology to, that employed in humans in Experiments 2 and 3. Differing groups of rats were presented with two items of competing mnemonic status (i.e. novel and familiar) in a standard spontaneous-object-recognition (SOR) task. The level of familiarity of the familiar item was manipulated across groups such that impact of the level of familiarity of the familiar item on the neural response, as indexed by *c-fos* expression, to the novel item was investigated. Furthermore, Albasser and colleagues (2010) have identified overlapping but distinct networks for the processing of novelty and familiarity. Thus, the effect presenting familiar items of differing memory strengths concurrently with a novel item was also investigated with regards to novelty and familiarity processing at a network level, using the networks identified by Albasser and colleagues (2010).

Surprisingly, in both Experiments 4 and 5, although animals displayed a novel item preference, exposure to a novel object in the SOR task did not lead to greater perirhinal cortex activity. Roloff and colleagues (2016) argue that the failure to find differences in the neural responses to novel and familiar items following an SOR task reflects a lack in the sensitivity as a consequence of the singe trial task used. Indeed, in a within-subject experiment using single unit recordings in perirhinal cortex neurons of rats, neurons with differences in their recognition-related neural responses were found during a visual-paired comparison tasks in which large numbers of stimuli were presented, but not in an SOR task in which the rat undertook a single trial with two object stimuli (see Section 1.4.2). Furthermore, recent research has suggested that novelty/familiarity processing in the perirhinal cortex is dependent upon firing frequency (Ho et al., 2015), with stimulation of the perirhinal cortex with frequencies of 30-40Hz causing rats to treat familiar images as novel, while frequencies of 10-15Hz causing rats to treat novel images as familiar. As both of these frequency ranges will cause action potentials and therefore IEG induction (Chaudhuri, 1997), unless a significantly different number of neurons are responding, these activities would appear identical using *c-fos* as a marker for neural activity. When taken together, this evidence suggests that the use of *c-fos* expression to investigate neural responses to novel and familiar items is not sensitive enough when paired with a behavioural methodology/manipulation consisting of a single tiral SOR, where *c-fos* may be used under methodological conditions in which a greater number of trials or stimuli is likely to drive the novelty response (Albasser et al., 2010), increasing the number of neurons responding to this (Roloff, Muller & Brown 2016). Hence, the sensitivity of the tools used to assess novelty and familiarity require consideration for future research within this field.

Furthermore, the neural networks outlined by Albasser and colleagues (2010) were overall ill-suited to represent the data obtained from the experimental groups in Experiment 5 of this thesis, in which the level of conflict between the novel and familiar item was highest (i.e. a novel item paired with either a highly familiar or a moderately familiar item). The data pertaining to the control group where rats were presented with two highly familiar items was well represented by the familiarity network established by Albasser and colleagues (2010). Furthermore, the data pertaining to the experimental group in which the rats were

presented with the least amount of familiarity (i.e. a novel and a low familiarity item) was well represented by both the novelty and the familiarity networks outlined by Albasser and colleagues (2010). Hence, the mnemonic status of the concurrent item did not uniformly or systematically affect the processing of the novel item, and thus the quality and extent of this disruption remains unclear. Of importance, the level of familiarity of the item concurrently presented with the novel item had no behavioural effect: exploration durations for novel and familiar items were equivalent regardless of the level of familiarity of the familiar item. The outstanding implications of this finding is discussed below (Section 6.3), in conjunction with findings from Experiment 6.

While a similar paradigm, in which a novel and a familiar item were presented concurrently, was employed to test recognition memory in both humans (Experiment 2 and 3) and rats (Experiments 4 and 5), an important consideration became apparent: were the same components of recognition memory being tested in these species? More specifically, does the novelty preference in the SOR capture both the component of sensitivity and bias known to contribute to human recognition memory? This was tested in humans (Experiment 6) and rats (Experiment 7). For human participants, the novelty preference in an eye-tracked visual-paired-comparison (VPC) task was considered an analogue for the novelty preference in the rodent SOR, and was found to positively correlate with measures of sensitivity, but have no relationship to measures of bias, as obtained on a standard single-item recognition task. Rats were trained on a single-item odour recognition task such that measures of recognition memory sensitivity and bias could be obtained and compared to novelty preference as obtained from a standard SOR procedure. While the rats were able to perform the single-item recognition task, the training requirement to establish this behaviour prevented more than six rats to be tested. As such no correlations were shown between the novelty preferences in the SOR and either sensitivity or bias. However, it is anticipated that with a larger sample size, the results in a rodent version of the experiment would parallel those found in Experiment 6 using human participants. The results from Experiment 6 validate the manner in which SOR is considered and discussed in the rodent literature. Interestingly, during the single-item-recognition task in humans, participants' memory sensitivity was correlated to their reaction times for correct rejections but not for

hits. Again the implication of this finding will be discussed in more detail below (Section 6.2).

6.2 Methodological Implications

One of the aims of this thesis was to better bridge and integrate the experimental methodologies used to test recognition memory in humans and rodents. This was directly addressed in Chapter 5 where it was established that the novelty preference measures obtained from the VPC as an analogue for the SOR are related to an individuals' memory sensitivity rather than bias, as estimated based on an equal variance signal detection model of recognition memory. Thus, the greater the novelty preference an individual displays, the greater their ability to discriminate old from new items in recognition memory. This is in keeping with the novelty-encoding hypothesis (see Section 1.1), which advances that novel items are allocated more cognitive processing such that these are encoded for better subsequent retrieval. Importantly, the relationship between novelty preference in the VPC task and recognition memory sensitivity in Experiment 6 is not causal, as recognition memory was not tested using the same exact stimuli as those presented in the VPC (see Section 5.1 for a discussion of the purpose of this). However, the hypothesis based on the novelty-encoding hypothesis would be that participants spending longer fixating upon novel items also encode them to a superior extent, and therefore have high recognition memory sensitivity. The lack of a relationship between novelty preference and bias is unsurprising as forced-choice tasks, in which participants are asked to identify the old (or new) item from a pair are considered bias-free (see Section 5.2). Participants do not have to identify the items as novel or familiar per se, but rather can identify the item which is relatively the oldest (or newest).

These findings validate the manner in which the SOR is discussed in the animal literature, such that deficits seen are considered to reflect primary memory impairments in the ability to discern novel from familiar items. Consequentially however, these findings reflect that the component of bias is unaccounted for in a significant portion of the animal literature. However, measures of bias were obtained from participants presented with two concurrent

objects in Experiments 2 and 3. Hence, for theoretical considerations concerning bias, the SOR could be amended to require responses from rats, such as knocking over a novel object but not a familiar one, for a target item presented alongside a concurrent item. Furthermore, where resources for extended training are available, a single item odour judgment task, as developed by Fortin and colleagues (2004) and implemented in Experiment 7, may be employed to obtain measures of sensitivity and bias in rats. The results from the human and rat analogues for the single item recognition and the SOR paradigm are well matched, and suggest these are tools of potential value for translational research.

Finally, although not questioning the methodological difference in human and animal recognition research directly, results obtained from the presentation of two items of varying mnemonic statuses used to test recognition memory in humans in Experiment 2 and 3, and in rats in Experiments 5 suggests some considerations for this manner of presenting stimuli. Indeed, the SOR is based upon this construct of presenting two items of differing mnemonic statuses at test, and is widely used in the animal recognition memory literature (see Section 1.2.1). However, when recognition memory was directly tested in human participants for an item paired with an irrelevant concurrent item, the presence and mnemonic status of this concurrent item interfered with recognition memory for novel items. This occurred differently for differing stimuli, and the results from Experiments 2 and 3 do not allow the nature of this interference to be outlined. Similarly, the manner in which engagement of the novelty and familiarity networks differed, but not systematically, between the experimental groups in Experiment 5 also suggests interference between the items concurrently presented. This is an important consideration when testing recognition memory in animals, where a deficit on the standard SOR but normal exploration of novel and familiar items when these are presented in mnemonically equivalent pairs (i.e. two novel or two familiar items; (Lisa Kinnavane et al., 2015; McTighe et al., 2010; Olarte-Sánchez et al., 2015)), may reflect an inability to resolve this interference.

6.3 Theoretical Implications

While not providing a cohesive understanding of the specificities of the novelty and familiarity processes, the findings from Experiments 1 – 5 all suggest discrepancies in how novelty and familiarity are processed. Thus, in line with findings from single unit recordings (e.g. Xiang & Brown, 1998), from fMRI experiments (e.g. Kumaran & Maguire, 2007), from analysis of neural networks (e.g. Aggleton et al., 2010), and from experiments using aged populations (e.g. Burke et al., 2011), the current findings challenge the assumption that novelty and familiarity are words pertaining to the same process, rather suggesting that these are differentiable. As outlined in Sections 1.2 of this thesis, this assumption pervades both the methodological constructs used to test recognition memory in animals and the theoretical models underlying the current understanding of recognition memory. The findings from the current thesis do not allow for characterisation of the differences between novelty and familiarity processing, although such differentiation at a neural level has been proposed in the literature (Roloff et al., 2016; Xiang & Brown, 1998).

Of significant interest in the current thesis are the findings from multiple experiments specifically highlighting novelty. In Experiment 1, the interaction between participants' bias and the test question was most apparent for judgements of high and medium confidence novelty. In Experiments 2 and 3 interference of concurrent items was present for the identification of novel but not familiar items. Furthermore, in Experiments 4 and 5 the presence of the novel item but not the familiarity of the concurrent item appeared to be driving rat's behavioural response in the SOR, while participants' recognition sensitivity was correlated with their reaction times for processing novel but not familiar items in Experiment 6. These effects specific to novelty occurring for differing methodologies and across different species suggest an important role for novelty processing specifically in recognition memory research. Indeed, while not all questions have been answered by the experiments reported in this thesis and some of the results provided by the same experiments are difficult to interpret, this combination of results suggests that novelty is processed in a differing way to familiarity and is both more susceptible to interference (Experiments 1, 2 and 3) and more highly related to behaviour (Experiments 4, 5 and 6) than

familiarity processing. To truly argue for separable novelty and familiarity processes a double dissociation between these is required. Historically, initial evidence towards a dissociation of processes emerges from clinical case studies where specific impairments are recoded in either process. Currently, such clinical case studies are limited with regards to selective impairments informing our understanding of novelty and familiarity processing. Indeed, as outlined in Section 1.4.1, a single case study provides evidence for a selective familiarity impairment (NB; Bowles et al., 2007; 2011), although this is framed in terms of a differentiation from recollection, where NB's responses to novel items was not specifically evaluated. Furthermore, to the author's knowledge, no case studies of specific novelty assessment deficits are recorded. Patients with deja-vecu (a chronic form of deja-vu) are reported to experience heightened levels of the feeling of familiarity for occasions which are truly novel (such as a friend's funeral; O'connor, Lever & Moulin, 2010), suggesting interference of novelty and familiarity processes, but not providing a dissociation of these. Without a double dissociation of either the cognitive processing or the neural processing of novelty and familiarity then these cannot be identified as unequivocally separable.

However, the evidence from the empirical chapters within this thesis highlight the need to question our assumption with regards to the processes involved in recognition memory. Indeed, the findings disputing the assumption that novelty and familiarity are a single process, and suggest a particularly influential role both of, and on, novelty processing in recognition memory. Thus, it is argued that that future research should investigates the differences between, and characteristics of, novelty and familiarity processing. The initial requirement for such research is a clearer definition of what is understood by novelty and familiarity. This is important at all levels of analysis, where greater effort should be made to identify definitions and conceptualisation of these processes which transcend levels of analysis and research in differing species. Indeed, when taking a larger frame of view it is acknowledged that, further research and subsequent data obtained which is difficult to interpret within the current framework of recognition memory processes (such as that from Experiments 2 and 3 of this thesis) may identify that it is not simply the addition of a process such as novelty within our understanding of recognition memory that is required, but rather that the current conceptualisation, definition and assumptions of recognition may require

consideration to allow for these data. Without the inclusion of novelty assessment in our understanding of recognition memory and the manner in which this is experimentally tested, a significant component of this crucial cognitive function will remain unaccounted for.

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APPENDIX A: Ethical Approval for Experiment 1

HUMAN EXP 1

University of St Andrews

University Teaching and Research Ethics Committee

20 March 2013

Ethics Reference No: *Please quote this ref on all correspondence*	PS9670
Project Title:	Effects of decision framing on a word judgement task
Researchers' Names:	Ifigagli Pit and Dr Akira O'Connor
Supervisor:	Dr Akira O'Connor

Thank you for submitting your application which was considered at the Psychology & Neuroscience School Ethics Committee meeting on the 27th February 2013. The following documents were reviewed:

1.	Ethical Application Form	08/03/2013
2.	Advertisement	08/03/2013
3.	Participant Information Sheet	08/03/2013
4.	Consent Form	08/03/2013
5.	Debriefing Form	08/03/2013
6.	Sample Test Materials	08/03/2013

The University Teaching and Research Ethics Committee (UTREC) approves this study from an ethical point of view. Please note that where approval is given by a School Ethics Committee that committee is part of UTREC and is delegated to act for UTREC.

Approval is given for three years. Projects which have not commenced within two years of original approval must be re-submitted to your School Ethics Committee.

You must inform your School Ethics Committee when the research has been completed. If you are unable to complete your research within the three year validation period, you will be required to write to your School Ethics Committee and to UTREC (where approval was given by UTREC) to request an extension or you will need to re-apply.

Any serious adverse events or significant changes which occur in connection with this study, and/or which may alter its ethical consideration, must be reported immediately to the School Ethics Committee and an Ethical Amendment Form submitted where appropriate.

Approval is given on the understanding that the 'Guidelines for Ethical Research Practice' (http://www.st-andrews.ac.uk/media/UTREC/guidelines%20Feb%2008.pdf) are adhered to.

Yours sincerely

Convenor of the School Ethics Committee

Cc: Dr A O'Connor (Supervisor)
School Ethics Committee

UTREC Co-ordinator, Maisie Reid, 3 St Mary's Place, St Andrews, KY16 9UY
Email: utrec@st-andrews.ac.uk Tel: 01334 462866

APPENDIX B: Ethical Approval for Experiment 2

University of St Andrews

University Teaching and Research Ethics Committee
Sub-committee

7 February 2014

Ethics Reference No: *Please quote this ref on all correspondence*	PS10773
Project Title:	Memory Experiment
Researcher's Name:	Magali Pitt
Supervisor:	Dr Akira O'Connor

Thank you for submitting your application which was considered at the Psychology & Neuroscience School Ethics Committee meeting on the 29th of January 2014. The following documents were reviewed:

1. Ethical Application Form 05/02/14
2. Participant Information Sheet 05/02/14
3. Consent Form 05/02/14
4. Debriefing Form 05/02/14
5. Data Management Plan 05/02/14

The University Teaching and Research Ethics Committee (UTREC) approves this study from an ethical point of view. Please note that where approval is given by a School Ethics Committee that committee is part of UTREC and is delegated to act for UTREC.

Approval is given for three years. Projects, which have not commenced within two years of original approval, must be re-submitted to your School Ethics Committee.

You must inform your School Ethics Committee when the research has been completed. If you are unable to complete your research within the 3 three year validation period, you will be required to write to your School Ethics Committee and to UTREC (where approval was given by UTREC) to request an extension or you will need to re-apply.

Any serious adverse events or significant change which occurs in connection with this study and/or which may alter its ethical consideration, must be reported immediately to the School Ethics Committee, and an Ethical Amendment Form submitted where appropriate.

Approval is given on the understanding that the 'Guidelines for Ethical Research Practice' https://www.st-andrews.ac.uk/utrec/guidelines/ are adhered to.

Yours sincerely

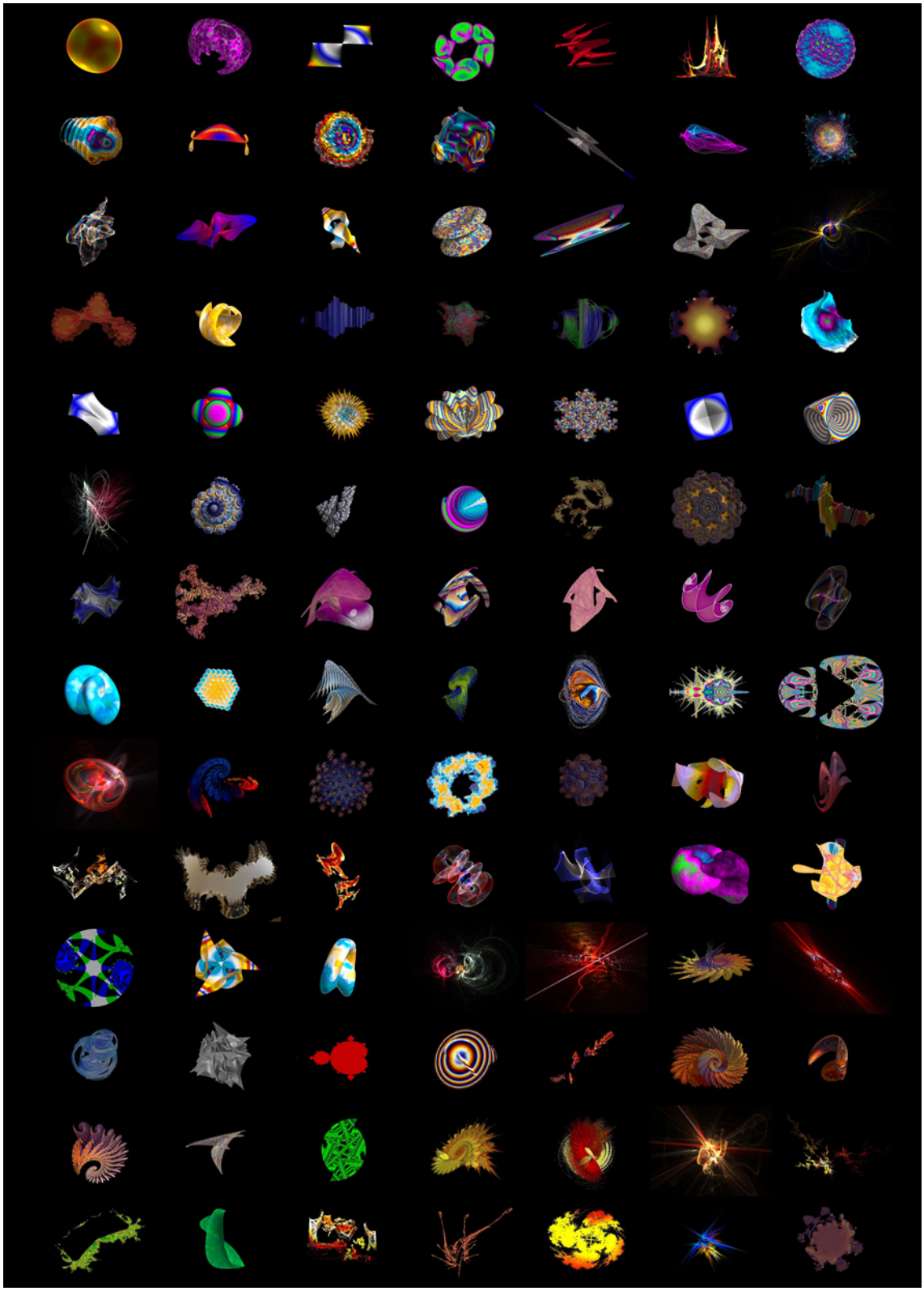
pp

Convenor of the School Ethics Committee

Cc: Dr Akira O'Connor *(Supervisor)*
School Ethics Committee

School of Psychology & Neuroscience, St Mary's Quad, South Street, St Andrews, Fife KY16 9JP
Email: psyethics@st-andrews.ac.uk Tel: 01334 462071

APPENDIX C: Fractal Stimuli Developed for Experiment 2



APPENDIX C continued: Fractal Stimuli Developed for Experiment 2

APPENDIX D: Ethical Approval for Experiment 3

University of St Andrews
from first to foremost

600 YEARS
1413 – 2013

Project Title	Memory Experiment
Researcher's Name	Magali Sivakumaran
Supervisor	Dr Akira O'Connor
Department/Unit	School of Psychology & Neuroscience
Ethical Approval Code (Approval allocated to Original Application)	PS10773
Original Application Approval Date	05 February 2014
Amendment Application Approval	29 October 2014

Ethical Amendment Approval

Thank you for submitting your amendment application which was considered at the Psychology & Neuroscience School Ethics Committee meeting on the 21st October 2014. The following documents were reviewed:

1.	Ethical Amendment Application Form	29/10/2014
2.	Advertisements	29/10/2014
3.	Participant Information Sheet	29/10/2014
4.	Consent Form	29/10/2014
5.	Debriefing Form	29/10/2014
6.	Example of stimuli	29/10/2014
7.	Questionnaire	29/10/2014

The University Teaching and Research Ethics Committee (UTREC) approves this study from an ethical point of view. Please note that where approval is given by a School Ethics Committee that committee is part of UTREC and is delegated to act for UTREC.

Approval is given for three years from the original application only. Ethical Amendments do not extend this period but give permission to an amendment to the original approval research proposal only. If you are unable to complete your research within the original 3 three year validation period, you will be required to write to your School Ethics Committee and to UTREC (where approval was given by UTREC) to request an extension or you will need to re-apply. You must inform your School Ethics Committee when the research has been completed.

Any serious adverse events or significant change which occurs in connection with this study and/or which may alter its ethical consideration, must be reported immediately to the School Ethics Committee, and an Ethical Amendment Form submitted where appropriate.

Approval is given on the understanding that the 'Guidelines for Ethical Research Practice' (http://www.st-andrews.ac.uk/media/UTRECguidelines%20Feb%2008.pdf) are adhered to.

Yours sincerely

Convenor of the School Ethics Committee

Cc: Dr A O'Connor (Supervisor)
School Ethics Committee

School of Psychology & Neuroscience, St Mary's Quad, South Street, St Andrews, Fife KY16 9JP
Email: psyethics@st-andrews.ac.uk Tel: 01334 462071

APPENDIX E: Recipe for Antifreeze Used in Experiment 4

Solution:

30% sucrose

30% Ethylene Glycol in 0.1 MSPB

Recipe:

300g sugar

500ml 0.2 PBS

300ml Ethylene Glycol

APPENDIX F: Ethical Approval for Experiment 6

University of St Andrews | FOUNDED 1413

University Teaching and Research Ethics Committee

22 December 2015

Dear Magali

Thank you for submitting your ethical application which was considered at the School of Psychology & Neuroscience Ethics Committee meeting on 15th December 2015; the following documents have been reviewed:

1. Ethical Application Form
2. Experimental Protocol
3. Advertisement
4. Participant Information Sheet
5. Consent Form
6. Debriefing Form
7. Data Management Plan

The School of Psychology & Neuroscience Ethics Committee has been delegated to act on behalf of the University Teaching and Research Ethics Committee (UTREC) and has granted this application ethical approval. The particulars relating to the approved project are as follows -

Approval Code:	PS11888	**Approved on:**	18/12/2015	**Approval Expiry:**	18/12/2020
Project Title:	Visual eye-tracking and word judgement				
Researcher:	Magali H. Sivakumaran				
Supervisor:	Dr Akira O'Connor				

Approval is awarded for five years. Projects which have not commenced within two years of approval must be re-submitted for review by your School Ethics Committee. If you are unable to complete your research within the five year approval period, you are required to write to your School Ethics Committee Convener to request a discretionary extension of no greater than 6 months or to re-apply if directed to do so, and you should inform your School Ethics Committee when your project reaches completion.

If you make any changes to the project outlined in your approved ethical application form, you should inform your supervisor and seek advice on the ethical implications of those changes from the School Ethics Convener who may advise you to complete and submit an ethical amendment form for review.

Any adverse incident which occurs during the course of conducting your research must be reported immediately to the School Ethics Committee who will advise you on the appropriate action to be taken.

Approval is given on the understanding that you conduct your research as outlined in your application and in compliance with UTREC Guidelines and Policies (http://www.st-andrews.ac.uk/utrec/guidelinespolicies/). You are also advised to ensure that you procure and handle your research data within the provisions of the Data Provision Act 1998 and in accordance with any conditions of funding incumbent upon you.

Yours sincerely

Convener of the School Ethics Committee

cc Dr Akira O'Connor (Supervisor)

School of Psychology & Neuroscience, St Mary's Quad, South Street, St Andrews, Fife KY16 9JP
Email: psyethics@st-andrews.ac.uk Tel: 01334 462071

APPENDIX F continued: Ethical Amendment for Experiment 6

University of St Andrews | FOUNDED 1413

University Teaching and Research Ethics Committee

24 June 2016

Dear Magali

Thank you for submitting your amendment application which comprised the following documents:

1. Ethical Amendment Application Form
2. Advertisement
3. Participant Information Sheet
4. Participant Consent Form
5. Participant Debriefing Form
6. Examples of stimuli
7. Questionnaire

The School of Psychology & Neuroscience Ethics Committee is delegated to act on behalf of the University Teaching and Research Ethics Committee (UTREC) and has approved this ethical amendment application. The particulars of this approval are as follows –

Original Approval Code:	PS11888	**Approved on:**	18/12/2015
Amendment Approval Date:	23/06/2016	**Approval Expiry Date:**	18/12/2020
Project Title:	Visual eye-tracking and image judgement		
Researchers:	Magali H. Sivakumaran and Imogen Callan		
Supervisor:	Dr Akira O'Connor		

Ethical amendment approval does not extend the originally granted approval period of five years, rather it validates the changes you have made to the originally approved ethical application. If you are unable to complete your research within the original five year validation period, you are required to write to your School Ethics Committee Convener to request a discretionary extension of no greater than 6 months or to re-apply if directed to do so, and you should inform your School Ethics Committee when your project reaches completion.

Any serious adverse events or significant change which occurs in connection with this study and/or which may alter its ethical consideration, must be reported immediately to the School Ethics Committee, and an Ethical Amendment Form submitted where appropriate.

Approval is given on the understanding that you adhere to the 'Guidelines for Ethical Research Practice' (http://www.st-andrews.ac.uk/media/UTREC guidelines%20Feb%2008.pdf).

Yours sincerely

Convener of the School Ethics Committee

cc Dr Akira O'Connor (Supervisor)

School of Psychology & Neuroscience, St Mary's Quad, South Street, St Andrews, Fife KY16 9JP
Email: psyethics@st-andrews.ac.uk Tel: 01334 462071