Schema and Memory Consolidation

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Abstract

The traditional view of systems memory consolidation is that it is a gradual process that takes place over days or weeks. Within this approach, the hippocampus (HPC) is thought to be involved in the rapid encoding of specific events, whilst neocortex is thought to be involved in slow learning. An idea posited recently is that systems consolidation can occur rapidly if an appropriate "schema" into which the new information can be incorporated has been previously created. Using a hippocampal-dependent paradigm, rats were trained to learn a schema involving 6 flavour-place paired-associates (PAs). Once the schema was acquired, relevant new information then became assimilated into extra-hippocampal regions and rapidly became hippocampal-independent. Building upon this foundation and the PAs schema paradigm, this thesis has explored several aspects of the neurobiology of schemas in animals. The first part of the thesis examined the importance of a relevant schema in new information processing. Rats were trained in both a consistent and inconsistent schema. In the consistent schema, rats could learn new PAs in a single trial; however, in the inconsistent schema, rats failed to learn the new PAs as they had not established an appropriate schema that could facilitate rapid learning. The second part of the thesis investigated the role of hippocampal NMDA receptors and dopamine receptors during encoding of new PAs. Bilateral hippocampal infusion of either the NMDA receptor antagonist D-AP5 or the D_1/D_5 dopamine receptor antagonist SCH23390 before encoding of new PAs resulted in impaired memory tested at 24 hr. This result suggests that the encoding of new PAs is dependent upon NMDA receptors in the HPC and also that dopamine is involved in the modulation of encoding new PAs. The final chapters of the thesis attempted to identify the extrahippocampal regions in which these new PAs are integrated with the schema during encoding. To identify the regions that may be involved, immediate early genes (*Zif268* and *Arc)* were used. In a group of cortical structures, including the prelimbic cortex, there was significantly higher Zif268 and Arc expression when encoding 2 new PAs compared to the reactivation of previously learned (original) PAs or the encoding of 6 new PAs. These findings indicate that the prelimbic cortex may be critical for rapid assimilation of new information into a pre-existing schema. Finally, the last experiment in the thesis investigated this finding using bilateral microinfusions of either the AMPA receptor antagonist CNQX or the NMDA receptor antagonist D-AP5 into the prelimbic cortex.

Infusions of CNQX and D-AP5 resulted in poor learning of the new PAs in the schema task. This indicates that parallel encoding of new PAs occurred in the prelimbic cortex and the HPC. The experimental results presented in this thesis suggest that the prelimbic cortex, in particular, plays a crucial role along with the HPC during encoding of new information in rapid memory formation.

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Declaration

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification except as specified.

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Abbreviations

AMPA	amino-3-hydroxy-5-methyl-isoxazole-4-propionate
ACC	anterior cingulate cortex
aCSF	artificial cerebrospinal fluid
AP	anterior-posterior
aRSP	anterior retrosplenial cortex
CA1	*Cornu Ammonis*1
CA3	*Cornu Ammonis*3
CC	caged control
CNQX	6-Cyano-7-nitroquinoxaline-2,3-dione
DA	dopamine
DAB	diaminobenzidine
D-AP5	D-(-)-2-Amino-5-phosphonopentanoic acid
DG	dentate gyrus
DV	dorsoventral
Es	experiments
Fs	flavours
fMRI	functional magnetic resonance imaging
HPC	hippocampus
IEGs	immediate early genes
IL	infralimbic cortex
Ins	insular cortex
LEnt	lateral entorhinal cortex
Ls	locations
mPFC	medial prefrontal cortex
ML	mediolateral
MTL	medial temporal lobe
NaCl	0.9% saline
NMDA	N-methy-D-aspartate
NPA	new paired-associates
NM	new map
Orb	orbifrontal cortex
OPA	original paired-associates
PAs	paired-associates
PBS	phosphate buffered saline
PFA	paraformaldehyde
pRSP	posterior retrosplenial cortex
PFC	prefrontal cortex

PL	prelimbic cortex
PTs	probe trials
REM	rapid eye movement
SCH23390	*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride
Ssp	somatosensory (barrel) cortex
SWS	slow wave sleep
vmPFC	ventral medial prefrontal cortex

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Chapter 1

Overview

We learn and remember better when new material can be related to what we already know. In 1932, the British psychologist Sir Frederick Bartlett found that people remembered new material relatively easily by using pre-existing knowledge structures to organise new material into meaningful and orderly patterns (Bartlett, 1932). According to Bartlett, a schema is a knowledge structure that is based on a set of similar past experiences and captures common features from these experiences. When a new situation is encountered, a schema helps to organise current knowledge and provide a framework for understanding.

The concept of schema is important in the psychological literature on human memory. However, it is not yet known in which areas of the brain schemas are stored, or what the underlying memory mechanisms behind their formation are. The neurobiology of schema has not yet been investigated, mainly because it is difficult to apply the concept in a biological setting (Squire, 2007). However, an animal model of schema formation has been proposed (Tse, Langston et al., 2007) that illustrates how the concept is relevant to the phenomenon of memory consolidation.

The standard view of systems memory consolidation refers to the gradual process of reorganisation by which new memories become remote memories (Squire and Alvarez, 1995; Frankland and Bontempi, 2005) and states that this usually occurs over a long time frame of weeks to months. According to this standard view, the learning of facts and events (that is, declarative memories) initially depends on the hippocampus (HPC), a structure deep in the temporal lobe of the mammalian brain. But as time passes after learning, the importance of the HPC gradually diminishes and

a more permanent memory is established in distributed regions of the neocortex (e.g. the medial prefrontal cortex, mPFC). The stabilisation of this memory trace in the brain is usually a slow process. There is an abundance of experimental evidence that investigated the hippocampal and the mPFC relationship, supporting this model of memory consolidation. This evidence includes studies that have used various experimented techniques, such as glucose uptake imaging (Bontempi et al., 1999), immediate early genes induction (Frankland et al., 2004; Maviel et al., 2004), *N*-methyl *D*-aspartate (NMDA) receptor activity (Takehara-Nishiuchi et al., 2006), expression of proteins involved in axonal and sprouting (Routtenberg et al., 2000), and formation of dendritic spines (Restivo et al., 2009).

However, Tse, Langston et al. (2007) suggested that the systems consolidation can occur as quickly as within 48 hrs of learning new information if the animal had previously acquired a relevant "schema" that enables the rapid incorporation of new information. In their study, rats were trained to associate 6 flavours with 6 places in an "event arena". Rats were first cued with a specific flavour of pellet in one of 4 start boxes and could then receive more of the same flavoured of pellet by going to the correct location in the arena. Rats learned a total of 6 flavour-place paired-associates (PAs) gradually over several weeks. Once the rats had acquired these 6 PAs and thus developed a schema, 2 new PAs were introduced, each in a single trial. Forty-eight hours after the learning of these new PAs, the entire HPC in half of these rats was removed. Perhaps the most surprising finding was that these rats with HPC lesions could successfully recall the newly learned correct locations when given a cued recall trial 2 weeks later. This indicates a direct link between the schema concept and rapid memory consolidation. In short, the neocortex was able to incorporate new information rapidly, which was unexpected, because evidence from many animal

experiments in the literature suggest that memory consolidation requires at least a month (Squire, 2004).

This thesis explored several aspects of the neurobiology of schemas in animals. It includes further behavioural studies establishing the phenomenon in the event arena setting and investigated the casual role of a schema in rapid learning. It also reported pharmacological interventions and immediate early genes mapping studies. Thus, the approach is interdisciplinary.

Specifically, the experiments to be described mainly focus on the rapid memory consolidation that occurs in the schema paradigm and the roles played by the HPC and mPFC. There are 4 main experiments intended to:

- **examine the causal role of schema in relation to rapid memory consolidation,**
- **investigate the role of dopamine receptor modulation in the persistence of hippocampal NMDA receptor-dependent PA memory,**
- **identify which extrahippocampal regions are activated by original and new PAs between flavours and locations using immediate early gene mapping, and**
- **examine whether selective inactivation of NMDA receptor-mediated synaptic plasticity and AMPA receptor-mediated transmission in previously identified cortical regions impacts on this rapid memory consolidation.**

The aim of the present work, therefore, is to join the current search for links between hippocampal and cortical memory consolidation, motivated by the belief that the finding of such links would represent an important step forward in the understanding of biological memory formation.

Chapter 2

Introduction

In this chapter, the basic concepts and key players in memory, memory consolidation and schema will be introduced. In addition, the chapter will give a brief summary of relevant theories and findings in this field before setting the aims of the thesis in their proper context.

2.1 Memory

Memory is not a single unitary phenomenon, but rather, it is composed of several different components, which are mediated by separate brain systems. There are many ways to investigate memory. Memory is often separated into the distinct stages of encoding, consolidation and retrieval of information. In addition, one can differentiate memory into declarative or non-declarative memory. Declarative memory is explicit memory of general facts and everyday life events, and non declarative memory is procedural memory for skills and the ability to respond to stimuli through practice. Declarative memory can be divided into two categories: episodic memory, which stores specific personal experiences; and semantic memory, which stores general factual information (Tulving, 1972). Episodic memories are unique, concrete, personal experiences from the rememberer's past, whereas semantic memory refers to a person's abstract, timeless knowledge of the world that he shares with others (Tulving, 1972). Memory consolidation reflects the dynamic relationship between episodic and semantic memory. This thesis outlines a new approach to the dynamic interactions between episodic and semantic memory during the encoding, consolidation and retrieval of memory.

2.2 Memory consolidation

2.2.1 A brief history of memory consolidation

Memory is known to be initially labile but over time it becomes more stable (Frankland and Bontempi, 2005). The French psychologist Theodule Ribot first introduced the idea that memory takes time to stabilise or consolidate (Ribot, 1881). He related memory loss after brain damage to the age of particular memories and pointed out that recently acquired information was more impaired than remote memories. This time-dependent process of memory reorganisation is known as the Ribot gradient. Some years later, Muller and Pilzecker introduced the term 'consolidation' (Müller and Pilzecker, 1900). According to Squire (1986), they were the first to propose that newly learned information remains sensitive to interference for a certain period of time until the point when the memory traces becomes 'consolidated', and thereby would be refractory to disruption. In 1903, Burnham proposed that consolidation involves a time dependent process in which organisation of newly obtained memories takes place through some combination of physical reorganisation and the psychological process of repetition and association. He proposed that retrograde amnesia was a consequence of interrupted organisational processing, which must normally occur for a considerable period of time after learning. In later years, more physiologically based concepts of memory consolidation followed. In 1950, Lashley, basing his observations on experiments where he removed different amounts of cerebral cortex in rats that he then ran on complex mazes, concluded that memories are not localised but rather, are widely distributed throughout the cortex. Hebb (1949) then suggested that assemblies of cells work together to represent information and that these assemblies are distributed over large areas of cortex. The idea of a distributed memory store was far sighted for his time.

One of the most convincing lines of evidence for a memory consolidation process is the temporal aspects of retrograde amnesia. Retrograde amnesia is typically reported to be temporally graded and characterised by a loss of recent memories whilst sparing remote memories- a phenomenon initially observed by Ribot (Bontempi and Durkin, 2007). A breakthrough in the understanding of memory occurred in the late 1950s when Scoville and Milner opened a new era in the search for the anatomical correlates of temporal graded retrograde amnesia. In the well known case, the patient Henry Molaison (H.M.) (Scoville and Milner, 1957) had parts of his medial temporal lobe (MTL) surgically removed to alleviate a severe form of epilepsy. This surgery produced a severe, temporally graded retrograde amnesia. However, H.M's remote memories, acquired during his youth, early adult life remained unaffected. The memory profile exhibited by H.M. and other similar case studies has been interpreted as evidence for a time-limited role of the hippocampus (HPC) and related structures of the medial temporal lobe in memory storage (Rempel-Clower et al., 1996). H.M. recently died on 2nd December 2008 and his brain was sectioned for analysis (http://thebrainobservatory.ucsd.edu/hm_live.php). In short, the evidence suggested that, once consolidated, memories that were initially involved in the HPC are later stored in the extrahippocampal areas.

There are numerous animal studies that were later successful in reproducing the temporally graded retrograde amnesia observed in humans. In addition, with refined animal models of memory in primates and rodents and of the use of lesion approaches, many researchers then succeeded in identifying the anatomical components of the MTL memory system. Brain structures that constitute this system are anatomically connected and are conserved across species such as humans, non-human primates and rodents (Amaral and Witter, 1989; Lavenex and Amaral, 2000;

Suzuki and Amaral, 2003). The structures include the HPC (CA1, CA3 and dentate gyrus), subicular complex, and also, entorhinal, perirhinal and parahippocampal cortices.

It has become clear that the MTL system is very important for declarative memory of the kind affected in patient H.M. (Milner et al., 1998). From all these findings, neurobiologists have differentiated three stages of memory formation on the basis of their timescales. The first stage is a labile and vulnerable form, named short term memory, which processes information from seconds to minutes. A second stage is a more stabilised form, known as long term memory, where information can be stored from minutes to several days. The third stage is an enduring form, named remote memory, which ensures storage for extended periods of time lasting from several weeks to a lifetime (McGaugh, 2000). These concepts have led to the distinction between 'cellular' and 'systems' memory consolidation (Dudai and Morris, 2000), which will be described in the following section.

2.2.2 Phases of memory consolidation: Cellular and systems memory consolidation

Memory consolidation can be conceptually divided into two phases on the basis of their specific temporal kinetics. These phases are 'cellular consolidation' and 'systems consolidation' (Dudai and Morris, 2000; Dudai, 2004). Cellular consolidation occurs within minutes to hours following an encoding experience and is triggered by a series of cellular and molecular cascades within individual neurons (hence the name) that include release of neurotransmitters, activation of central receptors, activation of various transcription factors and associated gene signalling pathways (Dudai, 2004). These, now well-characterised, metabolic events lead to the

synthesis of proteins that are directly responsible for the occurrence or stabilisation of morphological changes within localised neuronal networks, such as changes in synaptic strength and restructuring of existing connections (Kandel and Squire, 2000; Dudai, 2004; Frankland et al., 2004).

On the other hand, in systems consolidation, memories that are initially dependent upon the HPC undergo reorganisation and may become hippocampal-independent. It occurs over a larger timescale than cellular consolidation (Dudai, 2004; Frankland & Bontempi, 2005) and involves multiple brain regions (hence the name). Marr (1971) formulated the first model to account for systems consolidation. He proposed that the HPC rapidly stores the day's events before the information is transferred to the cortex for subsequent reorganisation and reclassification (Marr, 1971). The concept of systems consolidation was directly inspired by studies of retrograde amnesi a gradients which indicated that the HPC cannot be the permanent repository of long term memory (Zola-Morgan and Squire, 1990). This idea actually forms the central tenet of most contemporary views of systems consolidation; that is, the HPC acts as a temporary consolidation organising structure, but storage and retrieval of enduring memories depend on broadly distributed extrahippocampal networks, presumably in cortex.

This thesis will focus on systems memory consolidation.

2.2.3 Models of memory consolidation

Currently there are 2 main models of systems memory consolidation, namely, the standard consolidation theory and the multiple trace theory.

2.2.3.1 The standard model of memory consolidation

In the standard model of memory consolidation (Frankland & Bontempi, 2005), the encoding of perceptual, motor and cognitive information initially occurs in several specialised primary and associative cortical areas. As mentioned above, the MTL, (including the HPC) is believed to rapidly integrate and bind together information transmitted from distributed cortical networks that support the various features of a whole experience in order to form a coherent memory trace. Systems consolidation then occurs slowly over time and involves a hippocampal-cortical dialogue to gradually strengthen cortical-cortical connections. This suggests that, over time as memories mature, the role of HPC gradually diminishes, leaving extrahippocampal regions, presumably cortical areas, to become independently capable of sustaining permanent memory traces and mediating their retrieval (McClelland et al., 1995; Squire and Alvarez, 1995; Frankland and Bontempi, 2005). A key feature of this model is that changes in the strength of hippocampal-cortical connections are fast but short-lasting, whereas changes within cortical-cortical connections are slow but long-lasting.

The standard model of memory consolidation asserts that systems level consolidation takes place after learning which, by enabling an interaction between hippocampal and neocortical ensembles, secures the stabilisation of memory traces outside the HPC (Squire and Alvarez, 1995; Manns et al., 2003) (Figure 1.1A). There is a large body of

evidence from both human and animal studies that is consistent with this view (Squire, 2004; Bayley et al., 2005).

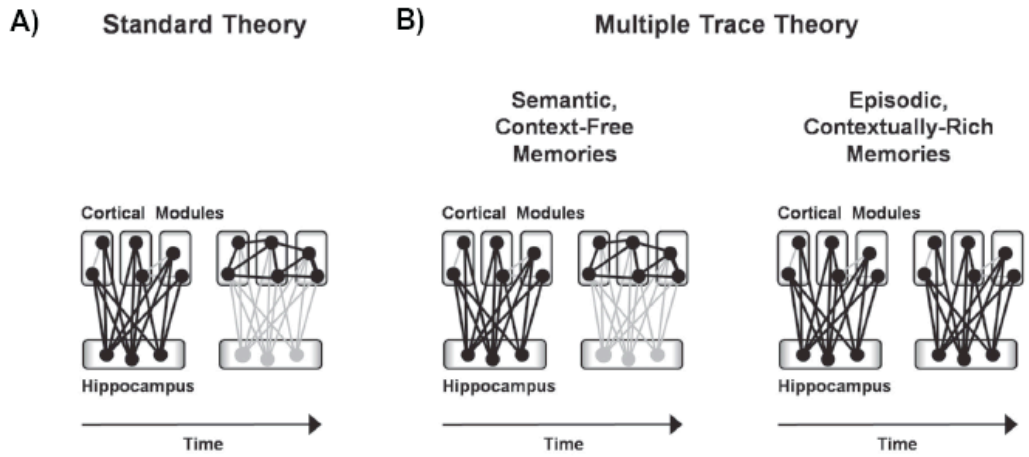

Figure 1.1 Two theories of time-dependent memory reorganisation in systems memory consolidation. (A) The standard consolidation theory proposes that, over time, memories become independent of the HPC. (B) The multiple trace theory proposes that semantic or context-free memories become independent of the HPC over time, but that episodic or contextually-rich memories always depend on the HPC (Figure reprinted from Akers and Frankland, 2009).

2.2.3.2 The multiple trace theory

An alternative view of memory consolidation is the multiple trace theory. The multiple trace theory suggests that the HPC retains a permanent role in memory storage and retrieval as long as memories exist (Nadel and Moscovitch, 1997). This theory is supported by 3 main lines of clinical and experimental observations.

Firstly, retrograde amnesia can, in some cases, be temporally ungraded (that is, it is flat), wherein both recent and remote memories have been reported to be similarly impaired (Cipolotti et al., 2001). *Secondly*, certain retrograde amnesia gradients have been reported to last for decades or up to almost the entire human life span in some amnestic patients, thus raising the question of the ethological value of such an extended period of consolidation. *Thirdly*, the observation of a retrograde amnesia gradient and its temporal extent may depend on the type of declarative memory to be consolidated (Moscovitch et al., 2006) (Figure 1.1B).

To account for these observations, the multiple trace theory proposes that, although experience is initially encoded in distributed hippocampal-cortical networks, the HPC is always required for rich contextual or spatial detail (Nadel and Moscovitch, 1997). According to the multiple trace theory, each consciously experienced event would consist of a cohesive hippocampal-cortical ensemble. Each time that a particular event is recalled, it would be recreated and recorded in the form of multiple and strong related memory traces dispersed over hippocampal-cortical networks. Therefore, the relative sparing of remote memories in amnesic patients would be a function of the extent of hippocampal damage. Limited damage is held to produce temporally graded retrograde amnesia, whereas extensive lesions would cause a flat gradient of retrograde amnesia. Nadel and Moscovitch (1997) posited that the recurrent creation of multiple hippocampal-cortical traces would predominantly favour the integration of information with pre-existing knowledge to form old semantic memories (memory for general knowledge of facts) whose retrieval could possibly occur without the contribution of the MTL memory system. However, in the case of remotely acquired episodic memories, which are autobiographical and richly detailed in nature, these authors postulated that retrieval would always require the contribution of hippocampal-cortical networks (Figure 1.1B).

2.2.3.3 Debate concerning these models

It must be noted that the results of some case studies, for example, the patient EP, who suffered large lesions of the MTL, has not been supportive of the multiple trace theory (Teng and Squire, 1999). Although having extensive hippocampal damage, he had excellent autobiographical memories from his youth and could accurately recall the spatial layout of the area where he grew up more than 50 years earlier. In addition,

he had good skills in mental navigation and in constructing novel spatial routes to access specific locations of his past neighbourhood. This shows that his spatial knowledge was likely extrahippocampal.

However, a more recent study of an experienced London taxi driver (patient TT) who acquired bilateral HPC lesion seems to support the multiple trace theory over the systems standard memory consolidation (Maguire et al., 2006). In this study, TT showed good knowledge of overall spatial relationships and landmarks in London. However, testing navigation *in situ* using a virtual reality representation of London revealed a deficit, including reliance on main routes. The authors suggest that EP likely had similar deficits but because his tests were static rather than the dynamic testing of TT, these deficits were not found.

This debate over the different models is still underway and there is evidence that supports both.

Both models, standard consolidation theory and multiple trace theory, have that the HPC and cortex are regions of high importance in memory consolidation. Specifically, standard memory consolidation theory states that the HPC and the prefrontal cortex are both integral structures in systems memory consolidation. The next section gives an anatomical overview of these 2 regions.

2.3 Hippocampus and prefrontal cortex anatomy

2.3.1 Hippocampal formation

The HPC is a critical brain structure that is involved in memory. The mammalian hippocampal formation is located within the forebrain and forms part of the temporal lobe of the cerebral cortex. The position of the HPC within the temporal lobe is such that it lies beneath the corpus callosum and stretches caudally from the septum, and latero-ventrally around the thalamus (Amaral and Witter, 1995). The hippocampal formation comprises of 3 distinct regions: the dentate gyrus, the subiculum and the HPC (or hippocampal proper). The HPC can then be divided into 3 subfields on the basis of the size of the cells and their distribution. These regions are CA1, CA2 and CA3 (Amaral and Witter, 1989). Pyramidal cells in CA3 receive a prominent mossy fibre input from the dentate gyrus, The other intrinsic connections are CA3 to CA1, and then CA1 to the subiculum.

2.3.2 Prefrontal cortex

The prefrontal cortex (PFC) of the rat is usually conceptually separated anatomically into medial, orbital and lateral areas (Ongur and Price, 2000). The medial PFC (mPFC) of rats can be subdivded into four main regions which, moving from dorsal to ventral, are the medial agranular cortex (AGm), the anterior cingulate (ACC), the prelimbic cortex (PL) and the infralimbic cortex (IL) (Berendse and Groenewegen, 1991). The mPFC is well recognised to be important for several higher order functions, including selective attention, visceromotor control, working memory, decision making and goal-directed behaviour (Vertes, 2006). Dorsal regions of the mPFC (AGm and ACC) have been implicated in various motor behaviours, while ventral regions of mPFC (PL and IL) have been associated with diverse emotional,

cognitive and mnemonic processes (Heidbreder and Groenewegen, 2003; Vertes, 2006). The present thesis focuses on the role of ventral regions (especially PL) of the rat mPFC in the memory consolidation aspects of behaviour.

In the ventral mPFC, the IL influences visceral/autonomic activity. IL stimulation produces changes in respiration, gastrointestinal motility, heart rate and blood pressure (Terreberry and Neafsey, 1983; Neafsey, 1990). In contrast, the PL has been implicated in cognitive process. PL lesions have been shown to results in deficits in delayed response tasks in rodents (Seamans et al., 1995; Floresco et al., 1997), and primates (Goldman-Rakic, 1994; Groenewegen and Uylings, 2000).

2.3.3 Hippocampal-mPFC circuitry in rats

In rats, hippocampal efferents to the mPFC arise from temporal aspects of CA1 and subiculum and terminate in the ventral mPFC, including the medial orbital area, IL and PL (Jay et al., 1989; Jay and Witter, 1991). There are no projections from CA2/CA3 or the dentate gyrus to mPFC (Jay and Witter, 1991). Interestingly, despite strong hippocampal to mPFC projections, there are no direct return projections from the mPFC to the HPC (Vertes, 2006). In the absence of such projections, the nucleus reuniens (RE) of the midline thalamus is an important relay structure in the transfer of information from the mPFC to the HPC. The mPFC distributes heavily to RE, and RE in return to the HPC (CA1/subiculum) (Jay & Witter, 1991).

2.4 Hippocampal-cortical interactions during systems memory consolidation

The first direct evidence for neocortical engagement during remote memory came from 2-deoxyglucose experiments. Mice were trained on a HPC-dependent spatial learning task and then monitored for brain activity [using (^{14}C) 2-deoxyglucose uptake] following retrieval of either recent (5 days) or remote (> 25 days) memory tests (Bontempi et al., 1999). Retrieval of recent spatial memories produced more robust HPC activation than retrieval of remote memories, a result consistent with the model that proposes progressive disengagement of the HPC during memory consolidation (Bontempi et al., 1999). The study went on to show that mPFC areas became more engaged as memories became remote. Greater cortical activation was observed at 25 days compared to the 5 days.

Frankland et al. (2004) found increases in neocortical immediate early gene expression following the retrieval of remote contextual fear memories in mice. This finding indicates that, while the HPC is engaged, contextual fear memories do not involve the mPFC, but with the progressive disengagement of the HPC, the mPFC becomes more involved in late-stage memory processes. Similarly, Maviel et al. (2004) showed increased IEG expression in neocortical regions following the retrieval of remote but not recent spatial memories in mice. Also, in a series of trace eyeblink condition experiments, Takehara-Nishiuchi et al. (2003, 2005) showed that mPFC is important for the neocortex dependent remote memory and that successful establishment of remotely acquired memory requires activation of NMDA receptors in the mPFC during at least the initial week of the post-learning period.

All of these studies point to the mPFC being involved in later stages of memory processing, including systems memory consolidation, but they do not directly address

what happens at the time of encoding (Takehara-Nishiuchi et al., 2003, 2005; Frankland et al., 2004; Maviel et al., 2004).

2.5 Sleep and memory – a dialogue between HPC and mPFC

As mentioned above, according to the systems memory consolidation theory, the HPC is necessary for the retrieval of recently encoding episodic memories. For remote memories, the mPFC seems to play a unique role in mediating retrieval of consolidated remote memories (Scoville and Milner, 1957; Squire, 1986; McGaugh, 2000; Frankland et al., 2004; Maviel et al., 2004; Takashima et al., 2006).

The transfer of memories from HPC to neocortex is widely believed to involve replay of neural patterns representing memory during sleep (McClelland et al., 1995; Buzsaki, 1998). It was suggested that slow wave sleep (SWS) provides an off-line mode of processing that enables the gradual incorporation of newly acquired hippocampal memories into neocortical networks for long-term storage (Marshall and Born, 2007; Molle and Born, 2009). Network oscillations in the mPFC (spindle waves) have a correlation with the high-frequency transient bursts of CA1 pyramidal cell activity (ripples) during SWS (Siapas and Wilson, 1998; Wierzynski et al., 2009). This indicates there may be a functional connection between the HPC and mPFC. In addition, fast forward playback of recent memory sequences was observed in mPFC during sleep (Euston et al., 2007).

A recent study (Diekelmann and Born, 2010) suggested a 2 stage memory system model that outlines the contribution of SWS and rapid eye movement (REM) sleep to memory consolidation (Figure 1.2). Here, information is initially encoded by both HPC and neocortex whilst awake. Then, during SWS, the HPC reactivates the new memories which drive reactivation in the neocortex which strengthens these new

connections. This, in turn, leads to the integration of new memories into the network of pre-existing long term memory in neocortex. During REM sleep, the HPC and neocortex are temporarily in a disconnected mode which allows for synaptic consolidation in neocortex.

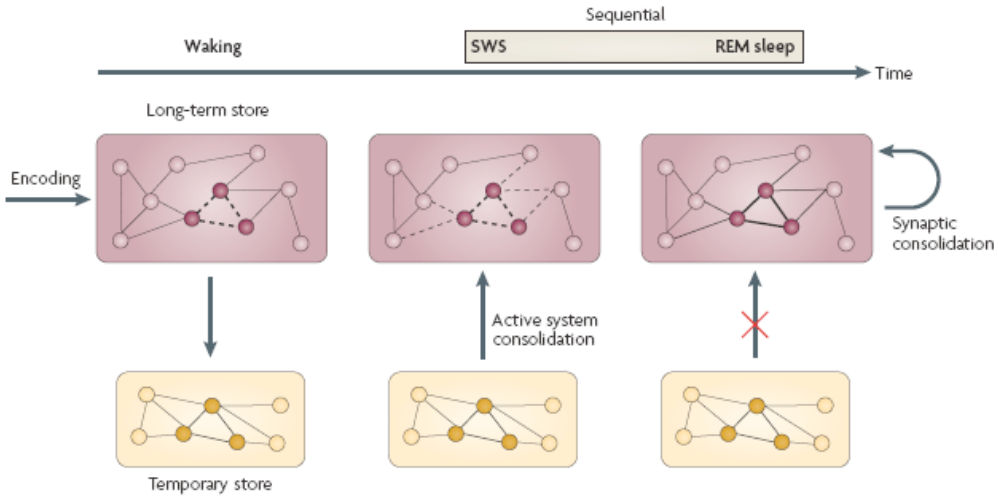

Figure 1.2 Contributions of slow wave sleep (SWS) and rapid eye movement (REM) sleep to memory consolidation in a two stage memory (Figure reprinted from Diekelmann and Born 2010).

2.6 Schema

2.6.1. What is a schema?

The English psychologist, Sir Frederick Bartletts introduced the term "schema" in 1932. Schema refers to pre-existing knowledge structures into which newly acquired information can be incorporated (Bartlett, 1932). Bartlett suggested that our knowledge of the world comprises a set of models or schemas that are based on past experience. When we attempt to learn something new, we base our learning on already existing schemas (Baddeley, 1976). Bartlett first introduced the schema concept to explain how it is that when people remember stories they typically omit some details and introduce rationalisations, reconstructing the story so that it makes more sense in terms of their own knowledge and experience (Cohen et al., 1993). The story is 'assimilated' into pre-stored schemas based on previous experience. Bartlett suggested that people remembered new material using mental structures through schemas.

An appropriate schema is needed

In 1969, Bower and colleagues conducted an experiment in which participants were shown a group of minerals presented either in an organised table (rare metals in one column, precious stones in another and so on or else arranged randomly). The group with an organised structure later recalled almost four times as many minerals as the group with random arrangement (Bower et al., 1969).

In another study by Bransford and Johnson (1972) in which people were shown a passage about sorting clothes, participants who were given a title of the passage in advance, and thus could fit the sentences into a meaningful schema, were able to recall far more of what they had read. Hence, from these studies, it is not the case that

any random schema helps when learning new information; it needs to be an appropriate schema.

2.6.2. Schema in animal models

The concept of schema is important in the psychological literature on human memory (Bransford, 1972). However, the neurobiology is unexplored. It is not yet known in which areas in the brain the schemas are stored or what the underlying neural mechanisms of their formation may be. The neurobiology of schema had not been investigated until recently (Tse, Langston et al., 2007) as it is difficult to apply the concept in a biological setting (Squire, 2007). Recently, an animal model of schema has now been proposed (Tse, Langston et al., 2007) which shows how the schema concept could be relevant to the phenomenon of systems memory consolidation in animal studies. Before going into the details of this animal model of schema, it is important to outline the paired-associations (PAs) task in the event arena used in this study.

Paired-associates in the event arena

Inspired by experiments using food caching behaviour to demonstrate episodic like memory in scrub jays (Clayton and Dickinson, 1998), Day et al. (2003) introduced the first study of flavour-place PAs learning task in the event arena. They developed a one-trial PAs task in which rats were given a specific flavour of cue pellet (e.g. banana) in the start box, and could find a reward pellet (e.g. banana) in the correct location in the event arena (Day et al., 2003). Every training day, rats were given 2 sample trials, separated by an interval of 5 min, and were presented with 2 different flavours and locations. In a single sample trial, rats left the start box to forage for flavoured food by locating the sand-well and digging for the food reward. Note that

there was no choice available for the rats during these sample trials. Twenty minutes after these sample trials, a choice trial was given. The rat was now given one of the 2 flavours that they experienced in the previous sample trials to eat in the start box. This constituted a recall cue. After the rat finished the cue pellet in the start box, it was allowed to run into the arena in which there were now 2 sand-wells available; however, only the correct one contained pellet rewards. Day et al. (2003) found that blockade of hippocampal NMDA receptors at the time of encoding impaired memory, whereas blockade of these same receptors at the time of retrieval did not impair memory. They also found that antagonising hippocampal AMPA receptors at the time of retrieval impaired memory.

Schema in the event arena

In order to investigate the neurobiology of schema, based on the flavour PAs paradigm (Day et al., 2003), rats were trained to learn 6 flavour place PAs concurrently (Tse, Langston et al., 2007), using different flavours of pellets and sand-wells located in the event arena. Unlike Day et al. (2003), PAs remained stable across days. The task was to learn which flavour was in which location such that, when cued with a specific flavour in start boxes at the side of the arena, the animals would be rewarded for going to the correct location by receiving more of the same food. The rats should be able to recall that chocolate flavoured food is at one location, strawberry flavoured food at another, and so forth.

The aim of this thesis is to investigate the neurobiology of the schema and so first of all, the original study will be described in detail to set the scene for the investigation.

The basic protocol for PAs schema training is as follows. A trial began when the rat was given a cue flavour pellet in the start box. After the rat finished the pellet, it was

allowed to enter the arena where there were 6 sand-wells of which only one contained flavoured pellet (which is the same cue flavour pellet that was given in the start box). The rats visited and sometimes dug at incorrect sand-wells, which did not contain food reward on that particular trial, until they found the new one. In every session, there were 6 trials. In each trial, a different sand-well in the arena was baited.

Following this basic schema protocol, a series of experiments was conducted (Tse, Langston et al, 2007). The first experiment was to establish whether the learning of PAs consisting of flavours and places requires the integrity of the HPC. Hippocampal lesions were made before training and after a month of training, sham-lesioned rats dug less frequently at incorrect sand-wells before going to the correct sand-wells whereas the hippocampal-lesioned rats did not improve. A single non-rewarded probe trial (PT) was conducted. This PT was to test whether the rats could recall the PAs. The sham-lesioned animals spent significantly more time digging at the cued location than at the other 5 incorrect ones, whereas the hippocampal-lesioned animals were at chance level (Figure 1.3).

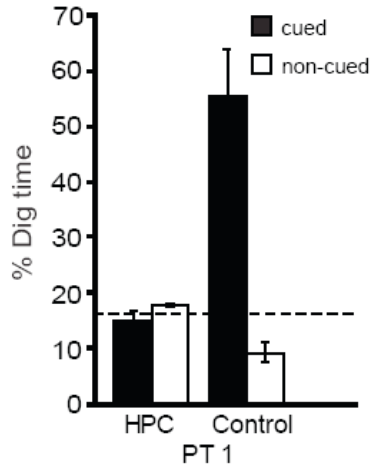

Figure 1.3 Preferential digging during a non-rewarded probe trial (PT1) by sham-lesioned but not hippocampal lesioned animals (Figure reprinted from Tse, Langston et al. 2007).

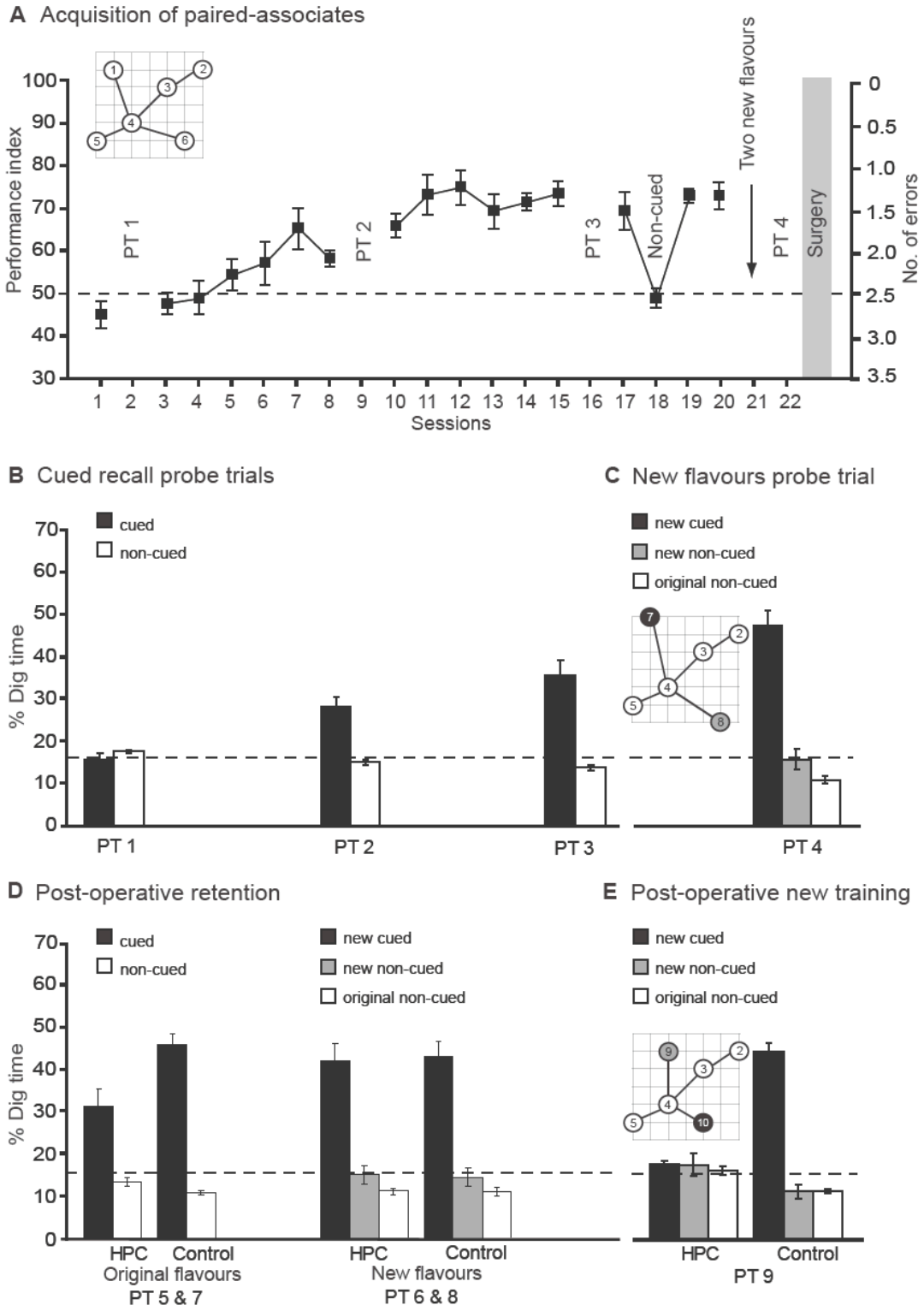

Figure 1.4 (A) Acquisition of PAs. The rats made fewer choice errors over training. (B) Cued-recall probe trials. Non-rewarded probe trials revealed a graded learning of the original PAs. (C) Effective cued recall of the newly learned location in PT4. (D) Postoperative retention. Both sham-lesioned and HPC-lesioned rats could effectively remember both original and the newly learned flavours locations. (E) Postoperative new training, Hippocampal lesions prevented the learning of new PAs. (Figure reprinted from Tse, Langston et al. 2007).

The second experiment was to investigate the properties of PAs learning and its consolidation in more detail. Rats were trained in a basic schema protocol.

Probe tests were scheduled at various stages before and after the sham or hippocampal lesions. The acquisition of sand-well choice behaviour during training was examined (Figure 1.4A). The animals made fewer choice errors as training progressed. In probe tests given at the beginning of the training session, the rats' performances were at chance level (Figure 1.4B), whereas after a month of training, the rats dug preferentially at the correct locations.

After the rats acquired these original PAs, and perhaps had developed a neocortical associative schema for this task, which might aid in the encoding of new PAs and rapid assimilation into a schema, a single training session of 6 trials was given. Two original PAs were replaced by 2 new PAs. Note the rats only received the 2 new PAs once, each in a trial. A probe trial was given 24 hours later to test memory for the new PAs. Preferential digging was observed at the correct new cued location relative to the new non-cued location (Figure 1.4C). The rapid acquisition of new PAs in a single trial and their retention over 24 hours are indications that the prior learning of an associative schema may aid the encoding and consolidation of new PAs.

Hippocampal or sham lesions were then made 48 hours after they learned the new PAs. After recovery from their surgeries (around 2 weeks), a series of probe tests were conducted to test if the rats remembered both the original PAs (which they learned a long time ago) and the newly learned PAs (which they learned in only a trial). Both sham-lesioned and the 48 hour HPC-lesioned rats could effectively remember both original and the newly learned flavours locations (Figure 1.4D). These findings imply that the memory trace for these new PAs is stored outside the

HPC, presumably neocortex; and consolidation of new PAs whose acquisition is mediated by the HPC takes place within 48 hours.

To establish that the learning of further new PAs still required the integrity of the HPC in these same animals, rats were introduced with 2 novel PAs. A probe trial conducted 24 hours later shows that sham-lesioned animals could readily learn and recall these new PAs, whereas the hippocampal-lesioned animals could not. This implies that HPC is always required for learning new PAs, but once the information is consolidated (48 hours), the HPC is perhaps not involved.

The third experiment was to examine whether there is a temporal gradient for neocortical consolidation of PAs memory over the range 3-48 hr. This was a very surprising result as the 48 hour HPC lesion group not only could successfully recall the original PAs learned over the previous month but, remarkably, could remember the newly acquired new PAs while the 3 hours HPC lesion group could not remember the newly learned PAs (Figure 1.5).

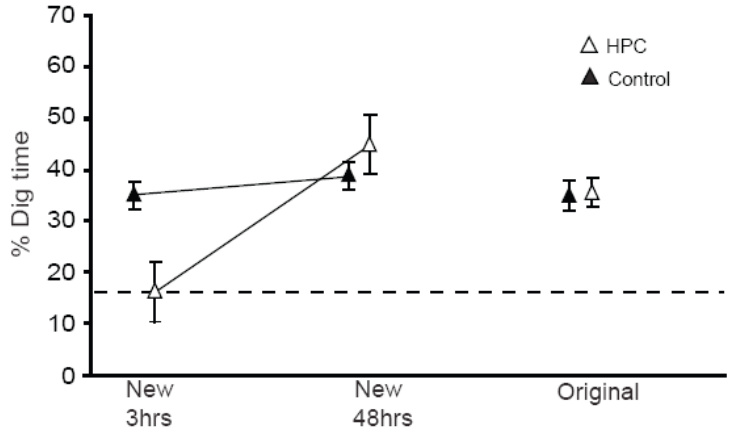

Figure 1.5 A temporal gradient of retrograde amnesia is observed in this paradigm. HPC lesions made 3 or 48 hours after training on the novel flavour tested 14 days later. The performance of the HPC 48-hour groups was significantly higher than the HPC (Figure reprinted from Tse, Langston et al. 2007).

2.6.3 Rapid memory consolidation

This new idea, distinct from the standard and multiple-trace models of consolidation, is that neocortical schema may enable rapid interleaving of new information with stability of trace memory over time. These findings indicate that the rate at which systems memory consolidation occurs in the neocortex can be influenced by prior knowledge (a schema). In the standard systems memory consolidation, the hippocampus is thought to be involved in the rapid encoding of specific events, whilst neocortex is thought to be involved in slow learning (Norman and O'Reilly, 2003). However, in this case, systems consolidation can occur rapidly if an appropriate schema into which new information can be incorporated has been previously created. Based on this interesting schema paradigm, the aim of this thesis is to further investigate the neurobiology of schema.

2.6.4 Functional magnetic resonance imaging (fMRI) in human schema task

Recently, a study by Van Kesteren et al., (2010a) took a step further to investigate whether the existence of a relevant prior associative network, schema, facilitates this crosstalk of HPC and mPFC in humans. They used fMRI and prior schema manipulation to track hippocampal connectivity during encoding and post-encoding rest. Participants were either exposed to the first part of a movie in its correct order (consistent group) or a scrambled order (inconsistent group). The next day, participants underwent fMRI while encoding the movie's final clip in original order, and subsequently, while resting. Interestingly, the result shows that the connectivity between the HPC and vmPFC was stronger for the inconsistent schema group both during encoding and postencoding rest. These findings suggest the possibility that crosstalk between HPC and vmPFC is required to compensate for the difficulty of

integrating novel information during encoding, and so provides support for the notion that functionally relevant hippocampal-neocortical crosstalk persists during off-line periods after learning (van Kesteren et al., 2010a).

Another schema study by van Kesteren et al., (2010b) showed that when new information is congruent with prior knowledge it is better remembered, most likely due to facilitated consolidation. They conducted a visuo-tactile learning paradigm in which participants studied visual motifs randomly associated with word-fabric combinations, representing everyday objects made of fabric and either congruent or incongruent with common knowledge, as assessed in an independent study (e.g. cotton-dress in contrast to leather sheets). The next day, participants were scanned using functional fMRI while recognising the visually presented motifs. van Kesteren et al., (2010b) found that the congruent associations were remembered better than incongruent ones, which is correlated with stronger retrieval-related activity in connectivity between medial prefrontal and left somatosensory cortex. This result shows the successful retrieval of congruent (consistent) compared to incongruent (inconsistent) visuo-tactile associations is related to enhanced processing in a medial prefrontal somatosensory network, suggesting optimised rapid memory consolidation by facilitated neocortical integration when new information fits a pre-existing schema.

2.7 Aims of the thesis

So far, the introduction has provided a background of different theories of memory consolidation, and the possible brain regions that are involved. The original study (Tse, Langston et al., 2007) of PAs schema set the scene for the further investigation of underlying neurobiology.

The **first** aim is to ensure that the rapid memory consolidation was induced due to an associated schema. This is addressed in Experiment 1 in chapter 4.

The **second** aim is to investigate the neurobiology of the HPC during encoding and retrieval of paired-associates in the schema paradigm. This is addressed in Experiment 3 in chapter 5.

The **third** aim is to investigate not just the HPC, but other possible brain regions (presumably cortical regions) involved in the encoding and retrieval of paired-associates in the schema paradigm. This is addressed in Experiment 4 in chapter 6.

The **final** aim is to investigate the neurobiology of those brain region(s) which are involved in the encoding and retrieval of paired-associates in the schema paradigm. This is addressed in Experiment 5 in chapter 7.

Chapter 3

General methods

3.1 Subjects and housing

The subjects were adult male Lister-hooded rats (Charles River, UK), aged 8 to 10 weeks at the start of experimentation and weighing 230 to 260 gm. They were housed in groups of 3 to 4 rats per cage [Experiments (Es) 1, 2, 3 and 4] or in single cages (E5). They had free access to water at all times and were maintained at 85-90% of their free-feeding weight during experiments (bodyweights were recorded weekly). Experiments were conducted on a 12 hr (on)/12 hr (off) light cycle, with training during the light phase (8 am-8 pm). A total of 60 rats were used (E1: n = 9; E2: n = 3; E3: n = 11; E4: n = 28; E5: n = 9). All procedures were compliant with the UK (Animals [Scientific Procedures] Act, (1986) and with the [European Communities Council Directive of 24 November 1986 (86/609/EEC)] legislation governing the maintenance of laboratory animals and their use in scientific experiments.

3.2 Surgery

Cannulae implantation (Experiments 2, 3 & 5)

Rats were anaesthetised with 5% isoflurane (Abbott, UK) and positioned in a stereotaxic frame (Kopf instruments, USA). The top surface of the rats' heads were shaved and cleaned with disinfectant wipes. Incisions were made and the skin and underlying tissues were retracted to expose the skull. Holes were drilled into the skull at 4 or 6 locations and small stainless steel skull screws (Fine Science Tools, UK) inserted at 4 points (Es2 & 3) or 6 points (E5) over the skull. Small holes were then drilled into the skull over the cannulae target sites. In experiment 2 & 3, guide

cannulae (Plastics One, Bilaney, UK) were implanted bilaterally into the dorsal HPC (coordinates relative to skull at Bregma: anterior-posterior (AP) = -4.5 mm; mediolateral (ML) = 3.0 mm; dorsoventral (DV) from dura = -2.5 mm). In E5, guide cannulae (Plastics One, Bilaney, UK) were implanted bilaterally into the prelimbic cortex (coordinates relative to skull at Bregma: AP = 3 mm; ML = 0.7 mm; DV (from dura) = -1.9 mm) and the somatosensory (barrel) cortex (coordinates from Bregma: AP = -1.8 mm; ML = 5 mm; DV (from dura) = -0.9 mm). The dura was pierced at these sites with a narrow gauge needle before the cannulae were placed. The AP length of the prelimbic cortex is -4.70 mm to 2.20 mm.

Dental cement (Kemdent, UK) was then sculpted around the guide cannulae, covering the screws. The screws were to provide anchorage for the cement, and the cement fixed the cannulae in position. This cement holding the cannulae create a "cap" over the exposed skull. Vaseline was applied into the cannulae holes to avoid dental cement getting into the brain. Solid stainless steel ("dummy") cannulae (Plastics One, Bilaney, UK) were inserted into the implanted guide cannulae to prevent infection or blockages. In Es 2 and 3, dummy cannulae for HPC (33 gauge; 0.5 mm protrusion from the end of guide cannulae) were inserted into the guides (Figure 3.1A & B). In E5, dummy cannulae for the prelimbic cortex (0.5 mm protrusion from the end of guide cannulae) and for the somatosensory cortex (0.5 mm protrusion from the end of guide cannulae) were inserted into the guides (Figure 3.1C & D).

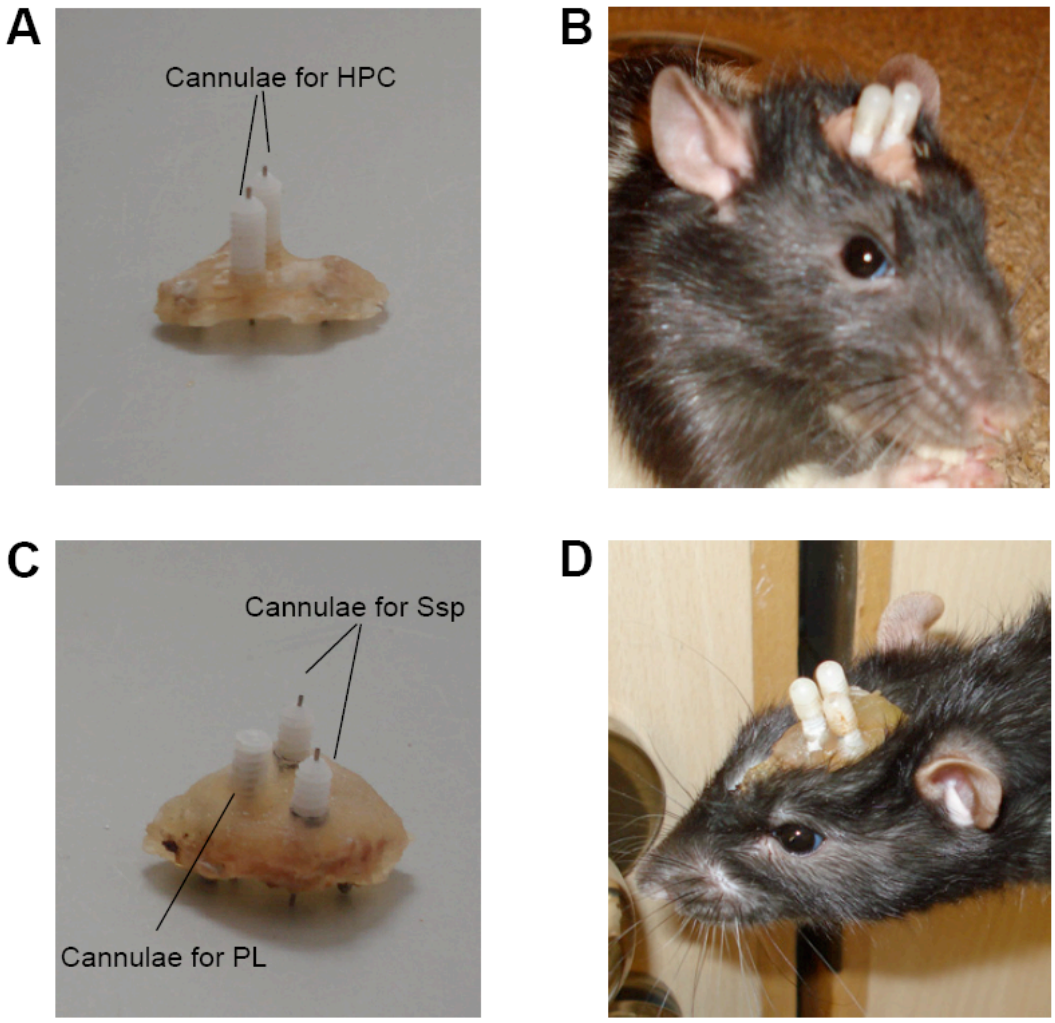

Figure 3.1 (A) Cannulae for hippocampus (HPC) on dental cement. (B) Rat with cannulae and dummy for HPC implant on its headcap. (C) Cannulae for the prelimbic cortex (PL) and the somatosensory (barrel) cortex (Ssp) on dental cement. (B) Rat with cannulae and dummy for the PrL and the Ssp implant on its headcap.

The dummy length and diameter of the dummy cannulae placed into the cannulae was the same length as the injection cannulae (Plastics One, Bilaney, UK). This design aimed to minimise tissue damage occurring at the time of infusions, when the injection cannulae were inserted into the brain.

Analgesia was administered by subcutaneous injection of Small Animal Rimadyl (0.08 ml/kg bodyweight) (Pfizer, UK) in 1ml sterile saline at the end of the surgical procedure. Large Animal Rimadyl (Pfizer, UK) was given at 1 ml/L in their drinking water in Es2 & 3 but not E5. All rats were allowed a recovery period of at least 7

days to allow them to regain their pre-surgery weights before food restriction and behavioural testing commenced.

3.3 Drugs and microinfusions

3.3.1 Drugs

Sterile 0.9% saline (NaCl) (Baxter, UK) and phosphate-buffered artificial cerebrospinal fluid (aCSF) were used as infusion vehicles and for dissolving drugs. Drug concentrations for infusions were: 5 mg/ml (15.4 mM) and 3 mg/ml (3 mM) of the D_1/D_5 antagonist SCH23390 [*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride, Tocris, UK], 5.9 mg/ml (30 mM) of the competitive NMDA receptor antagonist D-AP5 [D-(-)-2-Amino-5-phosphonopentanoic acid, Tocris, UK] and 0.89 mg/ml (3mM) of the competitive AMPA/kainate receptor antagonist CNQX (6-Cyano-7-nitroquinoxaline-2,3-dione, Tocris, UK). The pH of the drug solutions was adjusted to 7.2 by the addition of 1 M NaOH solution (for D-AP5) or of concentrated phosphoric acid (for CNQX). Drug solutions were prepared in larger quantities, divided into 50 μl aliquots, and kept frozen at - 20°C until use.

3.3.2 Mock infusion

Twenty four hours before the main infusions, there was a mock infusion session to habituate the rats for actual drug infusions. Rats were restrained manually and their dummy cannulae were removed. The injection cannulae were placed into the guide cannulae but no solutions were infused into the rats' brains. The injection cannulae were left in place for 5 min.

3.3.3 Main infusion

For bilateral infusions, rats were restrained manually and infusions into both hemispheres were performed simultaneously in a control testing room. Prior to infusion, the injection cannulae tips were dipped into alcohol and then rinsed in saline. The tips of these infusion cannulae protruded 0.5 mm from the ends of the guide cannulae within the brain, and were connected to SGE microsyringes (World Precision Instruments, USA) on a microinfusion pump (Sp200i syringe pump, World Precision Instruments, USA) via flexible polyvinyl chloride tubing (PKG tubing PE20, Plastics One, UK). The flexible tubing was rinsed through with bottled water for injections (Hameln, UK). In E2, NaCl (1 μl/hemisphere) was infused at a rate of 0.2 μl/min over 5 min, after which the infusion cannulae were left in place for a further 2 min to ensure all droplets of drug solution entered the brain. In E3, SCH23390, D-AP5 and NaCl (control infusion for SCH23390 & D-AP5) (1 μl/side) were infused at a rate of 0.2 μl/min over 5 min, after which the infusion cannulae were left in place for a further 2 min to ensure all drug solution entered the brain. In E5, D-AP5, CNQX and NaCl (control infusion for D-AP5 and CNQX) (0.5 μl/hemisphere or 2 μl/hemisphere) were infused at a rate of 0.4 μl/min over 1 to 5 min, after which the infusion cannulae were left in place for a further 1 min. The dummy cannulae were then rinsed with alcohol and saline and then placed back on the rats' headcap.

3.4 Behavioural training

3.4.1 Apparatus

An 'event arena' is a square shaped open field 'maze' in which event-context associations can be studied (Figure 3.2A). Two event arenas in which rats were trained to find flavoured food were made of plexiglass and placed in adjacent laboratory rooms containing a number of prominent and distinctive cues. They measured 1.6 m x 1.6 m and had floors containing a 7 x 7 grid of 49 circular holes (6 cm diameter) at 20 cm spacing. Six of these holes contained a sand-well and the remainder were covered with plastic lids. Sand-wells were placed into holes of different locations. The entire floor was covered with 3 cm of sawdust. Two distinctive landmarks (*Arena 1:* a glued stack of golf balls and a pyramid; *Arena 2:* a different pyramid shape, and a rectangular shape with a ball on top) were placed in 2 locations: row 4, column 2; and row 4, column 6. Rats had access to the arena from one of four plexiglass start boxes (25 cm x 25 cm x 25 cm). The boxes were covered with black paper to darken the interiors as rats prefer darker environments to bright ones. Each box had remote controlled sliding doors to allow the experimenter control over the rat's access to the arena. Inside each box, there were 2 sand-wells. One sand-well was for the flavoured cue pellet (Bioserv, USA) and the other sand-well contained water for the rat to drink.

The illumination of the laboratory room was maintained at a moderate level of brightness by wall mounted halogen lamps. The rats were observed by means of charge-coupled device (CCD) cameras connected to video recorders and their movements tracked by means of custom LabView (National Instruments, USA) computer software (P. Spooner, University of Edinburgh) which recorded the time

taken to locate the correct sand-well, the time spent digging at each sand-well and other performance parameters (Figure 3.2A & B).

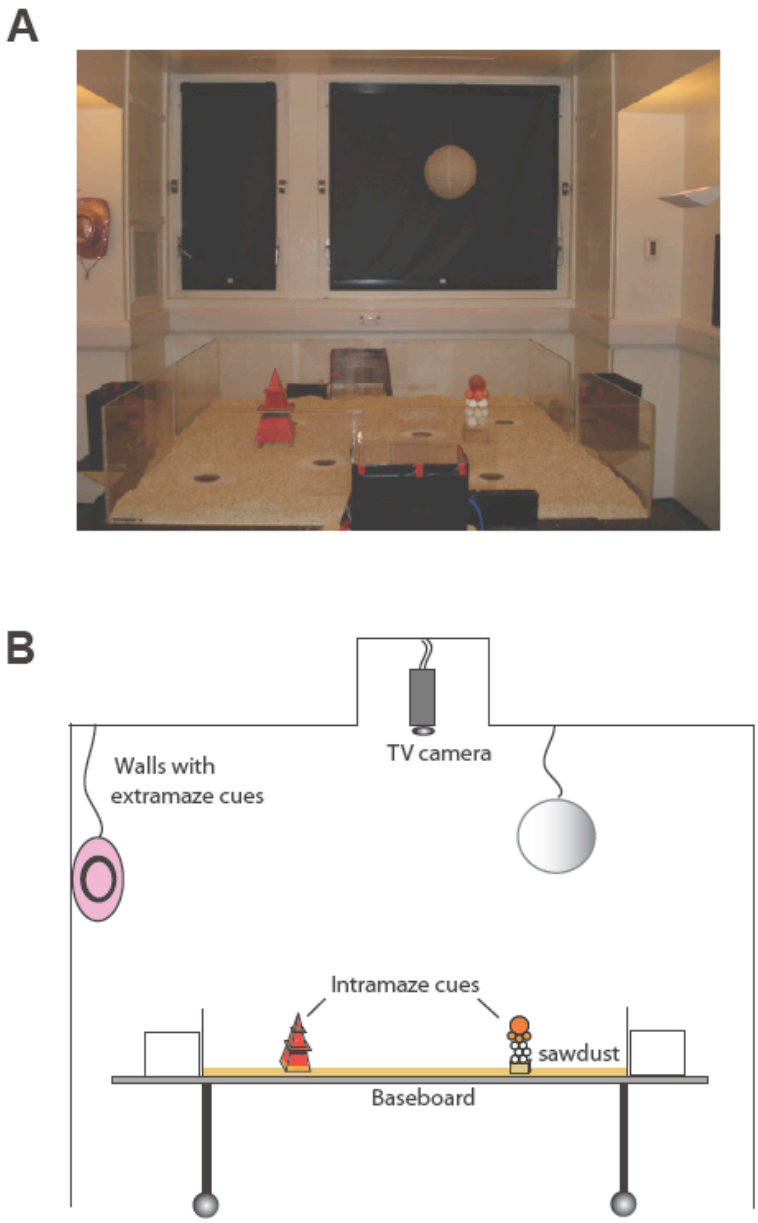

Figure 3.2 (A) Photograph of an event arena and experimental room. (B) Sideview schematic of an event arena and experiment room.

The plastic sand-wells that could be inserted into the holes in the arena floors had an inner diameter of 6 cm and a total depth of 5 cm. There were 3 main steps taken to ensure paired-associates (PAs) learning and recall in the absence of artifactual cues. *Firstly*, in both training and recall, the sand-wells contained a removable metal mesh grid 3.5 cm from the top, under which 1 gm of each of 6 flavour pellets were placed to provide comparable olfactory cues at each sand-well (Figure 3.3A).

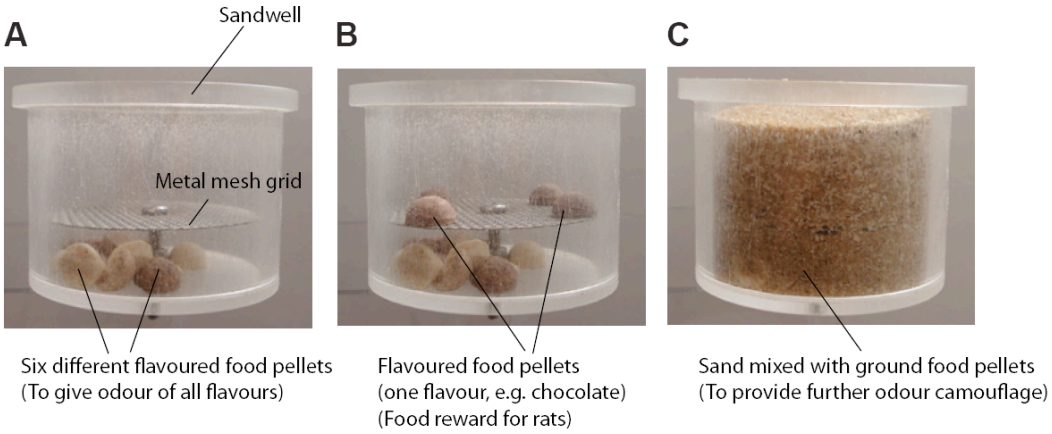

Figure 3.3 (A) An empty sand-well with a metal mesh grid. There were 6 different flavoured food pellets below the mesh to give odour of all flavours. (B) A sand-well with four 0.5 g flavoured food pellets (one flavour) on top of the mesh which are rewards for the rats. (C) A sand-well was filled with flavoured sand to provide further odour camouflage and to cover the rewarded pellets.

Secondly, the sand-well was filled with a mixture of 90 g sand plus ground-up food pellets (25 g per 2.5 kg sand), which included all the flavours used in the experiments (Figure 3.3C). *Thirdly*, reward was available during recall trials of training but in occasional 'probe trials', the flavoured food pellets above the metal mesh grid were omitted. In those trials, the duration of time spent digging was measured, as described in behavioural procedures. These olfactory control precautions were designed to ensure that when the rats entered the arena, their search was guided only by memory of the location of the flavour with which it was associated and not by odour emanating from the sand-well containing the food-reward.

To make food reward available in a sand-well, four 0.5 g pellets were hidden on the upper surface of the metal grid (Figure 3.3B) under the sand layer (Figure 3.3C). The

rats could then dig through the sand mixture to search for and retrieve each food pellet. The sand and food mixture was renewed every 1 to 2 sessions. The cue given to the rats to facilitate recall of correct sand-well was a flavoured food pellet, made available to the rats in their start-boxes.

3.4.2 Training protocol

Shaping, habituation and pre-training

On the day before habituation began, rats were shaped (trained) to dig for food in sand-wells in their home cages.

On session -8 of habituation (day 1), rats were put into the arena containing no sand-wells for 10 min to explore the arena and intramaze cues.

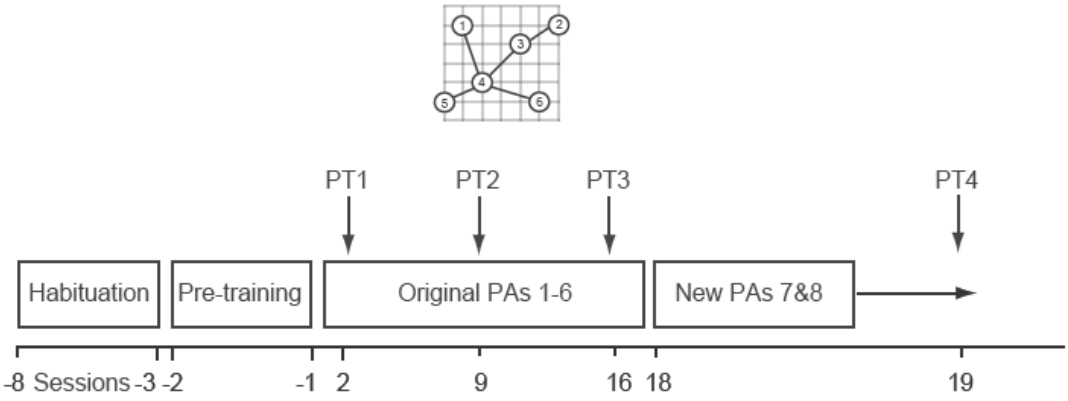

Figure 3.4 Experimental design of paired-associates (PAs) schema paradigm. After habituation and pre-training, rats were trained with original PAs and then new PAs. Several non-rewarded probe trials (PT 1-4) were scheduled to test the cued-recall of flavours of rats.

For sessions -7 to -3 (Figure 3.4), rats received one daily habituation trial. In this trial, rats were put in a startbox and were given a 0.5 g 'cue' control (non-flavoured) food pellet to eat. After the rats finished eating the pellet (typically around 30 s), they were allowed 10 min access to the arena. The rats started from a different startbox in each session. Rats were trained to search and dig for control food pellets in the sand-well in the centre location of the event arena. On session -7, one 0.5 g pellet was placed on

top of the sand-well. The rats collected the pellet and took it back to the start box. In the habituation trials that followed, one 0.5 g pellet was placed on top of the sand-well and the other buried in the middle of the sand-well. And on session -3, three 0.5 g pellets were at the bottom of the sand-well. By the end of habituation, all rats were running quickly into the arena, collecting pellets and returning to the start box to eat each pellet. Habituation normally lasted for 6 sessions (sometimes 7 sessions depending on the performance of the rat). This spontaneous food carrying behaviour is typical of rats (Maaswinkel and Whishaw, 1999). Also, as rats carry their pellets back and forth between the start box and sand-wells, this may help them to remember the route to retrieve the correct pellet location more efficiently.

The aim of the pre-training was to shape the rats to learn the PAs of different flavours in different locations. It consisted of 2 sessions with 3 out of the original 6 flavours introduced in each session. Rats were given one flavour per place PAs for 1 trial per session.

Acquisition of original schema of 6 flavour location paired-associates

Main Training (Sessions 1-13). The key feature of the protocol was the concurrent training of 6 flavour-place PAs each day. Each PA was presented to each rat for 1 trial/session [PA1 = flavour 1 (F1) at location 1 (L1); PA2 = flavour 2 (F2) at location 2 (L2); PA3 = flavour 3 (F3) at location 3 (L3); PA4 = flavour 4 (F4) at location 4 (L4); PA5 = flavour 5 (F5) at location 5 (L5); PA6 = flavour 6 (F6) at location 6 (L6)]. The arrangements of PAs were different in different experiments.

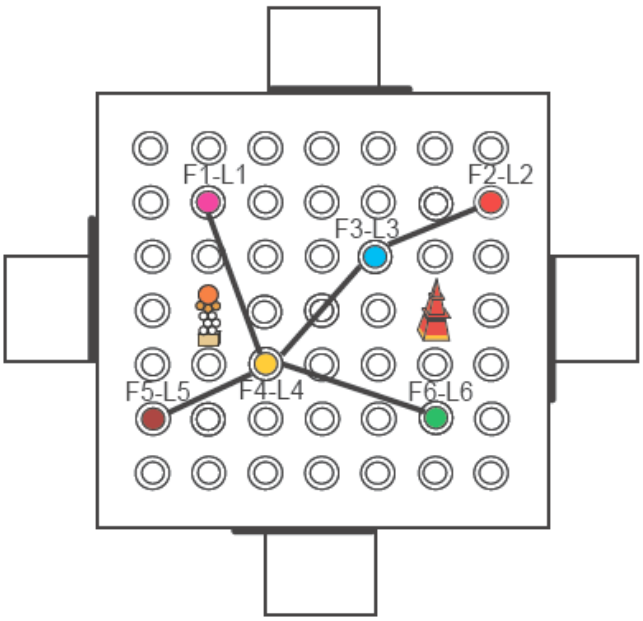

Figure 3.5 The spatial arrangement of the 6 PAs of the standard original schema (F, flavour; L, Location).

On any trial, all 6 sand-wells were accessible, but only one contained the appropriate flavoured reward, with the other 5 containing only the sand and masking flavours mixture. A trial began with the rat receiving a 0.5 g 'cue' flavoured pellet in one of the start boxes. After a period of 30 s in the start box during which the rats ate this cue pellet, the door was remotely opened (Figure 3.6A). Initially, the rats would run into the arena, and explore at one or more sand-wells until they find the correct location (Figure 3.6B) containing 3 food pellets of the same flavour as the cue (0.5 g each). These were typically carried back to the start box by the rat, pellet by pellet, and eaten in turn. After the rat returned to the start box with the 3rd pellet, the door was closed. A short period of eating time was allowed before the rat was carried back to its home cage. Rats typically trained on alternate days in two cohorts, with each cohort receiving 3 training sessions per week (cohort 1: Monday, Wednesday and Friday; cohort 2: Tuesday, Thursday and Saturday).

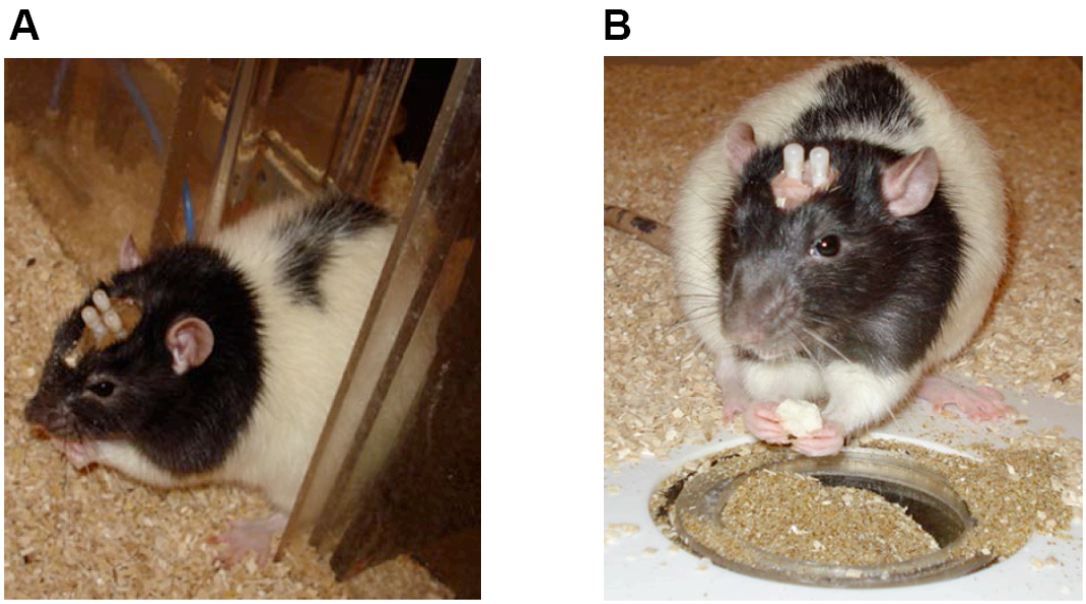

Figure 3.6 (A) A rat coming out from a startbox. (B) A rat retrieving a pellet in a correct sand-well location.

As training progressed, trials became quicker (reaching an asymptote of 3 to 4 min per trial). As all rats were trained consecutively, the inter-trial interval for an individual rat between successive flavour-place pairings was circa 45 min to 1 hr, resulting in a total daily session time of up to 6 h. Rats were returned to their home cage between trials. The various possible sequences of different flavoured pellets across 6 trials within a session were carefully counterbalanced across rats and sessions. On a given session, half the rats were cued from one start box (e.g. North) while the other half were cued from the opposite start box (e.g. South). The start locations were then pseudo-randomly assigned (North, South, East or West) across training sessions.

Non-rewarded probe tests

To examine cued-recall memory, several non-rewarded probe tests (PT 1-3) were scheduled. During these tests, all 6 sand-wells were open as usual, and the rats were free to dig in any of them, although none contained food reward. The rats were cued

with a single flavour as usual, and then allowed into the arena for a total of 120 s. Preferential digging at the cued location was used as an index of memory; better memory was indicated as time spent digging in correct location. The digging time at each of the sand-wells was measured using the computerised tracking system. As noted above, probe tests also served as a further control for artifacts and uncontrolled factors. The lack of reward at the target sand-well precluded any olfactory guidance to the correct location. Additionally, to prevent the rats using olfactory traces from a previous trial to locate the reward, the sand used in the wells in different locations was mixed between each trial to distribute any odours evenly around the arena and the sawdust surrounding each sand-well distributed around the arena between trials. None of these manipulations affected performance.

Performance measures

The rat's task was to locate, or choose the correct sand-well among the 6 sand-wells, and this 'choice' was a main dependent variable. During each trial of the experiment (training and probe tests), the experimenters recorded a 'choice' only when a rat placed its front paw on or into a sand-well. If a rat ran past or merely sniffed quickly in the vicinity of a sand-well, this was not considered as a choice. In rare cases, it was difficult to tell from the video monitors whether or not the rats had made a choice as defined above. In this case, when the experimenters entered the room at the end of a trial, they checked carefully if there were any traces of digging, that is, whether the sand had been displaced around the sand-well(s).

Several parameters were then measured.

1. Choice of sand-well(s) (the number of incorrect sand-wells that rats dug in before choosing the correct sand-well) up to and including the correct one.

2. Latency: Time before digging commenced at the correct well (second). The time was measured from when the rat left the start box until it reached the correct sand-well.

3. Performance index (PI): the number of errors converted to a PI score using 100-[100 × (errors/5)].

4. Digging time in PTs: the time spent digging at each of 6 sand-wells over 120 s. Although not rewarded *during* the 120 s probe test time, the rats were given 3 half pellets (correct flavour) in the correct location at the end of each probe test to limit extinction. If rats did not dig during the 120 s, the experimenter waited for 180 s before terminating the trial.

Rapid acquisition of novel flavour location paired-associates

Having learned 6 PAs in a schema, the rats were trained normally for 1 session before an investigation of whether they could acquire 2 new PAs in a single trial. During this, the sand-wells for PA1 and PA6 were closed, and replaced by 2 new paired-associates, PA7 (L7) and PA8 (L8) at neighbouring, but not identical, locations. The rats were trained for 6 trials in a single session with the 2 new PAs and the previous 4 PAs with the trial sequence positions of the new PAs counterbalanced across the rats. The reason for introducing 2 new PAs rather than just a single PA was to ensure that any preferential digging seen at the location of a new associate was not merely a novelty effect. If such an effect was due only to novelty, the rats would be expected to dig preferentially at both of the two new locations rather than the remaining original locations in a probe test carried out later. In contrast, if the rats rapidly learned each of the 2 new PAs, they would preferentially visit only the new location cued by the appropriate new flavour that was given in the start box. Thus, in the probe test

scheduled 24 hr later, the performance measures were designed to contrast digging time spent at the new cued location, with that for both the new non-cued location and the original non-cued locations.

Experiments 2, 3 & 5

3.5 Perfusion and Histology

All rats were terminally anesthetised with Euthanal (Merial, UK) and then perfused intracardinally with 0.9% saline, followed by 4% formalin. The brains were removed and stored in 4% formalin for several days. Coronal sections (30 μm) were cut using a cryostat with one in every 5 sections recovered for histological analysis. These sections were mounted on slides, stained with cresylviolet, and coverslipped using DPX (mixture of distyrene, tricresyl phosphate, and xylene). The sections were examined under a light microscope with 20-fold magnification to verify cannulae placements. For each brain, the infusion site was plotted by determining the deepest point at which tissue damage was evident and marking this location on the appropriate coronal sections from the Paxinos and Watson (1998) rat brain atlas.

Experiment 4

3.6 Immunohistochemistry

3.6.1 Tissue (sections) preparation

Ninety minutes following the training of the 5th trial in the schema task, rats were deeply anesthetised with Euthatal (Harlow, Essex, UK) and perfused transcardially with 0.1 M phosphate-buffered saline, pH 7.4 (PBS) and then 4% paraformaldehyde (PFA) in PBS. Brains were removed, fixed overnight in PFA and then transferred to

Falcon tubes containing 30% sucrose in PBS and kept at 4°C until they sank to the bottom. Coronal brain sections (40 μm) were cut on a freezing sliding microtome (Bright, UK) and divided into 6 sets. Each of these sets included sections at 240 μm intervals and collected into an antifreeze solution and maintained at -20°C for later processing.

3.6.2 Zif268 labeling

Free-floating sections were rinsed with PBS (2 × 10 min) washes to remove the antifreeze solution. Sections were then treated with 0.3% hydrogen peroxide in PBS for 30 min to inhibit endogenous peroxidase. Sections were then rinsed in PBS 3 times for 10 min each, after which they were incubated in Zif268 primary antibody (Santa Cruz Biotechnology, USA) diluted (1:3000) in blocking solution containing 2% normal goat serum, 0.2% Triton X-100 and 0.1% bovine serum albumin, all dissolved in PBS and stored overnight at room temperature. Sections were then rinsed in PBS 4 times for 10 min each, after which they were incubated with biotinylated goat anti-rabbit secondary antibody (Vector Laboratories, USA) diluted (1:2000) in blocking solution for 2 hr at room temperature. Sections were once again washed 4 times with PBS for 10 min each and incubated in avidin-biotinylated peroxidase complex solution (ABC Kit; Vector Laboratories, USA) for 1 hr at room temperature. Sections were then rinsed four more times in PBS for 10 min each. The reaction was then visualised using 3, 3'-diaminobenzidine (DAB substrate kit; Vector Laboratories, USA) until suitable staining developed. Sections were then give a final 3 rinses in PBS, after which they were mounted on slides (Superfrost Plus, VMR international, Belgium), dehydrated through a graded series of alcohols, and coverslipped.

Chapter 4

Experiment 1 - Causal role for schemas in learning paired-associates schema paradigm

4.1 Introduction

As mentioned in the introduction (Chapter 2), the original study of the series examining flavour-place paired-associates (PAs) schema investigated the possible influence of prior knowledge on the time course of systems consolidation for newly acquired information (Tse, Langston et al., 2007). When learning PAs, encoding and retrieval (initially) relies on the HPC. After rats had acquired 6 PAs and also perhaps a 'schema' of flavour-place PAs into which new PAs might be rapidly integrated, retrieval of this newly acquired knowledge became HPC-independent between 3 to 48 hours. This indicates that systems consolidation occurs very rapidly when new information can be readily integrated within 'schemas' of prior knowledge.

However, while consistent with the schema concept, the original study, at this juncture, had not addressed the question of whether a schema is necessary for this rapid memory consolidation. There are two distinct possibilities relating to this issue: (1) the rats find it increasingly easier over the course of training to encode, store and/or consolidate individual PAs as a result of increasing familiarity with the context of learning. Hence, in this case, the schema concept would be unnecessary, or (2) the rats require an activated schema to facilitate rapid memory consolidation of new PAs. To contrast these alternatives, rats were trained concurrently in 2 event arenas, each in a separate room. In one room, rats experienced a "consistent" set of PAs (schema) and in the other room, they experienced an "inconsistent" set of PAs. In the consistent

schema room, the flavours and location of each PA over sessions was kept constant; but for the inconsistent schema room, the location of each flavour changed every 2 sessions. That is, in the inconsistent schema room, the animals experienced the same 6 flavours and visited the same 6 locations in the arena, but the matching of flavour to places was inconsistent across sessions. The experimental hypothesis was that when rats experience a "consistent" schema, they will learn new PAs more readily than if they experience an inconsistent one. The alternative hypothesis was that the experience of learning PAs and familiarity with the 2 rooms will result in rapid learning in both environments.

This study has been published as part of Tse, Langston et al. (2007), a manuscript of which is provided in Appendix 2.

4.2 Materials and Methods

4.2.1 Subjects

Nine adult male Lister-hooded rats (Charles River, UK) were used in this experiment as described in General Methods (Chapter 3).

4.2.2 Apparatus

Two event arenas in which rats were trained to find flavoured food are described in General Methods (Chapter 3).

4.2.3 Behavioural training

The time course of this experiment is shown in Figure 4.1. After habituation and pre-training, rats were trained in consistent (C) and inconsistent (I) schema.

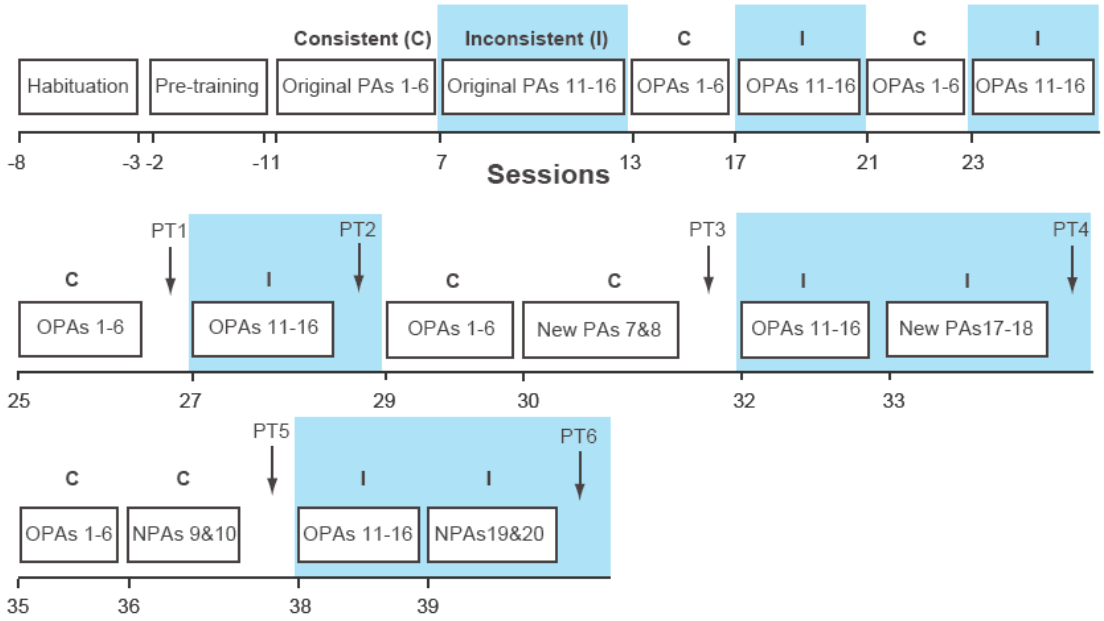

Figure 4.1 Timeline showing the design of Experiment (E)1: Schema training and learning new paired-associates in consistent and inconsistent schema conditions. (C, consistent schema; I, inconsistent schema; OPAs, original paired-associates; NPAs, new paired-associates. PT, probe trial)

4.2.3.1 Habituation and pre-training

The procedures for habituation (Sessions -8 to -3) and pre-training (Sessions -2 to -1) of the rats are described in General Methods (Chapter 3).

4.2.3.2 Training of consistent and inconsistent PAs schemas

Initial training (Sessions 1-24). Rats were trained concurrently on the 2 event arenas in 2 separate rooms. On Sessions 1-6, in one arena, rats were trained on a "consistent" schema in which flavours 1 to 6 were placed consistently at locations 1 to 6 respectively (Figure 4.2A). The 6 flavour-location PAs used were: PA1 (Rum in L1), PA2 (Strawberry in L2), PA3 (Ginger in L3), PA4 (Banana in L4), PA5 (Chocolate in L5) and PA6 (Bacon in L6). The rats were trained with these PAs for 6 sessions (Figure 4.2C). On Sessions 7-12, these same rats were then introduced to another event arena ("inconsistent schema", in another room but with the room allocation counterbalanced) in which they were confronted with another set of 6 flavours in a different geometric arrangement of 6 locations (Figure 4.2B). In this room, however, the mapping of flavours to locations was changed every 2 days (Figure 4.2D). Each flavour was assigned to one location for 2 sessions, and then pseudo-randomly swapped between the 6 other locations for successive sets of 2 sessions. Briefly, rats were trained in Sessions 7 & 8 with PA11 (Pineapple in Location (L)11), PA12 (Brandy in L12), PA13 (Coconut in L13), PA14 (Very Berry in L14), PA15 (Paprika in L15) and PA16 (Butter in L16); in Sessions 9 & 10, the mapping was changed such that Paprika (used to be in L15) was moved to L11, Very Berry in L12, Brandy in L13, Butter in L14, Pineapple in L15 and Coconut in L16; in Sessions 11&12, the mapping of flavours to locations was switched again. This constant re-mapping using a common set of 6 flavours and 6 locations constituted an inconsistent PAs schema.

The initial 12 sessions of training (6 on the consistent schema followed by 6 on the inconsistent schema) were followed by another 4 sessions on each type of schema, then 2 sessions on each type of schema. This training ensured that, even in the inconsistent schema room, there was no violation of the principle that PAs assignments could be stable for a period of time (2 days of training).

On Session 25, rats were trained in the consistent schema. Twenty-four hours later a probe trial (PT) was conducted. Then, on Session 27, rats were trained in the inconsistent schema.

4.2.3.3 Probe tests for the original flavour-place PAs

PTs were conducted on Sessions 26 (PT 1) and 28 (PT 2) to test whether the rats could recall the PAs in both schemas (Figure 4.1).

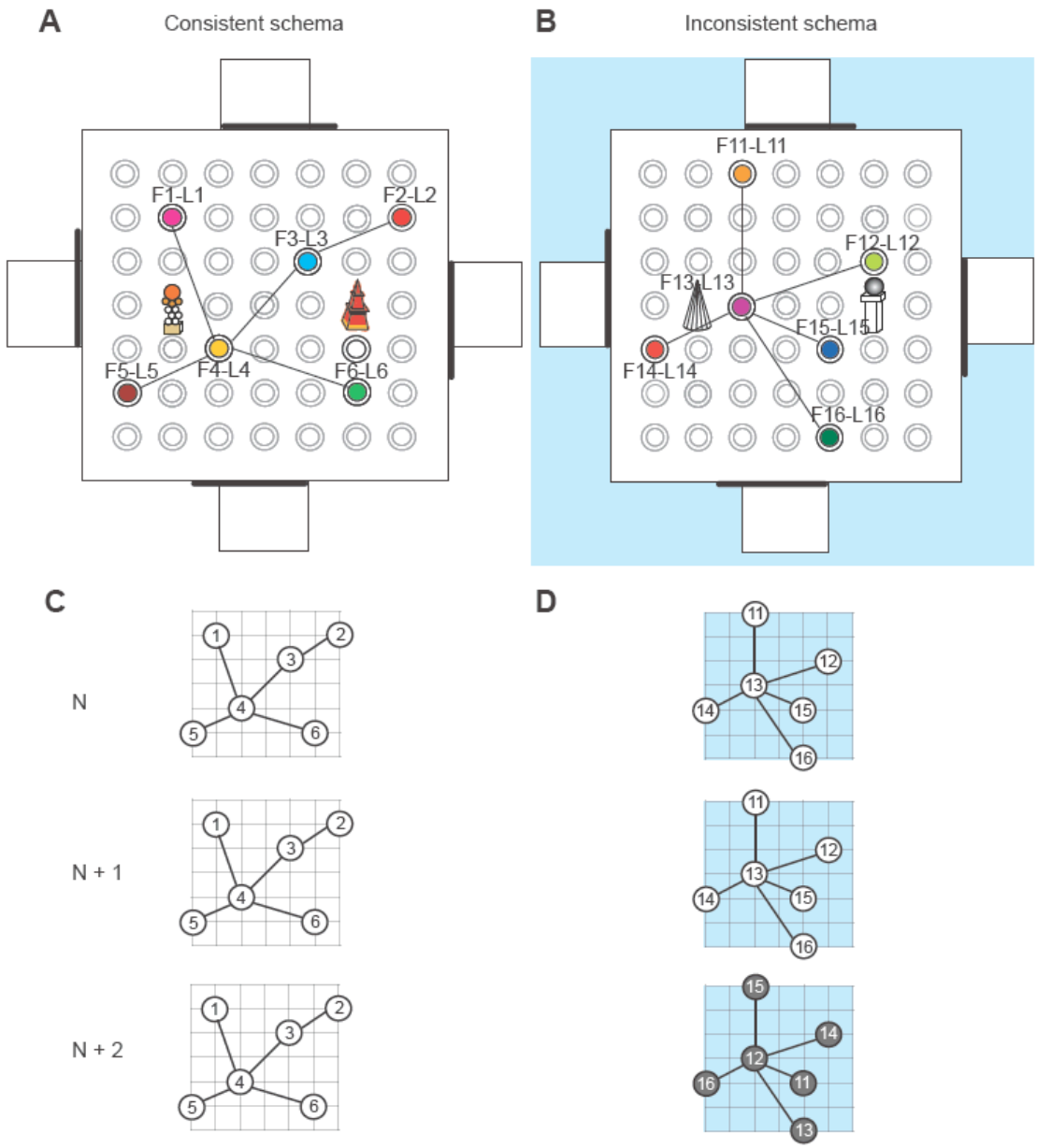

Figure 4.2 (A) (B) Consistent and inconsistent schema layout. (C) With the consistent schema, the mapping of flavours to places is consistent across sessions (N = session). (D) inconsistent schema used a common set of 6 flavours and locations that were associated for 2 sessions but then changed every third session (see N + 2, shaded blue).

4.2.3.4 Acquisition of novel PAs in consistent and inconsistent schemas

Training and PTs for novel PAs (Sessions 29-40). The next step was to establish whether or not the rate of learning new PAs differed when it occurred in the consistent compared to the inconsistent schema. In this phase, which began in Session 29, the animals had been trained on 4 successive sequences of 3 training sessions (Figure 4.1).

On Session 29 (consistent schema), rats were further trained in the consistent-context of PAs 1 to 6. On Session 30, the sand-wells for PA1 (Rum in L1) and PA6 (Bacon in L6) were closed, and replaced by 2 novel flavours: PA7 (Marshmallow in L7) and PA8 (Apple in L8) at neighbouring but not identical locations (Figure 4.3). The rats were then trained for a session consisting of only 2 trials, each with one novel PA. The order of presentation of the 2 new PAs trials was counterbalanced across rats. On Session 31, a non-rewarded PT was conducted for these novel PAs.

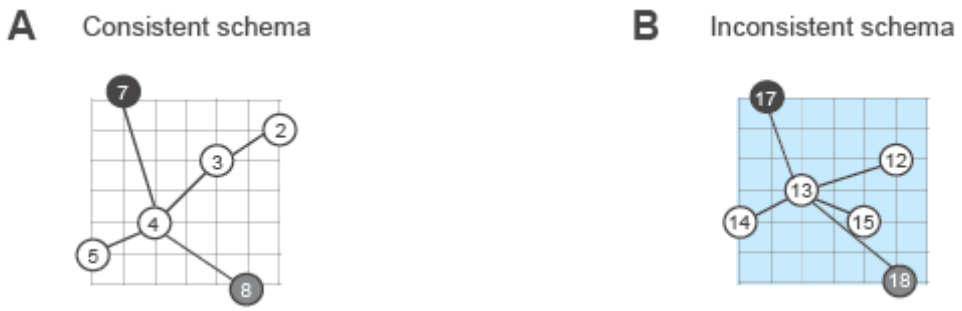

Figure 4.3 New PAs in the consistent and inconsistent schema layouts. In the consistent schema, the sand-wells for PA1 (Rum in L1) and PA6 (Bacon in L6) were closed, and replaced by two novel flavours: PA7 (Marshmallow in L7) and PA8 (Apple in L8) at neighboring but not identical locations. In the inconsistent schema, the sand-wells for PA11 (Anise in L11) and PA16 (Orange in L16) were closed, and replaced by two novel flavours: PA17 (Marshmallow in L17) and PA18 (Apple in L18) at neighbouring but not identical locations.

This 3 session sequence was then repeated in the inconsistent context (sessions 32-34) using PAs 1-16. On Session 32 (inconsistent schema), rats were trained with further inconsistent context of PAs 11-16. On Session 33, the sand-wells occupying L11 and L16 were closed, and 2 novel PAs were created: Anise at L17 and Orange and L18

(Figure 4.3) at neighbouring but not identical locations. The rats were then trained for only 2 trials, which consisted of just the 2 new PAs. The order of presentation of these 2 new flavour trials was counterbalanced. The row and column locations of L7 and L8 in the consistent event arena and of L17 and L18 in the inconsistent arena were identical (Figure 4.3). On Session 34, a non-rewarded PT was conducted for these novel associates. This 3 session sequence was repeated in the consistent and then the inconsistent with PAs 9 and 10 and PAs 19 and 20 (Figure 4.4). The locations used for these 2 new PAs in both consistent and inconsistent schema were identical in each arena. The sequence ended with PT6 on Session 40 (Figure 4.1).

Figure 4.4 New PAs in consistent and inconsistent schema layout. In the consistent schema, the sand-wells for PA1 (Rum in L1) and PA6 (Bacon in L6) were closed, and replaced by two novel flavours: PA9 (Grape in L9) and PA10 (Chocolate and mint in L10) at neighboring but not identical locations. In the inconsistent schema, the sand-wells for PA11 (Anise in L11) and PA16 (Orange in L16) were closed, and replaced by two novel flavours: PA19 (Almond in L19) and PA20 (Brandy in L20) at neighbouring but not identical locations.

4.2.4 Statistical analyses

Several measures of performance were assessed. These were: number of errors in main training, converted into a performance measure, and time spent digging in each sand-well during probe trials. Differences between the groups were analysed using independent t-tests and ANOVAs analyses, where appropriate.

4.3 Results

4.3.1 Differential acquisition of consistent and inconsistent schemas

Choice performance (performance index) gradually improved in the consistent schema room but not in the inconsistent schema room (Figure 4.5). Above chance performance was consistent from session 15 onward ($p < 0.01$ for each comparison with chance level of performance). Those same animals failed to learn PAs (on some sessions performance was above chance, e.g., Session 27, but performance never rose above 60% correct) when in inconsistent schema room.

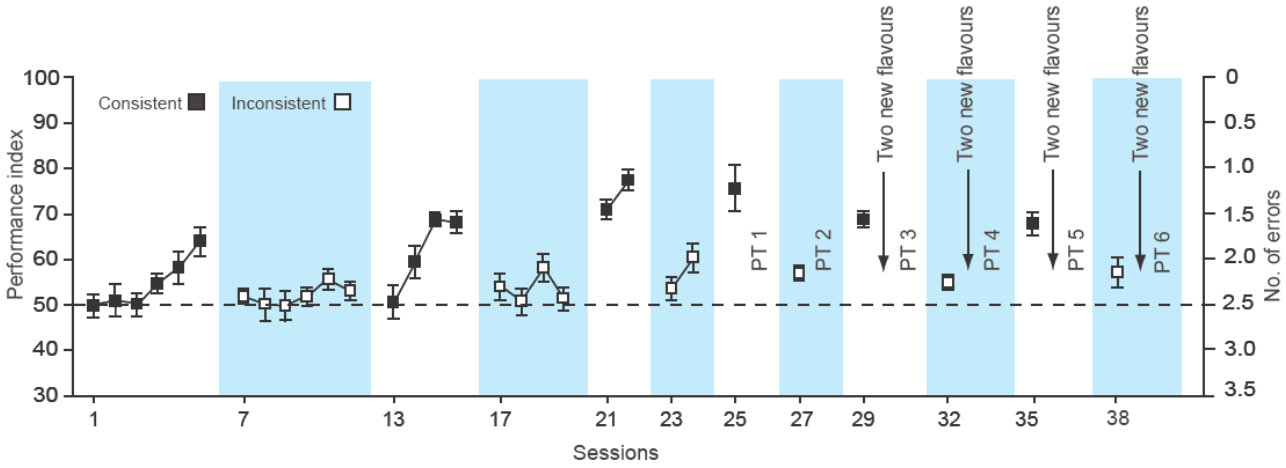

Figure 4.5 Differential acquisition of consistent and inconsistent schema. Effective acquisition by rats occurred when mapping of flavours to places remained consistent, with 6, 4, 2, and then single sessions (session 1 to 40; white background). Rats failed to learn a series of inconsistent schemas (blue-background).

4.3.2 Cued recall probe trials of original PAs

The rats showed effective cued recall of the appropriate location when given an original flavour cue in the consistent schema (PT1: $t = 10.9$, df = 8, $p < 0.001$), but performed at chance level (PT2: $t < 1$) in the inconsistent schema (Figure 4.6).

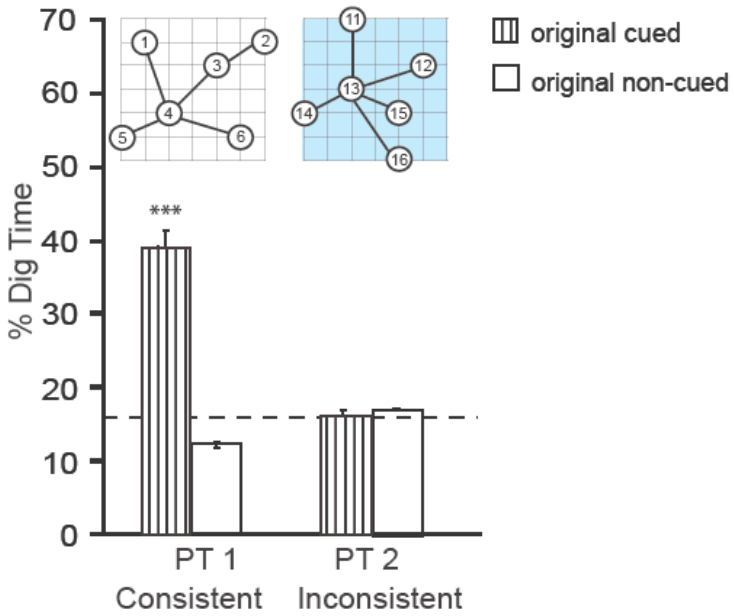

Figure 4.6 Cued recall PTs in consistent and inconsistent schemas. Rats displayed preferential digging in the PTs at the cued locations for the consistent schema but not for the inconsistent schema. ***$p < 0.001$

4.3.3 Acquisition and cued recall probe trials of newly learned PAs

The rats showed successful acquisition and 24 h retention of the new PAs only when encoding occurred in the consistent schema context (PTs 3 & 5) but not in the inconsistent schema (PTs 4 & 6; Group x Location interaction: F = 13.92, df = 1.64/26.30, $p < 0.001$) (Figure 4.7).

Approach latencies from the start box to the correct sand-well during these probe trials were equivalent in the consistent (20.9 ± 1.9 s) and inconsistent (20.0 ± 2.5 s) contexts, indicating comparable motivation to perform each task.

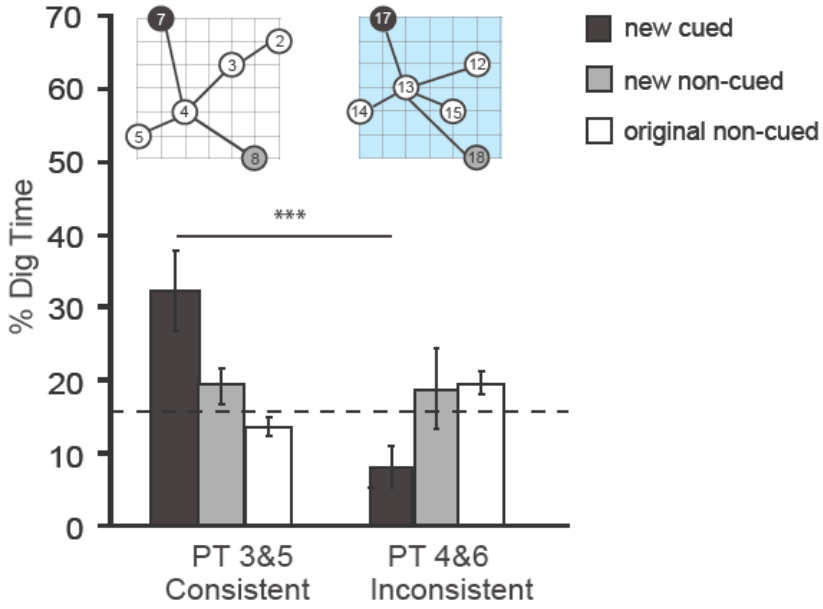

Figure 4.7 New PAs cued recall probe trials. Performance 24 h after exposure to the two new PAs when the animals would be encoding information using a consistent activated schema (PTs 3 & 5) was consistently good to the cued new location, whereas performance after use of an inconsistent schema was not (PTs 4 & 6). ***$p < 0.001$

4.4 Discussion

4.4.1 Causal role of schemas in learning

As mentioned in the introduction, there are 2 methods that the rats could possibly use to learn PAs in the experiment. They are: (1) context familiarity, or (2) a mental schema.

If rats learn new PAs as isolated events and their speed of learning increases context familiarity as training in this protocol proceeds, their ability to learn new PAs in the 2 contexts (consistent and inconsistent) should be the same. However, if the animals can bring an appropriate activated schema to bear on the process of learning, a difference between the two contexts might be observed. The consistent schema would only be activated in its appropriate context. From the results of the cued recall of new PAs, we observed that rats show successful acquisition and 24-hour retention of new PAs only when encoding occurred in the consistent-schema context. Hence, this experiment strongly suggests that the possession of an activated schema is important in the acquisition of new PAs.

4.4.2 Consistent and Inconsistent schema like study in humans

Bransford and Johnson (1972) demonstrated the idea that merely processing a schema was insufficient; it had also to be activated. Participants in that study were asked to read a passage about procedures of sorting clothes. They found that the first group of participants had considerable difficulty in both understanding and remembering the passage (Bransford and Johnson, 1972). A second group was given the title of the passage, “Washing Clothes” after they had read it. With this title in mind, the passage suddenly made sense, but this group still had great difficulty in recalling what they had read. A third group, however, was given the title before reading the passage and

they remembered almost twice as much as the other groups. This is probably because they could fit the sentences of the passage into a meaningful schema, which enabled them to recall far more of what they had read.

Based on the idea that when new information is congruent with prior knowledge it is better remembered, a recent study by van Kesteren et al. (2010b) attempted to demonstrate the consistent and inconsistent schema concept in humans. They conducted a visuo-tactile learning experiment in which participants learned visual motifs which were randomly associated with word-fabric combinations, representing everyday objects made of fabric. These combinations were either congruent (consistent) or incongruent (inconsistent) with common knowledge, as assessed in an independent study. The following day, participants' brains were scanned using fMRI whilst they recognised the visually presented motifs. They found that congruent associations were remembered better than incongruent ones. They demonstrated that successful retrieval of consistent compared to inconsistent visuo-tactile associations is related to enhanced processing in a medial prefrontal somatosensory network (van Kesteren et al., 2010b). These studies lend support to the results presented in this chapter (E1), which is rats learned new PAs readily in consistent schema but not inconsistent schema.

This chapter highlights the importance that an appropriate, activated schema can have in facilitating the encoding of new information.

Chapter 5

Experiments 2 and 3 - Contributions of hippocampal NMDA and dopamine receptors in novel paired-associates schema memory

This chapter describes 2 experiments (Es); E2 was a pilot experiment to examine if multiple microinfusions into HPC would cause any physical damage to brain tissue. This was a necessary step before undertaking experiments that required multiple infusions, such as these used in E3 and E5. E3 examined the possible roles of hippocampal plasticity in paired-associates (PAs) learning: a role at encoding for NMDA receptors and a role at encoding for dopamine D1/D5 receptors in determining the persistence of memory. These roles were investigated pharmacologically by microinfusion of relevant antagonists.

Experiment 2

5.1 Introduction

The aim of this study was to investigate whether multiple intrahippocampal (iHPC) microinfusions of saline would cause physical damage to the HPC. Before embarking on an experiment involving multiple injections into the brain (Chapters 5 and 7), a simple pilot experiment was undertaken to compare multiple versus single infusions of saline into the HPC. Assuming that saline contributions to any such damage are minimal, by comparing 16 infusions with one injection, it should then be possible to see if there is any mechanical or other physical damage caused by the injections process itself.

5.2 Materials and methods

5.2.1 Subjects

Three adult male Lister-hooded rats (Charles River, UK) were used as described in General Methods.

5.2.2 Surgery

Rats were implanted bilaterally with infusion guide cannulae in the dorsal HPC, as described in the General Methods. After surgery, rats were given a recovery period of 9 days before the start of the infusions.

5.2.3 Drugs and microinfusions

Twenty four hours prior to the first infusions, there was a mock infusion session in which rats were habituated to the restraint necessary for a successful hippocampal infusion. The injection cannulae were placed into the guide cannulae but no solutions were infused into the rats' brains. Each mock infusion lasted 5 min.

Sterile 0.9% saline (NaCl) was used as an infusion vehicle into the HPC. For each rat, one hemisphere was infused once, while the other hemisphere was infused 16 times. For the hemisphere with 16 injections, 2 infusions occurred per session and there were a total of 8 sessions at 48 hr intervals. The infusion procedures were described in detail in the General Methods.

5.2.4 Perfusion and histology

The detailed methods of perfusion and histology were described in General methods.

5.3 Results

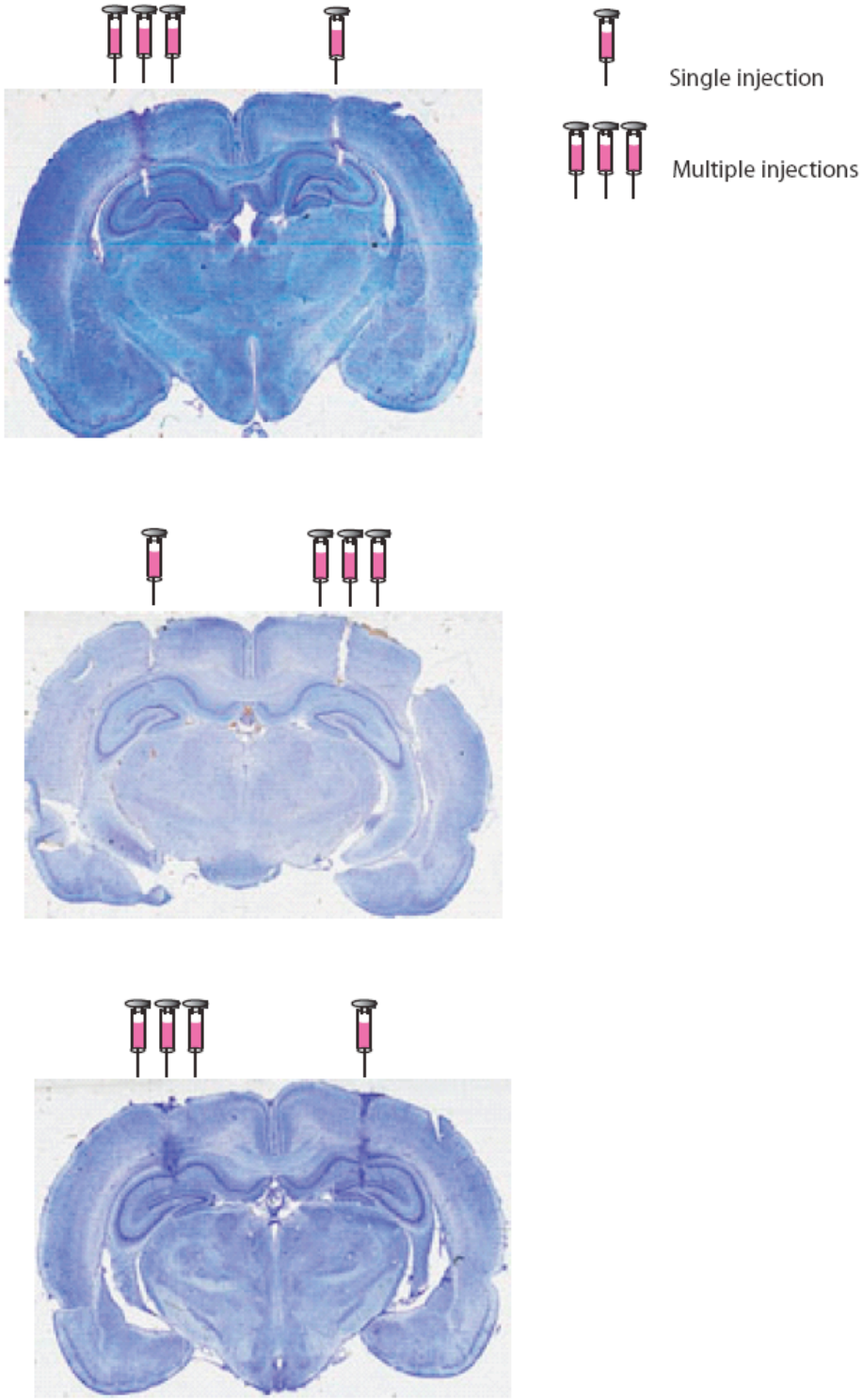

Figure 5.1 Nissl-stained section showing 3 examples of representative cannulae tracks in the dorsal HPC in both hemispheres. Three syringes represent multiple injections, while one syringe represents a single injection.

From the 3 representative sections, it is apparent that those hemispheres that had multiple injections have slightly more damage than those that had only a single injection.

5.4 Discussion

Further experiments including multiple injections were deemed suitable since multiple injections did not appear to cause extensive damage to the brain.

Experiment 3

This experiment was conducted by the author and a previous postdoctoral research associate at the University of Edinburgh, Dr Ingrid Bethus (now at the University of Marseilles). Joint contributions were made towards the surgeries, behavioural training and analysis.

5.5 Introduction

In the original schema study (Tse, Langston et al., 2007), hippocampal dependence for incorporating memory of new PAs into a schema was discovered. However, the mechanisms by which the HPC accomplishes this had not been uncovered.

There were 2 main aims of the study. The first was to examine the possible role of hippocampal plasticity in PAs learning by examining the impact of iHPC infusion of the NMDA antagonist D-AP5 at the time of novel PAs encoding. The second aim was to distinguish 2 possible functions of dopamine (DA) release in the HPC during PAs learning. Two hypotheses were considered: one is that hippocampal DA is critical for encoding and the other is that it is critical for persistence of memory, with the relevant signal transduction events occurring at the time of memory encoding.

5.5.1 NMDA receptors, memory consolidation and schema

The N-methyl-D-aspartate receptor is a subtype of excitatory amino acid receptors widely distributed throughout the mammalian central nervous system (Monaghan and Cotman, 1985; Davis et al., 1992). NMDA receptors in rodent HPC have been shown to be essential for spatial learning and memory (Morris et al., 1986), and also for the induction of long-term synaptic plasticity at various hippocampal synapses (Collingridge et al., 1983; Nakazawa et al., 2004).

5.5.2 Dopamine and Novelty

Dopamine (DA) is a neuromodulator that originates from small groups of neurons in the mesencephalon; the ventral tegamental area (VTA), the substantia nigra and in the diencephalons (Scatton et al., 1980). DA functions as a neurotransmitter, activating the five types of dopamine receptor - D1, D2, D3, D4 and D5, and their variants. Dopaminergic projections are in general very diffuse and reach large portions of the brain. DA has long been implicated in certain forms of conditioning in the striatum. In addition, activity of dopaminergic neurons in the VTA occurs under circumstances of novelty and error detection. A study emphasises that DA transmission is central to learning, because learning occurs best when the stimuli involved are novel (Bassareo et al., 2002).

Dopamine and the HPC

The HPC receives dopaminergic inputs, which come from the VTA and the substantia nigra (Scaton et al., 1980). As the computation of novelty requires the comparison of incoming information with stored memories, this computation might be expected to occur in the HPC. HPC and VTA form a functional loop designed to detect novelty (Legault and Wise, 2001; Lisman and Grace, 2005) and to use this novelty signal to control the entry of behaviourally significant information into hippocampal long-term memory store. This suggests that the HPC may be linked in both directions to the midbrain DA system, which may be a functional link involving the analysis of novelty. In the model suggested by Lisman and Grace (2005), they developed the concept that the HPC and the midbrain dopaminergic neurons of the VTA form a functional loop. The effect of hippocampal dopamine on neuronal plasticity and long term memory formation is mainly mediated by D1 receptors (Jay, 2003; O'Carroll et

al., 2006). A PAs schema task was used to test whether D1 receptors are involved in encoding or persistence of long term memory.

This study has been published as part of Bethus et al. (2010), a manuscript of which is provided in Appendix 3.

5.6 Material and methods

5.6.1 Subjects

Eleven adult male Lister-hooded rats (Charles River, UK) were used in this experiment as described in General Methods.

5.6.2 Surgery

Rats were implanted bilaterally with infusion guide cannulae in the dorsal HPC, as described in General Methods. After surgery, rats were given a recovery period of 7 days before the start of the behavioural procedures.

5.6.3 Drugs and microinfusions

The D_1/D_5 antagonist SCH23390 was used at concentrations of 5 μg/μl (15.4 mM) and 1 μg/μl (3 mM) and the competitive NMDA antagonist D-AP5 was used at a concentration of 5.9 μg/μl (30 mM). SCH23390, D-AP5 and NaCl (control vehicle for SCH23390 & D-AP5) (1 μl/side) were infused at a rate of 0.2 μl/min over 5 min, after which the infusion cannulae were left in place for a further 2 min to ensure all drug solution entered the brain. The infusion procedures were described in detail in the General Methods.

5.6.4 Apparatus

The event arena in which rats were trained to find flavoured food, as described in General Methods, was used.

5.6.5 Behavioural training

The time course of this experiment is shown in Figure 5.2.

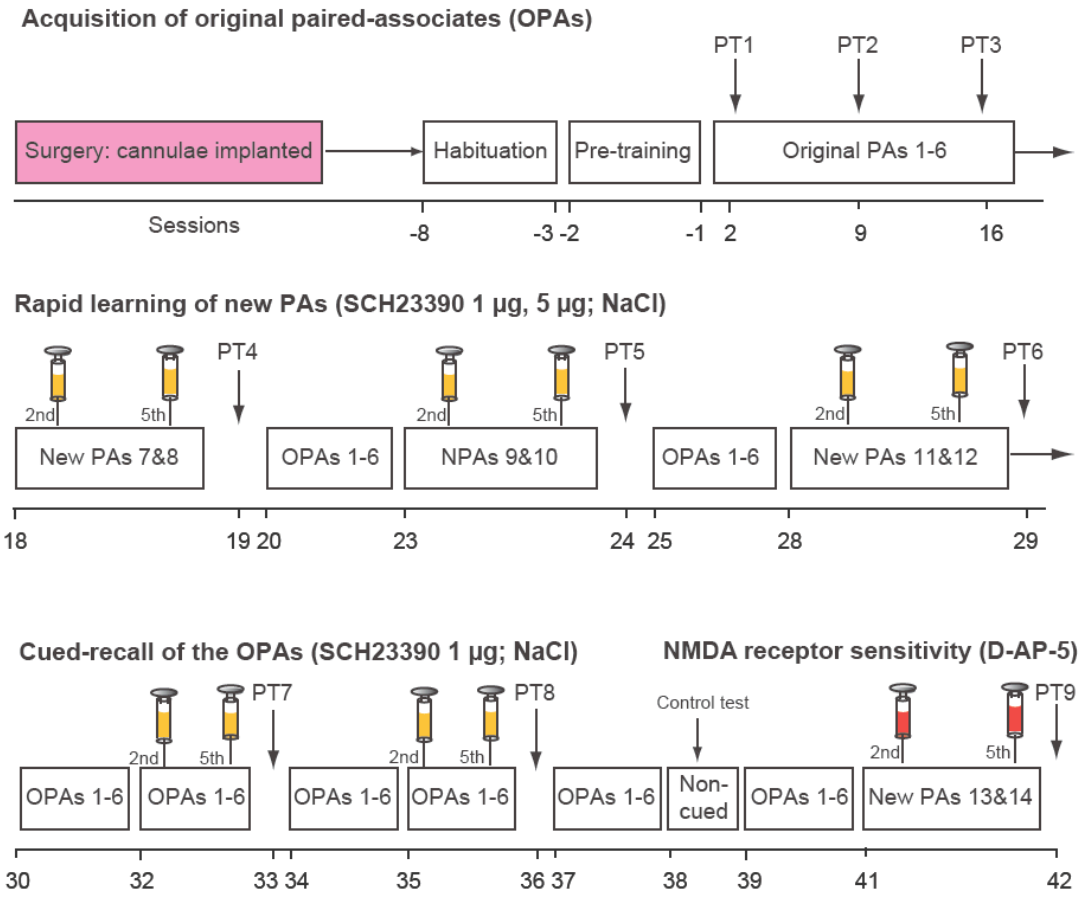

Figure 5.2 Timeline showing the design of E3: The acquisition of original PAs, rapid learning of new PAs, cued recall of original PAs and NMDA receptor sensitivity. The syringes indicate the bilateral iHPC infusions (before the beginning of the second and fifth trials on the training day of the new PAs). The yellow syringe indicates the infusion of SCH23390 while the red syringe indicates the infusion of D-AP5. PT = Probe trial.

5.6.5.1 Shaping, habituation and pre-training

Rats were shaped, habituated and pre-trained in event arena as described in General Methods.

5.6.5.2 Training of original PAs schema (sessions 1-17)

The experiment was a within-subjects, repeated-measures design, consisting of a series of initial training sessions, new PAs learning, cued recall of original PAs and drug infusions sessions (Figure 5.2). The original arrangement of PAs 1-6 is shown in Figure 5.3. During the experiment, rats were trained on alternate days in 2 cohorts of up to 6 rats with each cohort received 3 training sessions per week (cohort 1: Monday, Wednesday, and Friday; cohort 2: Tuesday, Thursday, and Saturday).

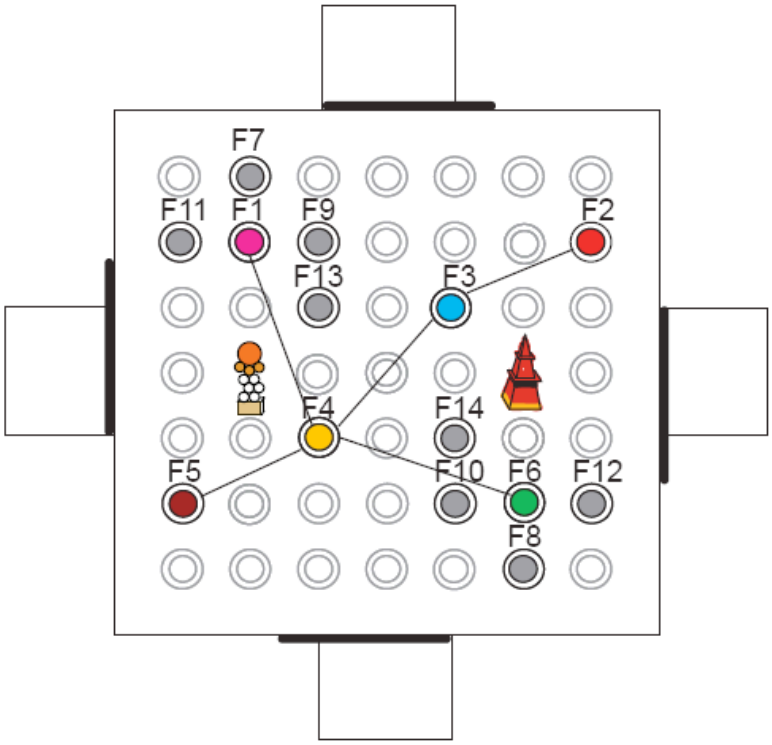

Figure 5.3 Spatial arrangements of the PAs in the schema task.

5.6.5.3 Non-rewarded probe tests (Sessions 2, 9, 16)

To examine cued-recall memory, several non-rewarded probe tests (PTs 1-3) were scheduled. During these tests, all 6 sand-wells were open as usual and the rats were free to dig in any of them, although none contained any food reward. The rats were

cued with a single flavour as usual, and then allowed into the arena for a total of 120 s. Digging time was used as an index of memory strength.

5.6.5.4 Training and probe tests (Sessions 18-29): Impact of SCH23390 on new PAs

Having learned 6 PAs in a schema paradigm, the next step was to investigate the impact of intrahippocampal infusion of the D1/D5 antagonist SCH23390 on the acquisition of new PAs (Sessions 18, 23 & 28). On this first change in sand-well arrangements within the event arena, the wells for PA1 and PA6 were closed, and replaced by others containing 2 novel flavours at neighboring locations: PA7 (Almond) and PA8 (Paprika). In one session, rats were trained for a total of 6 trials ; 1 trial only to each of the 2 novel flavours and the 4 remaining trials to PAs of the original schema (i.e. PAs 2-5). Drugs (3 conditions: SCH23390 1 µg, SCH23390 5 µg and NaCl) were infused 20 min before trial 2 and again before trial 5 (approximately 3 hr later), with 1/3 of the rats receiving each drug per session in counterbalanced order. Within-subject counterbalancing across the 3 drug conditions (1µg and 5 µg SCH23390 and NaCl) required the creation of 4 further novel PAs (PA9 = Bacon; PA10 = Cinnamon; PA11= Cherry; and PA 12 = Ginger). Probe tests (PTs 4, 5 and 6) and were scheduled 24 hr after each of the relevant new PA training sessions for PAs 7 and 8, PAs 9 and 10, and PAs 11 and 12, with 3 interposed normal training days (Sessions 20-22 and 25-27).

5.6.5.5 Training and probe tests (Session 30-36): Impact of SCH23390 on original PAs

The next test examined the impact on later recall of SCH23390 infused prior to the regular daily training of 2 of the original PAs. No effect of these procedures was anticipated, therefore, this testing of original PAs was expected to serve as a control for the mere administration of SCH23390. The rats were given further training on the original set of PAs for 2 sessions (30 and 31). On Session 32, SCH23390 (1 μg) or NaCl was infused 20 min before trial 2 and again before trial 5 with original flavours being introduced on these trials rather than new ones (Figure 5.2). A probe trial (PT 7) was conducted 24 hr later to test cued-recall memory for these original PAs. After 2 further sessions of training, the two other original PAs (Sessions 35, 36) were repeated to counterbalance the different conditions across the full set of 12 rats.

5.6.5.6 Non-cued control test

Another control test, on Session 38, was conducted to test whether the cue flavour given in the start box actually did guide the rat's search, rather than any cryptic olfactory cue at the goal. A standard session of 6 daily rewarded trials were conducted in which the successive set of 6 separate cued pellet in the start box were absent. The performance index measure for retrieval of the first cue pellet should then fall to chance if this start box cue pellet is critical for memory retrieval.

5.6.5.7 Training and probe tests (Session 41-42): Impact of D-AP5 upon learning

The next set of sessions was a critical test of hippocampal dependence of new PAs learning by examining the impact of the NMDA receptor antagonist D-AP5. Two new PAs (PA 13 = Strawberry; and PA 14 = Butter) and 4 original PAs (PAs 2-5) were

introduced on session 41. The rats were trained for 6 trials in one session with original PAs 2-5 introduced on trials 1, 3, 4 and 6, and PAs 13 and 14 on trials 2 and 5. Drugs [two conditions: D-AP5 (30 mM, 1 µl) and aCSF (1 µl)] were infused 20 min before trial 2 and again before trial 5. A probe trial (PT 9, Session 42) was scheduled 24 h later. Half of the rats were given D-AP5 and the others were given vehicle.

5.6.6 Perfusion and Histology

Detailed methods of perfusion and histology are described in General methods. Inspection of the location of the tips of the cannulae in coronal sections of the brain of the rats revealed these to be located in dorsal HPC, in the CA1 region of the HPC (Figure 5.4).

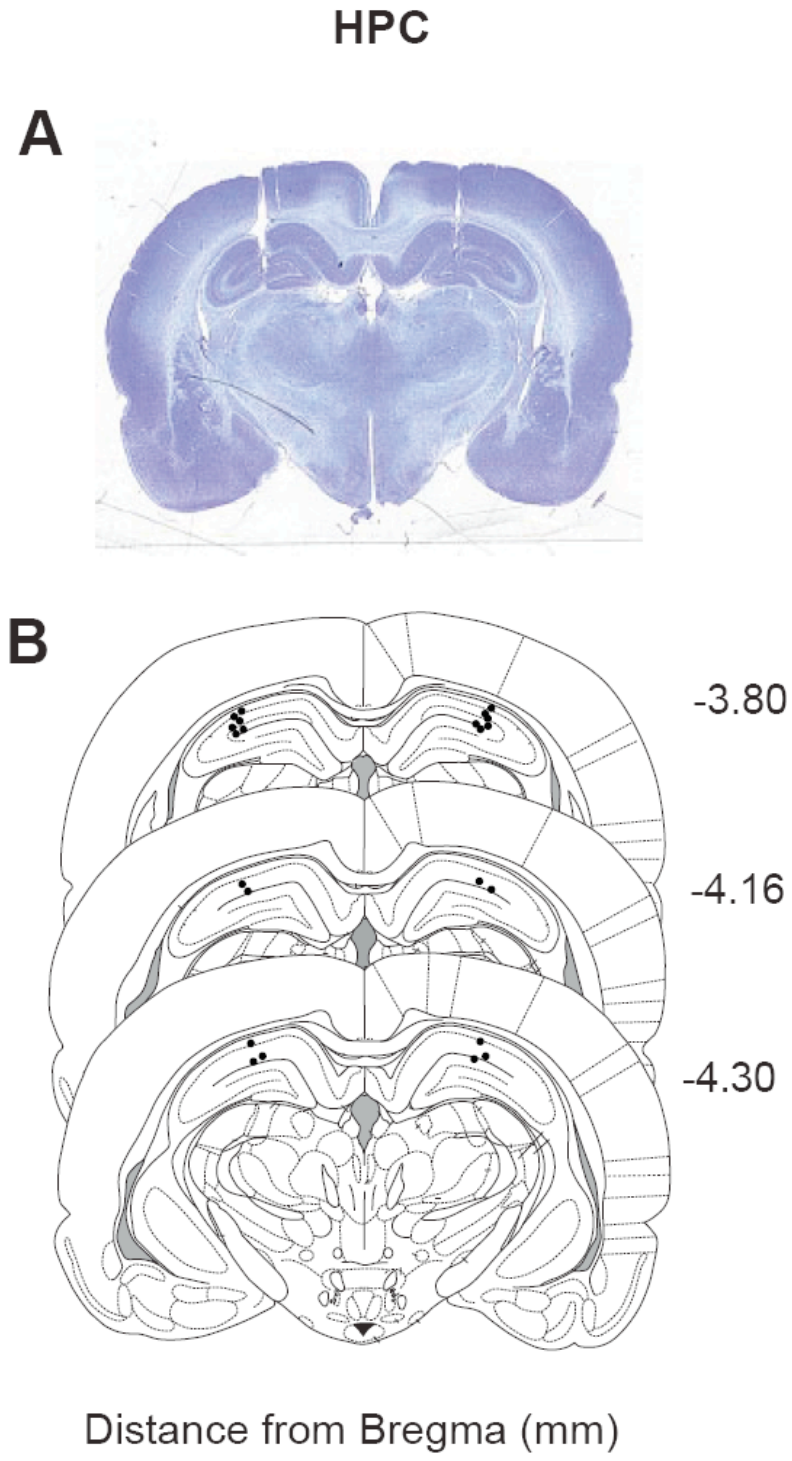

Figure 5.4 (A) The top shows Nissl-stained section showing examples of representative cannulae tracks in the dorsal HPC in each hemisphere of the brain. (B)The bottom shows plots of the locations of cannulae tips (n =11 per HPC). Infusion sites are marked on the appropriate section of a stereotaxic brain atlas (Paxinos and Watson, 1998).

5.7 Results

5.7.1 Initial training, probe tests and non-cued test (Sessions 1-17, 38)

Rats were trained on a set of 6 original PAs from session 1 to 17. As shown in Figure 5.5, the rats were initially at chance levels in the first 4 sessions ($t > 3.71$, df = 10, $p < 0.001$), but they improved across 15 sessions to a 'performance index' (PI) score of between 70 to 80% (details of calculating the performance index from the number of errors were described in General methods). A repeated measures ANOVA of the PI scores for all the rats showed that performance improved monotonically across all training sessions ($F = 11.36$, df = 4.8/48, $p < 0.001$).

During the training phase, 3 initial non-rewarded probe trials (PT1-3) were scheduled as shown in Figure 5.5 to examine acquisition of PA memory. Figure 5.6 represents percentage of the dig time at the cued location (filled bars) relative to that at the non-cued locations (white bars). These probe trials revealed, as expected, a graded learning of the original PAs from sessions 1-16 ($F = 23$, df =1.75/17.51, $p < 0.001$). T-tests results indicate that digging for cued PA was above chance levels in PT3 ($t = 7.76$, df = 10, $p < 0.001$).

To exclude the possibility that an olfactory cue in the correct sand-well guided the rat's choices on training days, a single session of 6 trials in which the daily protocol was unchanged, except that no cue flavours were offered in the start box, was carried out. PI score fell to chance (Figure 5.5, Session 38), returning to above chance levels on the next normal session (Session 39). It follows that animals were likely not being guided to the correct sand-well by odours emanating from it or scents left by previous rats, but instead, by cued-recall.

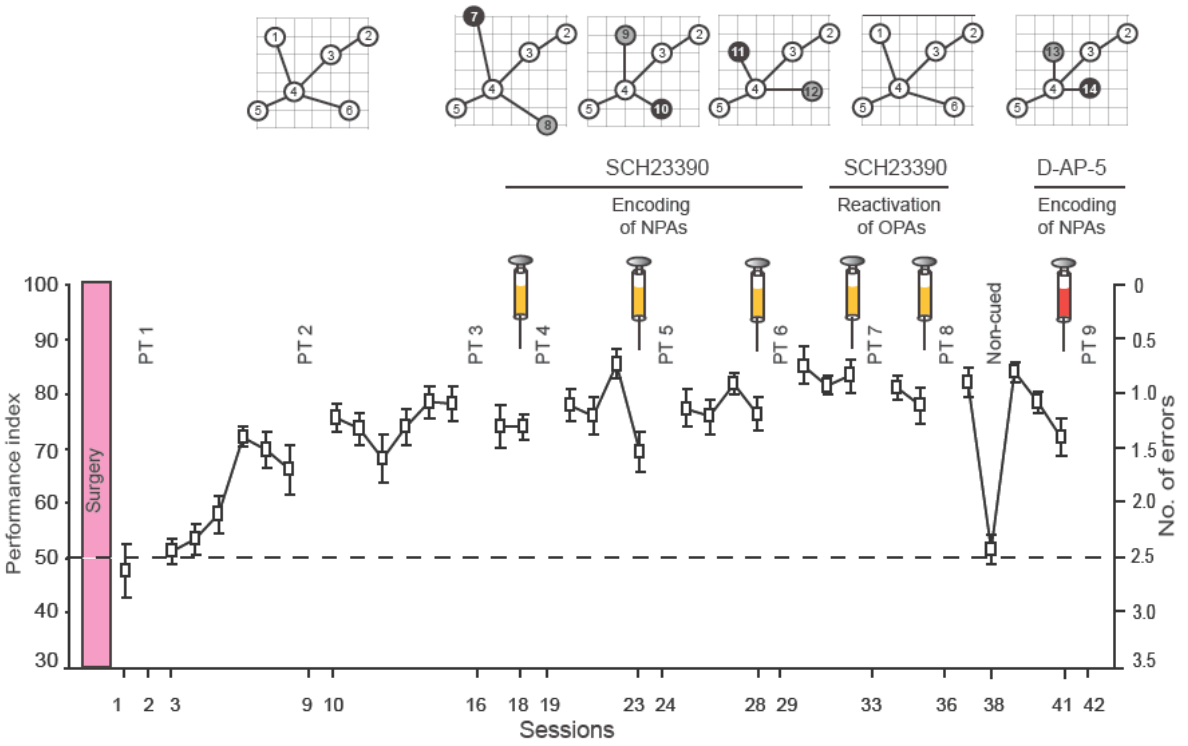

Figure 5.5 Acquisition of the original 6 PAs (sessions 1-17). Single days of training of new PAs alternated with 3 days of the original schema (sessions 18-42) in different drug conditions. Performance rose to a stable level of 80% with minimal variability. Removing cue flavours from the start box on session 38 results in performance dropping to chance and then returning to 70% correct on a succeeding normal session (session 39).

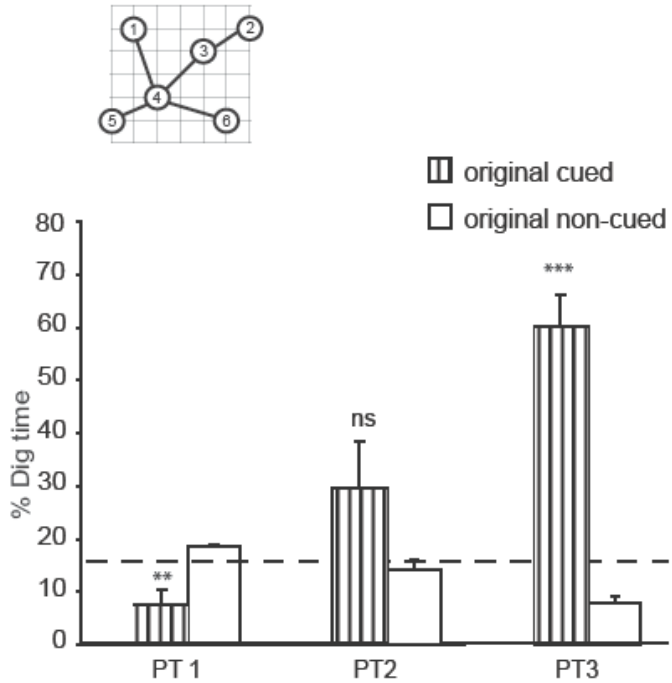

Figure 5.6 Three cued-recall PTs for the acquisition of the original schema. The graph represents percentage of dig time at the cued locations (striped bars) relative to that at the non-cued locations (white bars). ns, nonsignificant. $^{**}p < 0.01$; $^{***}p < 0.001$

5.7.2 NMDA dependency of novel PAs acquisition (D-AP5) (Sessions 41– 42)

Having learned 6 PAs in a single schema paradigm, the next step was to examine the possible role of hippocampal plasticity via iHPC infusion of the NMDA antagonist D-AP5 at the time of encoding of novel flavour place PAs. Figure 5.7 presents dig time data of this probe test in session 42. The ANOVA revealed an interaction between drug (a between subjects factor) and paired-associates (F = 10.01, df = 1.14/9.15, p < 0.01). A pairwise comparisons analysis with Bonferroni's corrections for multiple comparisons showed a significant difference between the control group (aCSF) and the D-AP5 group on % digging time at the correct new cued PAs (Figure 5.7, black bars) (p < 0.05). Additional t tests compared the proportion of time spent digging for the new cued PA relative to chance, and revealed that digging was above change for the control group (aCSF, t = 3.14, p < 0.035) but not for the D-AP5 group (D-AP5, t <1, NS). This finding suggests that novel PA acquisition requires HPC dependence and indicates that it involves local NMDA receptor activation.

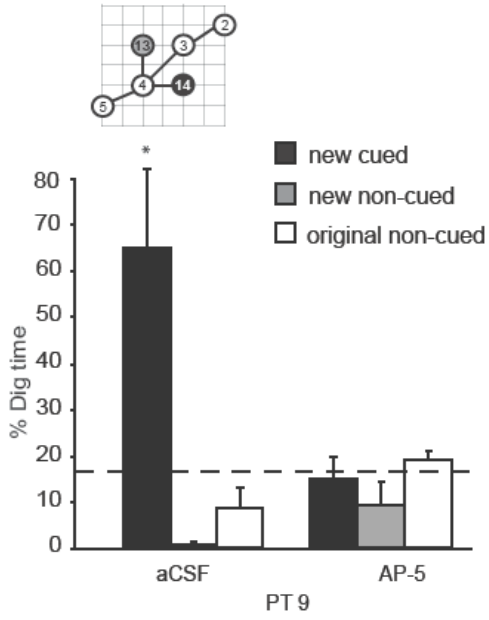

Figure 5.7 Hippocampal NMDA receptor dependence of the acquisition of the new PAs. The graph represents percentage dig time at the new cued location (black bars) and the new non-cued location (gray bars), as well as the average of the 4 original PAs (white bars) for PT 9 in the control (aCSF) and drug (D-AP5) conditions. *p < 0.05

5.7.3 Dopamine dependency of novel PAs acquisition (SCH23390) (Sessions 18-36)

The second aim of this study was to investigate the role of dopamine in HPC, via iHPC infusion of the dopamine antagonist SCH23390 during encoding of new PAs. Figure 5.8 presents dig time data of the relevant probe tests (Sessions 19, 24 and 29). An ANOVA revealed an interaction between drug and digging locations (F =8.5, df = 2.49/24.9, $p < 0.001$) (Figure 5.8). Pairwise comparisons, with Bonferroni's corrections for multiple comparisons, and focusing exclusively on digging time at the new cued location across drug conditions (i.e., black bars, Figure 5.8), showed significantly less digging when animals were treated with SCH23390 (1 μg, $p < 0.05$; 5 μg, $p < 0.01$) than with saline. Similarly, t tests that compared the proportion of time spent digging at the cued location indicated above chance performance in the control condition (t = 3.42, df =10, $p < 0.01$) but not for the SCH23390 1 μg condition (t = 1.02, df = 10, NS). There was less digging by chance in the SCH23390 5 μg condition (t = 9.80, df = 10, $p < 0.05$). As animals treated with 1 μg of SCH23390 had a deficit in encoding new PAs, only this lower concentration (1 μg) was used in later experiments.

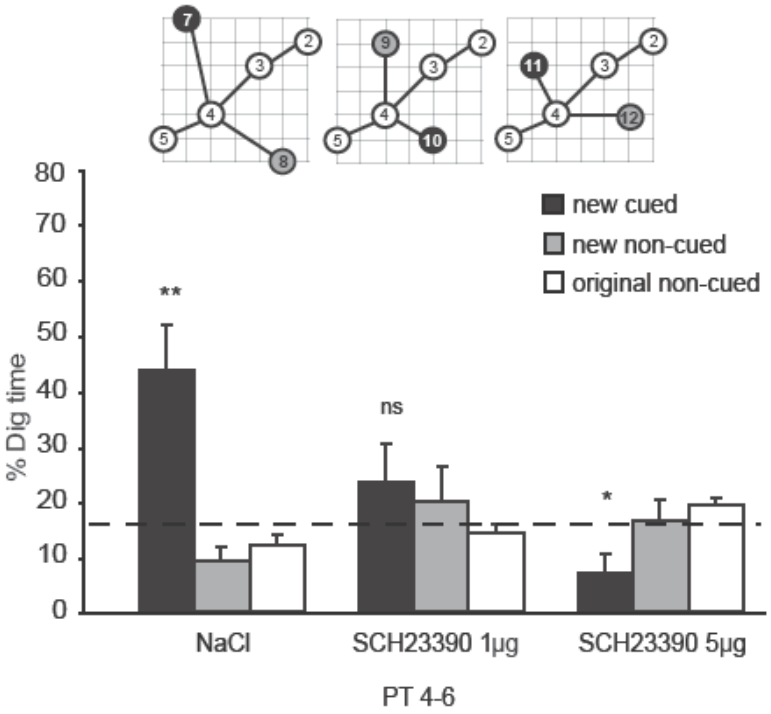

Figure 5.8 Dopamine dependency of encoding of new PAs. Percentage dig time in all 3 conditions (NaCl, SCH23390 1 μg, and SCH23390 5 μg) across the 3 counterbalanced probe tests (PT4-PT6). ns, nonsignificant. *$p < 0.05$; **$p < 0.01$

5.7.4 Latency to dig at correct SW on the day of drug infusions (SCH23390)

A concern with drugs affecting dopamine is the possibility of motor effects. One concern may be that those rats treated with SCH23390 were perhaps slower at retrieving the pellets and that consequently, this may have adversely affected the result. Figure 5.9 shows a within-session comparison of the latency to dig at the correct sand-well for new PA trials (in the presence or absence of the drug) with the average latency on the remaining original PA trials without the drug. There appears to be a trend for the latency to dig at the correct sand-well to be slightly slower for new PA trials in the presence of SCH23390 (54.6 s ± 16 for SCH23390 1 μg and 45 s ± 11 for SCH23390 5 μg compared to 15.8 s for NaCl). This may be the case because animals that were still under the effects of the drug took longer to go to the correct location. However, an ANOVA of these latency scores revealed no overall interaction between drug and trial ($F = 2.5$, df = 1.72/17.2, $p > 0.1$).

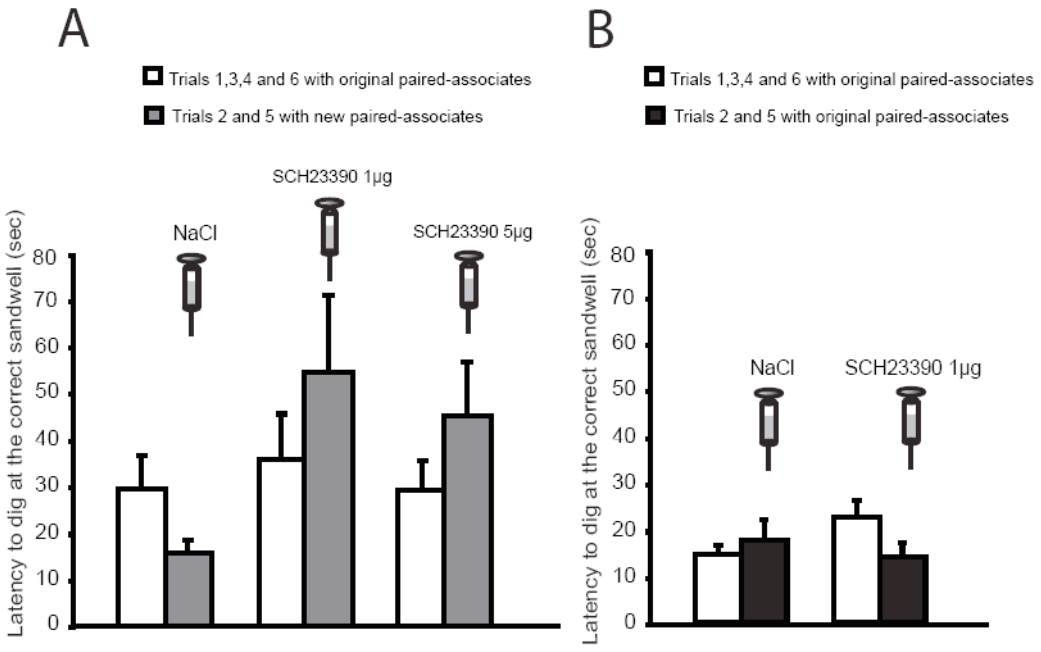

Figure 5.9 Latency to dig at correct sand-well on the day of drug-infusions. (A) New paired-associates: Latencies on the two new PA trials 20 min after drug infusion (grey bars) compared with latency on the four remaining original PA trials without drug (white bars). (B) Original paired-associates: Latency to complete original PA trials in presence of drug (NaCl vs SCH23390) compared with latency to complete original PA trials without any drug.

5.7.5 Dopamine is not required in recall of previously trained original PAs

Figure 5.10 shows the lack of an effect of SCH23390 on memory of previously trained original PAs. An ANOVA revealed a significant difference in dig time at the cued and non-cued locations (F = 44.80, df = 1/10, p < 0.01) but no significant interaction between drug and cue location (F <1). T tests comparing the proportion of digging time for the cued PA relative to chance were significant for both conditions (NaCl; t = 4.12, df = 10, p < 0.001; SCH23390 at 1 μg; t = 9.88, df = 10, p < 0.001).

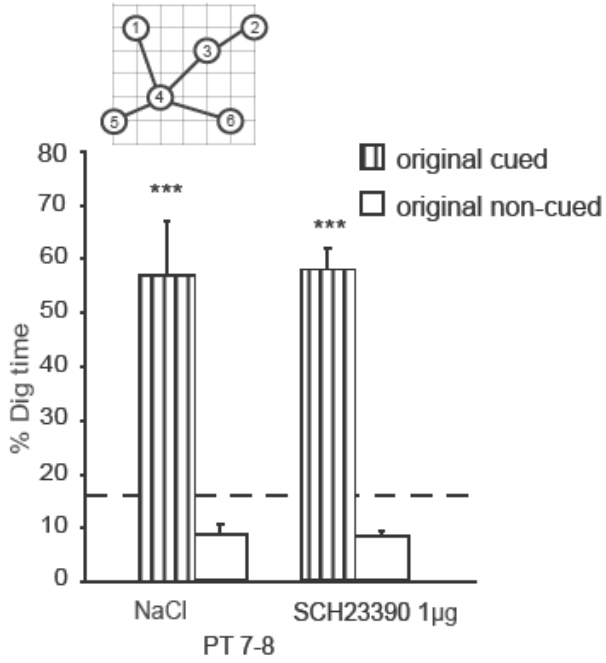

Figure 5.10 SCH23390 and previously trained memories (original PAs). Percentage dig time for the original PAs in 2 conditions (NaCl and SCH23390 1μg). ***p < 0.001

5.8 Discussion

The main findings of this chapter are as follows: (1) Intrahippocampal infusion of D-AP5 at the time of encoding of new PAs impaired later memory; (2) Intrahippocampal infusion of SCH23390 impaired long term 24 hour memory for new PAs in an episodic-like memory task in which rats had previously acquired a flavour-place schema; (3) similar infusions of SCH23390 had no effect on the memory of previously established PAs.

5.8.1 The hippocampal dependence of the PAs task

This is the first time that the PAs schema task has been used to examine the neurobiology of one trial new PAs encoding in a pharmacological within-subjects design. The PAs schema task has been demonstrated using a hippocampal lesions design (Tse, Langston et al., 2007), and this new data further establishes the rapid one trial memory consolidation. In addition, this present study shows that bilateral iHPC infusions of D-AP5 before the encoding of new PAs results in memory loss when tested 24 h later.

In the previous HPC lesion study (Tse, Langston et al., 2007), it was shown that the acquisition of the new PAs is HPC-dependent as revealed by HPC lesions. The present data strongly suggests that the acquisition of PAs is NMDA receptor-dependent within HPC, at the time of encoding, possibly involving associative synaptic potentiation (Bliss and Collingridge, 1993).

In addition, in the previous HPC lesion schema study, there was a concern of whether ibotenic acid lesions might result in aberrant neocortical activity after the lesion (Rudy and Sutherland, 2008). With the present pharmacological study, the concern is reduced as iHPC infusion of D-AP5 is not known to have such effect on neocortex.

The present study suggests that the encoding of new PAs critically involves an NMDA receptor-dependent mechanism in HPC, perhaps followed by cellular and then rapid systems consolidation.

5.8.2 The role of hippocampal D1/D5 receptors in encoding of new PAs

The main finding from the present study is that intrahippocampal infusion of the D1/D5 dopaminergic antagonist SCH23390 before encoding of new PAs caused impaired memory 24 later but that SHC23390 had no effect on the memory that had previously been established.

However, at this juncture, there are still 2 possibilities. One is that hippocampal DA is critical for encoding, and the other is that it is critical for persistent memory. To distinguish between these 2 possibilities, a further experiment was conducted (Bethus et al., 2010) which compared the impact of SCH23390 on short term and long term memory. In this study, a cohort of rats was trained to learn 6 PAs schema, SCH23390 was infused into the HPC 20 min before encoding of the new PAs. Rats were then tested after either a short (30 min) or long (24 hr) delay. Memory for the new PAs was impaired at 24 hr but intact at 30 min. This indicates that hippocampal DA is critical for the persistence of memory rather than the encoding.

A similar result was found in a study that showed DA memory storage (Rossato et al., 2009). In this study, they showed that DA in CA1 contributes to the maintenance of long term memory 12 hours after the acquisition.

It would be interesting to investigate the role of DA in extrahippocampal structures that may be involved in the encoding and storage of new PAs in a schema task.

Chapter 6 – Experiment 4

Using immediate early genes mapping to identify cortical regions involved in encoding of new paired-associate in schema

6.1 Introduction

As detailed in the introduction (chapter 2), the original hippocampal lesion study in the paired-associates (PAs) schema paradigm suggested that systems memory consolidation can occur quickly (within 48 hrs) after learning new information, if the rats had previously acquired a schema allowing for incorporation of new information (Tse, Langston et al., 2007). Using this hippocampal-dependent paradigm, rats were trained to learn a schema involving 6 flavour-place PAs. In the previous chapter (E3), it was shown that the encoding of new PAs is dependent upon NMDA receptors in the HPC and also that dopamine is involved in the persistent of memory. It is known that, once a schema has been acquired, relevant new PAs become assimilated into extra hippocampal regions and rapidly become hippocampal-independent. A next sensible step was therefore to identify these extra-hippocampal regions involved in rapid memory consolidation. This was addressed by comparing neuronal activation in several brain areas simultaneously by measuring expression of the inducible immediate early genes (IEGs) *Zif268* and *Arc*. These IEGs are involved in synaptic plasticity and memory formation and are commonly used as an index of neuronal activation (Jones et al., 2001; Maviel et al., 2004). This 'IEGs study' is described in this chapter.

6.1.1 Immediate early genes

IEGs are the primary response genes rapidly induced following cellular stimulation. IEGs are useful markers for neuronal activity. One reason for this is because this class of gene is rapidly and transiently activated in response to neuronal activation (Davis et al., 2003); another reason is due to their widespread expression in brain. Traditional lesion studies do not directly measure the contribution of a target region; rather, they measure how the rest of the brain functions in its absence, in other words, they reveal the necessity for a brain area. As a consequence, there is a need to complement lesion data with data from techniques examining normal brain tissue. IEGs studies can reveal the differential involvement of several areas. IEGs expression, has been used to examine the patterns of activation in multiple brain areas during both spatial and non-spatial tasks as a complement to lesion studies (Tischmeyer and Grimm, 1999). Measuring the activity of IEGs provides a novel form of functional imaging that permits activity in many regions to be compared simultaneously in the brain (Herdegen and Leah, 1998).

There are many studies showing that IEGs are involved in synaptic plasticity and memory formation (Dragunow, 1996; Guzowski, 2002; Frankland et al., 2004; Maviel et al., 2004; Ross and Eichenbaum, 2006). Using IEGs mapping may be a suitable way to identify cortical regions involved in the encoding of new PA information. The reason for choosing encoding of new PAs and retrieval of well-learned PAs as a starting point was because studies of consolidation require the comparison of recent or remote memory time-points that would make for a much more complex experimental design.

Different IEGs are used in different studies; this is because different genes have different properties. IEGs can be divided into 2 functional classes: (1) regulatory

transcription factors that influence the activity of other "downstream" genes (e.g. *Zif268*), and (2) effector genes that directly affect cellular physiology (e.g. *Arc*) (Herdegen and Leah, 1998; Lanahan and Worley, 1998; Guzowski et al., 2001). As both *Zif268* and *Arc* are known to be involved in synaptic plasticity (Guzowski et al., 2001; Davis et al., 2003; Bramham et al., 2008), these are good candidate genes to use to identify cortical regions involved in encoding of new PAs in the present schema task. In practice, the protein products of gene activation using immunohistochemistry were measured in this study, as in situ hybridisation of mRNA expression is both more complicated and little used in earlier studies.

The inducible expression of protein and mRNA products of the regulatory IEG Zif268 is used as a marker of neural activity for mapping studies of the nervous system. The different time courses of mRNA and protein expression of Zif268 are described in Figure 6.1 (Zangenehpour and Chaudhuri, 2002).

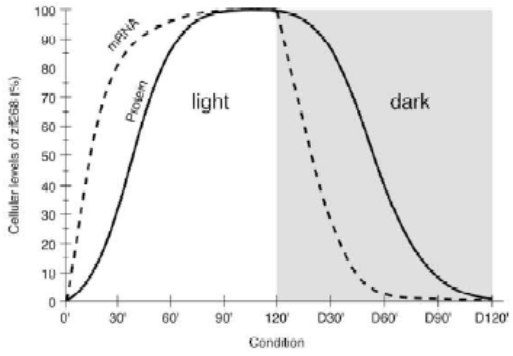

Figure 6.1 The cellular induction and decay profiles of both mRNA and protein levels of Zif 268 in primary visual cortex (Figure reprinted from Zangenehpour and Chaudhuri, 2002).

The baseline levels Zif268 mRNA/protein expression was estimated from animals that received no visual stimulation after 72 h of dark adaptation. Both of the mRNA and the protein levels reach their highest peak at around 90 min. Previous study (Ramirez-Amaya et al., 2005) has provided that Arc protein expression in cortex was elevated between 0.5 to 2 h after a novel exploration event, returning to the baseline level at

the time point ranging from 3 to 6 h. Knowing the timing of the IEGs peak levels can help when designing experiments; in particular, to identify the most suitable time to perfuse the animals for visualisation.

6.2 Material and methods

6.2.1 Subjects

Twenty eight adult male Lister-hooded rats (Charles River, UK) were used in this experiment as described in General Methods. Two cohorts of rats were used, each with 14 rats.

6.2.2 Apparatus

The event arena was used as described in General Methods.

6.2.3 Behavioural training

The time course of this study is shown in Figure 6.2. The rats first underwent *habituation*, *pre-training* and then *original PAs schema training* (Sessions 1-17). On the critical session (S18), the animals were divided into 3 groups:

- **new paired associates (NPA) group (encoding and retrieval)**
- **original paired associates (OPA) group (retrieval only)**
- **new map (NM) group (encoding only)**

There was also a:

- **Home cage control (CC) group (no memory process activation)**

The purpose was to use IEGs to map possible locations in the brain where memory reactivation and/or new encoding may be occurring during the 5th and 6th trials of the critical session.

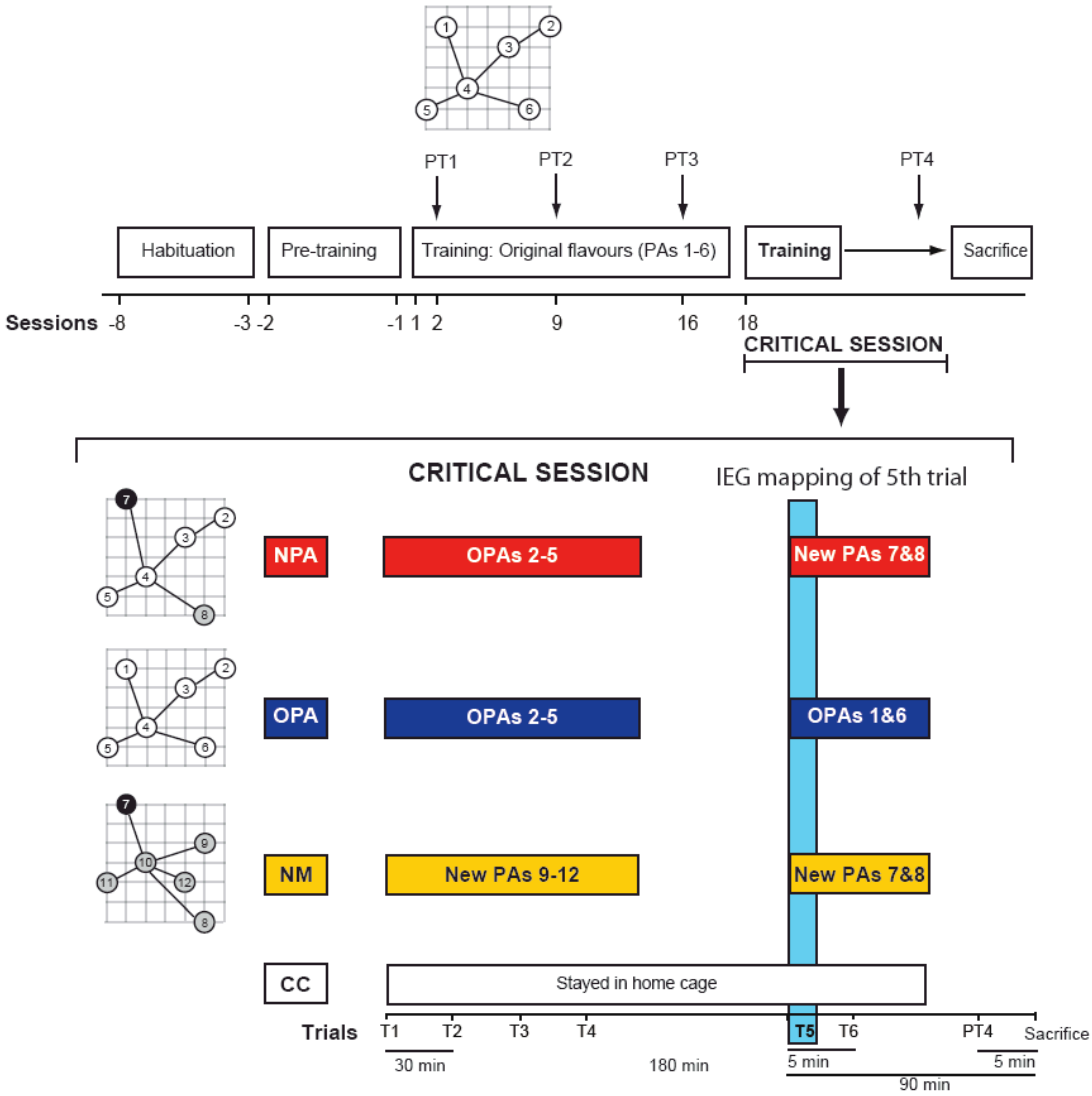

Figure 6.2 Timeline showing the design of E4: the acquisition of original PAs and probe trials (sessions 1-17). A caged control (CC) group stayed in a home cage throughout the experiment. On the critical session 18, rats were divided into 3 groups: new paired associates (NPA) group, original paired associates (OPA) group and a new map (NM) group.

6.2.3.1 Caged control group

Twenty-eight rats were used in this study, 7 of which were allocated to a caged control group (CC). This group of rats were brought from the animal house to the event arena control room every session (as were the experimental groups). However, this caged control group stayed in the home cage at all times and never entered the event arena (Figure 6.4). The reason for group CC was to compare the baseline IEG

activity of brain regions with that of the other experimental groups. The remainder of the rats (n=21) underwent behavioural training.

6.2.3.2 Shaping, habituation and pre-training

Rats (n=21) were shaped, habituated and pre-trained in the event arena as described in General Methods.

6.2.3.3 Training of original PAs (session 1-17)

The spatial arrangement of the original PAs 1-6 is shown in Figure 6.3. Each PA (consisting of a specific flavour and its location) was presented for 1 trial/session.

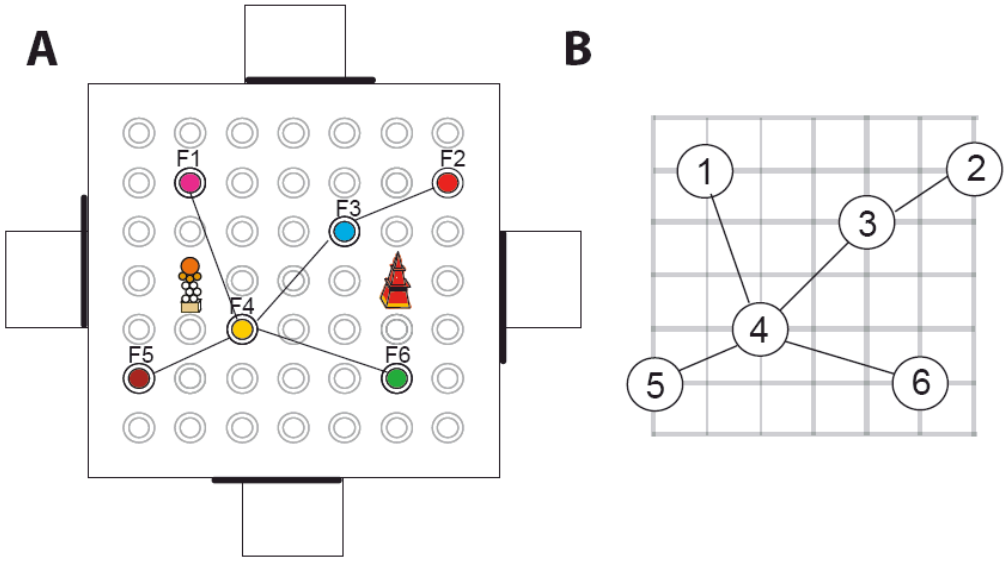

Figure 6.3 (A) The arrangement of the original PAs of a schema (F1-6, flavours of food at specific location). (B) Diagram of original schema.

6.2.3.4 Non-rewarded probe tests (sessions 2, 9, 16)

To examine cued-recall memory, 3 non-rewarded probe tests (PTs1-3) were conducted. During these tests, all 6 sand-wells were open as usual and the rats could dig in any of them, but none contained any food pellets as reward. The rats were cued

with a single flavour as usual, and then allowed into the arena for a total of 120 s. Digging time was used as an index of memory strength.

6.2.3.5 Critical session (session 18)

Training of PAs

Once the rats had learned 6 PAs in the schema paradigm, the next step was to investigate which hippocampal or extrahippocampal (i.e. cortical) areas might be involved in the encoding of new PAs in the schema paradigm. This examination was 'hypothesis-free' and involved the analysis of multiple brain areas.

Rats were divided into 3 groups, with each group having 7 rats: new paired-associates (NPA) group, original paired-associates (OPA) group and new map (NM) group (Figure 6.2).

- **The NPA group's training consisted of 4 original PAs and 2 new PAs.**
- **The OPA group's training consisted of 6 original PAs.**
- **The NM group's training consisted of 6 new PAs.**

A summary of important events during the critical session experienced by different groups of rats can be found in Figure 6.4.

Events during critical session	NPA	OPA	NM	CC
Brought to the event arena control room	+	+	+	+
Running in the event arena	+	+	+	-
Eating pellets in the event arena	+	+	+	-
Learned an original schema	+	+	+	-
Activate an original schema	+	+	N/A	-
Learning new PAs	+	-	+	-
Learning new PAs in original schema	+	-	-	-

Figure 6.4 Events during the critical session.

The reason for introducing an NPA group was to investigate encoding and assimilation of new PAs. The reason for introducing an OPA group was to examine whether the expression observed in the NPA group was merely caused by activating a schema or also represented new encoding (Figure 6.4). If the IEG expression of the NPA group was due only to activating a schema, both the OPA and NPA groups would have similar activation. The reason for introducing a NM group was to examine whether the expression observed in the NPA was merely a novelty effect of learning new PAs. If the IEGs expression of the NPA group was due only to novelty, both the NPA and NM groups should have similar activation. In contrast, if the activation pattern is due to learning and assimilating new PAs into the original schema, the regional pattern of IEG activation should be different (Figure 6.4). Finally, the value of the CC group was to provide a point of reference for all the groups that were required to display behaviour of some kind in the event arena.

The critical session consisted of 6 trials, each trial with a different PA. For the first 4 trials, both the NPA and OPA groups were presented with original PAs 2-5 and the NM group was presented with new PAs 9-12. The inter-trial time was 30 min (Figure 6.2).

After the fourth trial, all rats were placed back to home cage for 180 min. The reason for this wait was because of the temporal dynamics of Zif268 expression. As noted in the introduction, the cellular induction and decay profiles of protein levels of Zif268 in the primary visual cortex shows that the protein levels reach peak levels at 90 min and start to decay at 120 min (Zangenehpour and Chaudhuri, 2002). By 180 min, the protein level of Zif268 is much lower. The aim of this experiment was to map the IEG activity induced on the 5th and 6th trials of the training without major influence from trials 1 to 4; hence, the 180 min wait was necessary.

On the 5th and 6th trials, the OPA group was presented with original PAs 1 & 6. The NPA and NM groups were presented with new PAs 7 & 8. The inter-trial time was 5 min (Figure 6.2). The animals were then returned to their home cages for 85 min.

Probe trials

Five minutes before the rats were sacrificed a probe trial was performed to test if the rats remembered the PAs with which they had been presented with on the 5th trial.

6.2.4 Immunohistochemistry

Ninety minutes following the training of the 5th trial in the schema task, rats were deeply anesthetised with Euthatal (Harlow, Essex, UK) and perfused transcardially. The tissue preparation and immunohistochemistry procedures were described in detail in General Methods. Zif268 immunohistochemistry was conducted at the University

of Edinburgh, while arc immunohistochemistry was conducted at the University of Tokyo by Dr Masaki Kakeyama.

6.2.5 Image capturing and analysis

6.2.5.1 Images capturing

Images of the sections were captured on a PC running Image Pro Plus (version 6.2; Media Cybernetics, USA), using a microscope (Leica DMRB, Germany) with a 10× objective equipped with a QICAM camera (QImaging, Canada). The regions of interest for each section were montaged using Image Pro Plus software. For each region of interest, images were captured from 3 consecutive sections from both hemispheres.

Regions of interest

The regions analysed are depicted in Figure 6.5 and their abbreviations are explained in Figure 6.6.

The HPC (CA1, CA3 and DG) was of interest because learning of PAs in the schema task is dependent on the HPC (Tse, Langston et al., 2007) (Figure 6.5). Prelimbic cortex, infralimbic cortex, anterior cingulate cortex, anterior retrosplenial cortex and posterior retrosplenial cortex were of interest because previous studies have shown their importance in either recent or remote memories (Bontempi et al., 1999, Frankland et al., 2004; Maviel et al., 2004). Insular cortex was of interest because it is the primary taste cortex (Verhagen et al., 2004), lateral entorhinal and orbitofrontal cortices were of interest because these cortices were related to the information processing for smell (Verhagen et al., 2004). Somatosensory cortex (barrel cortex)

was included as a control region because it is not known to have a specific role in flavour, spatial or taste memory (Vann et al., 2000).

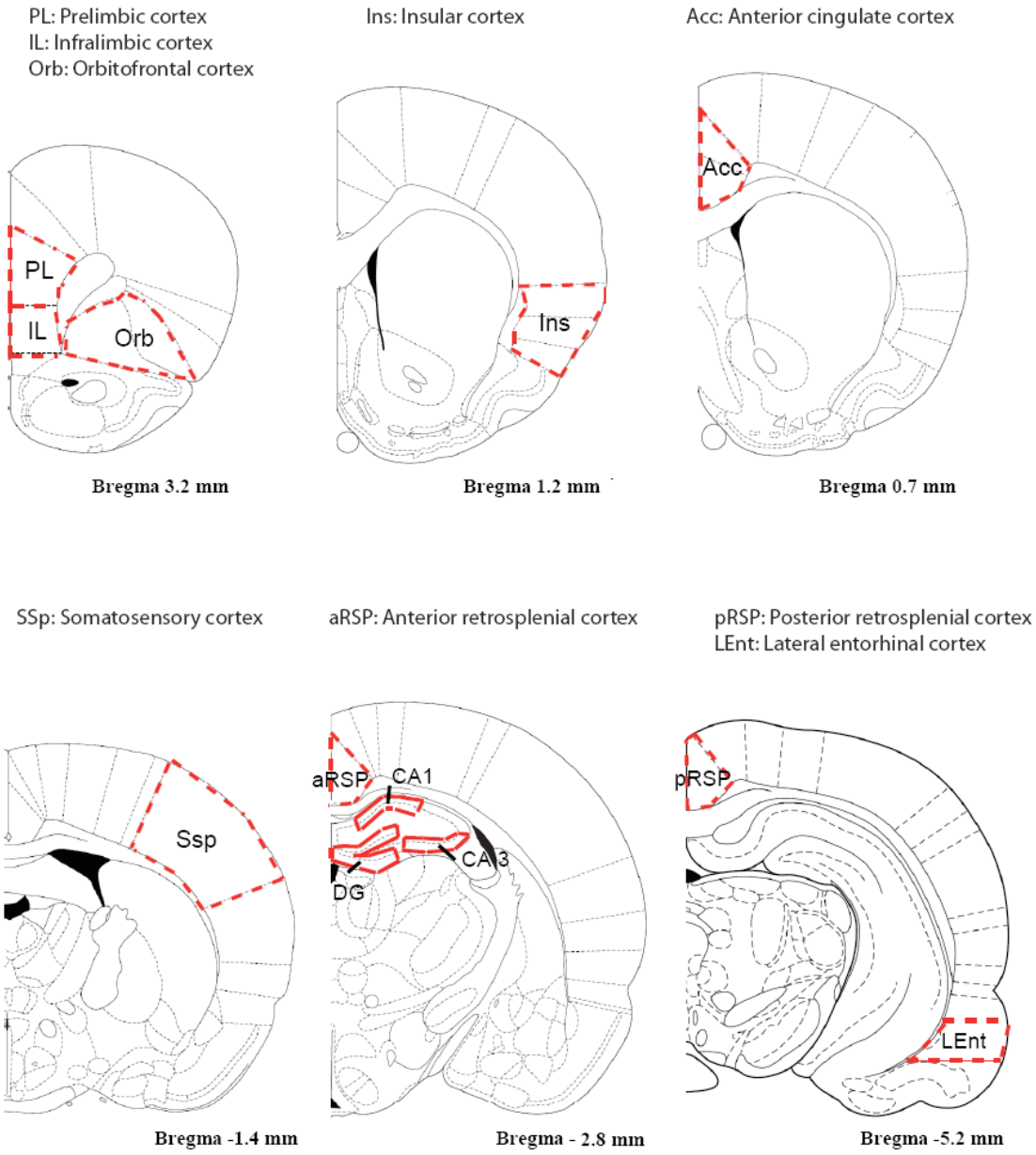

Figure 6.5 Areas examined for Zif268 and Arc expression. Red dotted areas show the areas where images were captured and counted for Zif268 and Arc expression. The numbers beneath each picture represent AP coordinates from bregma from Paxinos and Watson (1998).

Abbreviation	Brain region
Prefrontal and retrosplenial cortices	
PL	Prelimbic cortex
IL	Infralimbic cortex
ACC	Anterior cingulate cortex
aRSP	Anterior retrosplenial cortex
pRSP	Posterior retrosplenial cortex
Sensory cortices	
Orb	Orbitofrontal cortex
Ins	Insular cortex
Ssp	Somatosensory cortex
HPC & parahippocampal cortex	
CA1	*Cornu Ammonis*1
CA3	*Cornu Ammonis*3
DG	Dentate gyrus
LEnt	Lateral entorhinal cortex

Figure 6.6 Table show abbreviations of brain regions used in figures and texts.

6.2.5.2 Image analysis

After image processing, counts of stained nuclei were carried out using Image Pro Plus. Hippocampal and cortical areas were assessed using counts of nuclei labelled above threshold. For all regions of interest analysed, all 6 cortical layers were included. In CA1/CA3, the pyramidal cells and the stratum oriens layers were included; while in dentate gyrus, the molecular and the granule cell layers were included. The image analysis of individual sections was always conducted 'blind' with respect to groups assignment.

Threshold setting

The threshold was always set using a standard formula derived from the level of illumination of the background of the particular section being counted. The formula was **threshold = mean of background + standard deviation of background * (*constant*),** where the background was calculated using layer 1 of the section in

cortical areas and the stratum oriens layer for CA1/CA3 and molecular layer for dentate gyrus.

To find the value for the constant, several values were used to generate different thresholds. Then, these threshold values were used to generate cell counts which were compared to manual counts of the section. The constant that generated the cell counts that matched the manual counts the closest was than selected to be the constant for all the counts for all the sections. In this case, the constant value was 3.75. One point to note is the manual counts were conducted separately by 2 independent experimenters. Using this 'objective' method to set the threshold, different experimenters could, in theory, come up with the same cell counts for the same section. This ensures a high level of objectivity.

Parameters for the measurement

Two parameters for cell counting were used.

- Size of the neurons (width and length). The minimum size counted by the software was set to 7 μm to reduce false background signal.
- Watershed split selection was enabled to automatically split any clustered cells into single cells.

Steps for cell counting

First, a colour image montage (Figure 6.7A) including the region of interest was converted to a grey scale pixel image (Figure 6.7B). Then, in order to measure the background (layer 1 in cortical areas, stratum oriens layer for CA1/CA3 and molecular layer for dentate gyrus) on each section, 10 squares (Figure 6.7C, red boxes) were drawn *manually* using image pro plus software. The mean and the standard deviation of the background boxes were calculated using a custom built macro for image pro plus software (see appendix 1). Next, the region of interest

(Figure 6.7D, green line) was highlighted manually. The macro then calculated the threshold value taking into the account the parameters described above. The macro counts then displayed the stained nuclei were displayed (Figure 6.7E, red dots), and the counts were summed and the area of region of interest was calculated (Figure 6.7F, red area).

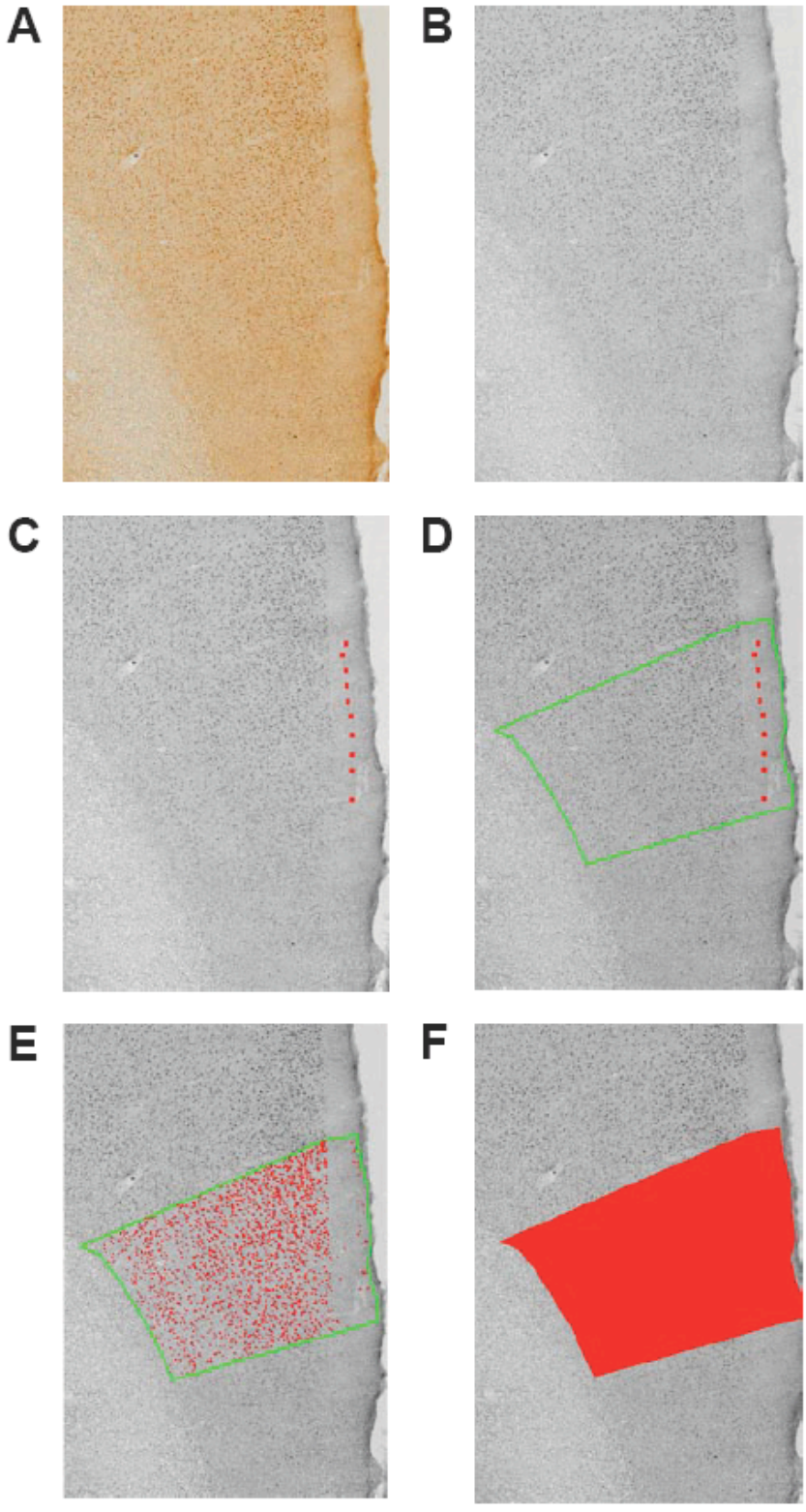

Figure 6.7 Procedure for cell counting.

6.2.6 Data analyses

6.2.6.1 Raw counts

For each brain region analysed, the number of immunoreactive neurons of Zif268 and Arc were counted using 3 consecutive sections from each hemisphere of the brain slices. This means that in each region there are 6 data points per rat. In each section, 2 values were abstracted: (1) the number of immunoreactive neurons and (2) the area of the region of interest. The raw cell count for each section was arrived at by dividing the number of immunoreactive neurons by the area of the region of interest. The means of these counts were then averaged within each rat and then within each group (OPA, NPA, NM and CC).

6.2.6.2 Normalised counts

For each brain region, the mean raw counts for the OPA, NPA and NM groups were normalised using the mean raw counts from the respective control group within each cohort. Within each group, the raw count of each section was divided by the mean raw count of the CC group and were then averaged within each rat and then within each group and expressed as a percentage.

6.2.7 Statistical analyses

6.2.7.1 Behavioural

Several measures of performance were assessed. These were: number of errors in main training, converted into a performance measure, and time spent digging in each sand-well during probe trials. Differences between the groups were analysed with independent t-test and ANOVA analysis, where appropriate.

6.2.7.2 Cell counting

A mixed factorial ANOVA was used to determine differences between regions and groups in Zif268 and Arc cell counting. One-way ANOVA was used to determine differences between groups with respect to this cell counting. Simple main effects were analysed further with Ryan-Einot-Gabriel Welsch Range (REGWR) *post-hoc* test.

6.3 Results

6.3.1 Behavioural performance

6.3.1.2 Initial training and probe tests of original schema (Sessions 1-17)

Rats were trained on a set of 6 original PAs from session 1 to 17. Figure 6.8A shows the acquisition curve of this original PAs memory. The performance index scores for all the rats showed that performance improved across all training sessions and a repeated measures ANOVA showed that this was significant (F = 20.32, df = 6.75/134.92, p < 0.001). Performance index score was computed as 100-[100×(error/5)]. From sessions 6 onwards, the performance index score was between 70% and 80%, this is significantly different from chance (ts > 9.43, df = 1/20, p < 0.001).

During the training phase, 3 non-rewarded probe trials (PTs 1-3) were scheduled as shown in Figure 6.8A to examine acquisition of PAs memory. Figure 6.8B represents the percentage of dig time at the cued location (striped bars) relative to that at the non-cued locations (white bars). These probe trials revealed, as expected, a graded learning of the original PAs from sessions 1-17 (F = 10.95, df = 1.78/35.50, p < 0.001). T-test results indicated that digging at the cued location was above chance in PT2 (t = 2.77, df = 20, p < 0.05) and PT3 (t = 6.41, df = 20, p < 0.001).

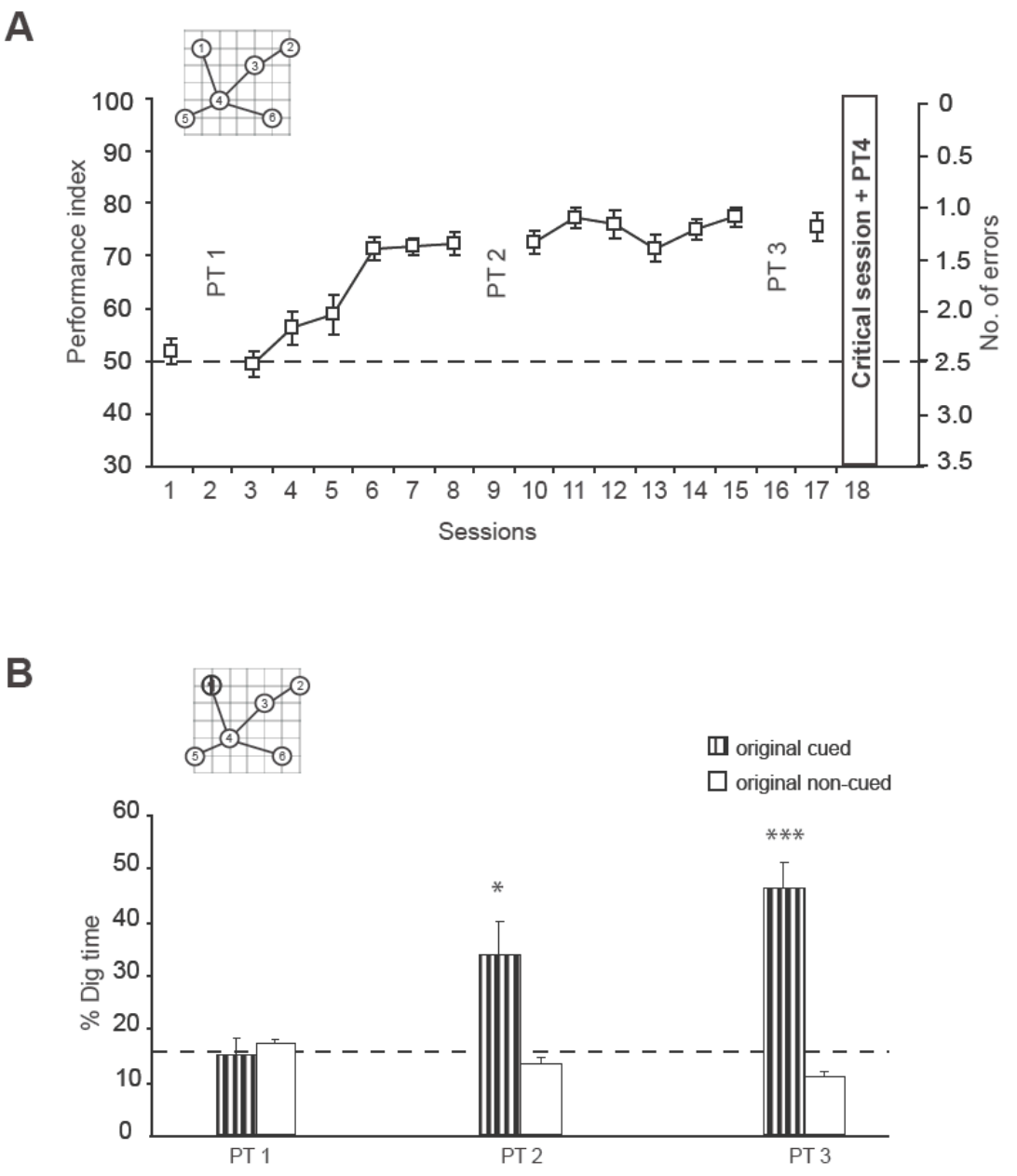

Figure 6.8 (A) Acquisition of the original 6 PAs (schema) (sessions 1-17). Performance rose to a stable level of 70% with minimal variability (from session 6 onwards). (B) Three cued-recall probe tests for the acquisition of the original schema. Non-rewarded probe tests revealed a graded learning of the original PAs (striped bars = % dig time spent in correct cued location) across sessions 2, 9 and 16. *$p < 0.05$; ***$p < 0.001$

6.3.1.2 Performance index and latency to dig at the correct sand-well on the critical session

On the critical session (18), rats were divided into 3 groups: new PAs (NPA) group, original PAs (OPA) group and new map (NM) group. Figure 6.9A shows the performance index of these 3 groups in the critical session (18) on trials 1 to 4, and trials 5 and 6. The ANOVA revealed an interaction between trials and groups (F = 4.52, df = 2/18, $p < 0.05$).

On trials 1 to 4 (white triangles, Figure 6.9A), the OPA and NPA groups experienced the well-learned original PAs and their performance indices were at 70%, which was significantly above chance (OPA group, t = 4.076, df = 6, $p < 0.05$; NPA group, t = 5.284, df = 6, $p < 0.05$). The NM group experienced new PAs and their performance index was, as expected, at chance level ($t < 1$, NS).

On trials 5 and 6 (black triangles), the OPA group experienced the well-learned original PAs, and the performance index was at 80% which was significantly different from chance (t = 4.90, df = 6, $p < 0.05$). The NPA and NM groups experienced new PAs and their performance indices were at chance level ($t < 1$, NS).

Figure 6.9B shows the latency to dig at the correct sand-wells of all OPA, NPA and NM groups on the critical session on trials 1 to 4 and trials 5 and 6. There is a trend for the latency to dig at the correct sand-well to be slightly slower for NPA and NM groups in searching for the new PAs than the OPA group searching for the original PAs in trials 5 and 6. This may be because NPA and NM groups experienced new PAs while OPA group experienced well-learned original PAs. However, an ANOVA of these latency scores revealed no overall interaction between trials and groups (F = 2.19, df = 2/18, $p > 0.05$).

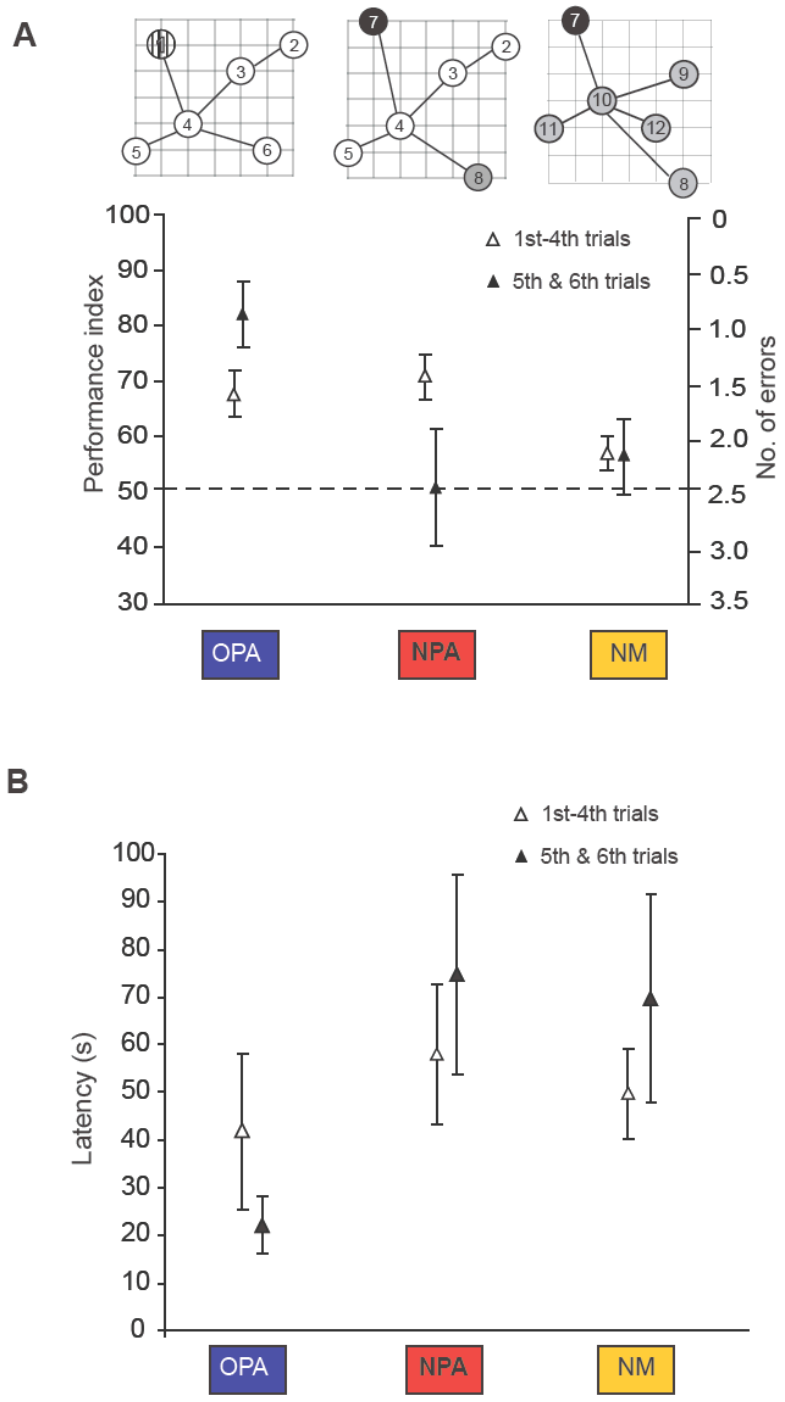

Figure 6.9 (A) Performance index on the critical session 18. On trials 1-4 (white triangle), OPA and NPA experienced original PAs and performance index were at 70%, while NM experienced new PAs and therefore performance index was at chance level. On trials 5 and 6 (filled triangle), OPA experienced original PAs and performance index were at 80%, while NPA and NM experienced new PAs and performance index were at chance level. (B) Latency to dig at the correct sand-well on the critical session 18. On trials 1-4 (white triangle), there is no difference between the OPA, NPA and NM groups. On the trials 5 and 6 (filled triangle), there is no difference between the OPA, NPA and NM groups.

6.3.1.3 Cued recall probe trial on critical session

Five minutes before the rats were sacrificed, a probe trial was performed to test if the rats remembered the PAs with which they had been presented on the 5th trial. Figure 6.10 shows the percentage of dig time at the cued location relative to the non-cued location. One-sample *t* tests compared the proportion of time spent digging in the original cued group (OPA), the new cued group (NPA) and the new cued (NM) to chance, and revealed that digging time was above chance for the OPA group ($t = 2.55$, $p < 0.05$) and the NPA group ($t = 6.19$, $p < 0.001$) but not the NM group ($t < 1$, NS). This finding suggests that both the OPA and NPA groups remembered the PAs that were presented to them on the 5th trial, while NM group could not remember the newly learned PAs.

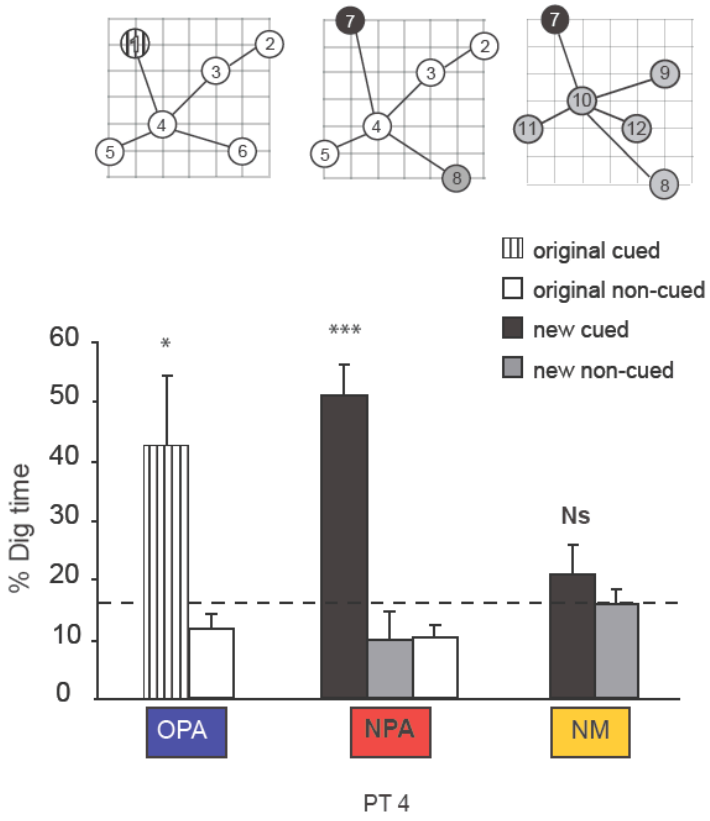

Figure 6.10 Cued recall probe test on critical session (5 min before perfusion). Both OPA and NPA groups remembered the cue correct location, while NM group could not remember the correct cued location. Ns, nonsignificant. *$p < 0.05$; ***$p < 0.001$

6.3.2 IEG analysis

The Zif268 immunohistochemistry was conducted at the University of Edinburgh, while the Arc immunohistochemistry was conducted at the University of Tokyo by Dr Masaki Kakeyama. The Zif268 data is therefore the focus of this thesis, but the Arc data will be briefly mentioned also.

6.3.2.1 Zif268 expression in hippocampus and various cortices

The expression of IEGs is correlated with levels of neuronal activity and can be used to track changes in the organisation of memories (Teixeira et al., 2006). To identify which hippocampal and extrahippocampal regions are involved in rapid memory consolidation, the expression of the Zif268 in the brain was measured.

Various regions

Figure 6.11A represents the normalised Zif268 counts across all the brain regions that were measured. These regions consist of PL, IL, ACC, aRSP, pRSP, Orb, Ins, Ssp, HPC and LEnt. There was a trend for the NPA group to have higher Zif268 counts than the OPA and NM groups, but this did not reach statistical significance [$F(3,20) = 2.91$, $p > 0.05$]. The NPA group's Zif268 count was, nonetheless, significantly higher than the CC group's zif count ($p < 0.05$, REGWR).

However, it may be the case that differential patterns of IEG activation are seen across the different groups, therefore, a regional breakdown of the Zif268 counts was performed.

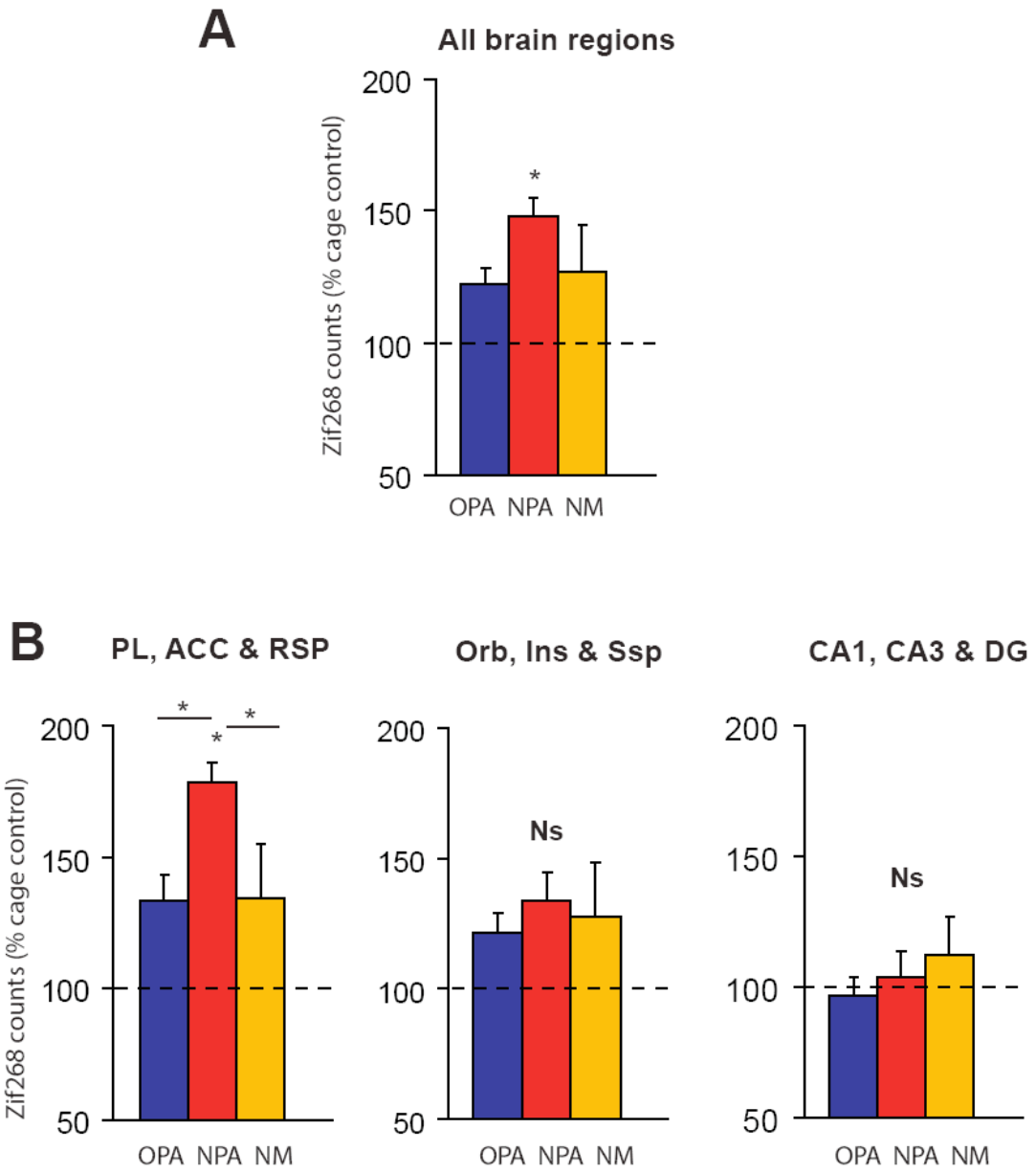

Figure 6.11 (A) Normalised counts of Zif268 positive nuclei across all the brain regions. (B) Normalised counts of Zif268 positive nuclei in different brain regions.
Ns, nonsignificant. *$p < 0.05$

Different Zif268 expression patterns in different regions

Figure 6.11B represents the normalised Zif268 counts for different regions showing different patterns.

In the PL, ACC, anterior and posterior RSP, an ANOVA revealed a significant difference in the normalised Zif268 counts in these regions between the OPA, NPA, NM and CC groups [$F(3,20) = 5.65$, $p < 0.01$]. The NPA group's Zif268 count was significantly higher than OPA, NM and CC's Zif268 count ($p < 0.05$ each, REGWR). When comparing these regions to the hippocampal formation (CA1, CA3 & DG), a two way ANOVA revealed that there was an interaction between region and group [$F(3,20) = 6.0$, $p < 0.01$]. This shows that the expression pattern observed in these cortical regions is significantly different from the expression pattern observed in CA1, CA3 & DG.

In the primary and secondary sensory cortices (Orb, Ins & Ssp), there were no significant differences in the normalised Zif268 counts between the OPA, NPA, NM and CC groups [$F(3,20) = 0.936$, $p > 0.05$]. They also did not differ from the level seen in the CC group.

There was a slight trend for the NPA and NM groups having higher Zif268 counts than OPA group in hippocampus (CA1, CA3 & DG); however, this did not reach statistical significance [$F(3,20) = 0.274$, $p > 0.05$].

Prefrontal and retrosplenial cortices (PL, IL, ACC, anterior and posterior RSP)

Figure 6.12 represents the normalised Zif268 counts for PL, IL, ACC, aRSP and pRSP.

In PL, a one way ANOVA revealed a significant difference in the normalised Zif268 counts in these regions between the OPA, NPA, NM and CC groups [$F(3,20) = 6.24$, $p < 0.01$]. The NPA group's Zif268 count was significantly higher than NM and CC's Zif268 count ($p < 0.05$ each, REGWR) but not the OPA's Zif268 count. In IL, there were no significant differences in the normalised Zif268 counts between the OPA, NPA, NM and CC groups [$F(3,20) = 0.976$, $p > 0.05$].

In contrast, in ACC, a one way ANOVA revealed a significant difference in the normalised Zif268 counts between the OPA, NPA, NM and CC groups [$F(3,20) = 3.83$, $p < 0.05$]. The NPA group's Zif268 count was significantly higher than CC's Zif268 count ($p < 0.05$ each, REGWR) but not higher than the OPA and NM group counts.

In aRSP, there were no significant differences in the normalised Zif268 counts between the OPA, NPA, NM and CC groups [$F(3,18) = 0.85$, $p > 0.05$].

A one way ANOVA revealed a significant difference in the normalised Zif268 counts in pRSP between the OPA, NPA, NM and CC groups [$F(3,20) = 6.64$, $p < 0.01$]. The NPA group's Zif268 count was significantly higher than OPA, NM and CC's Zif268 count ($p < 0.05$ each, REGWR).

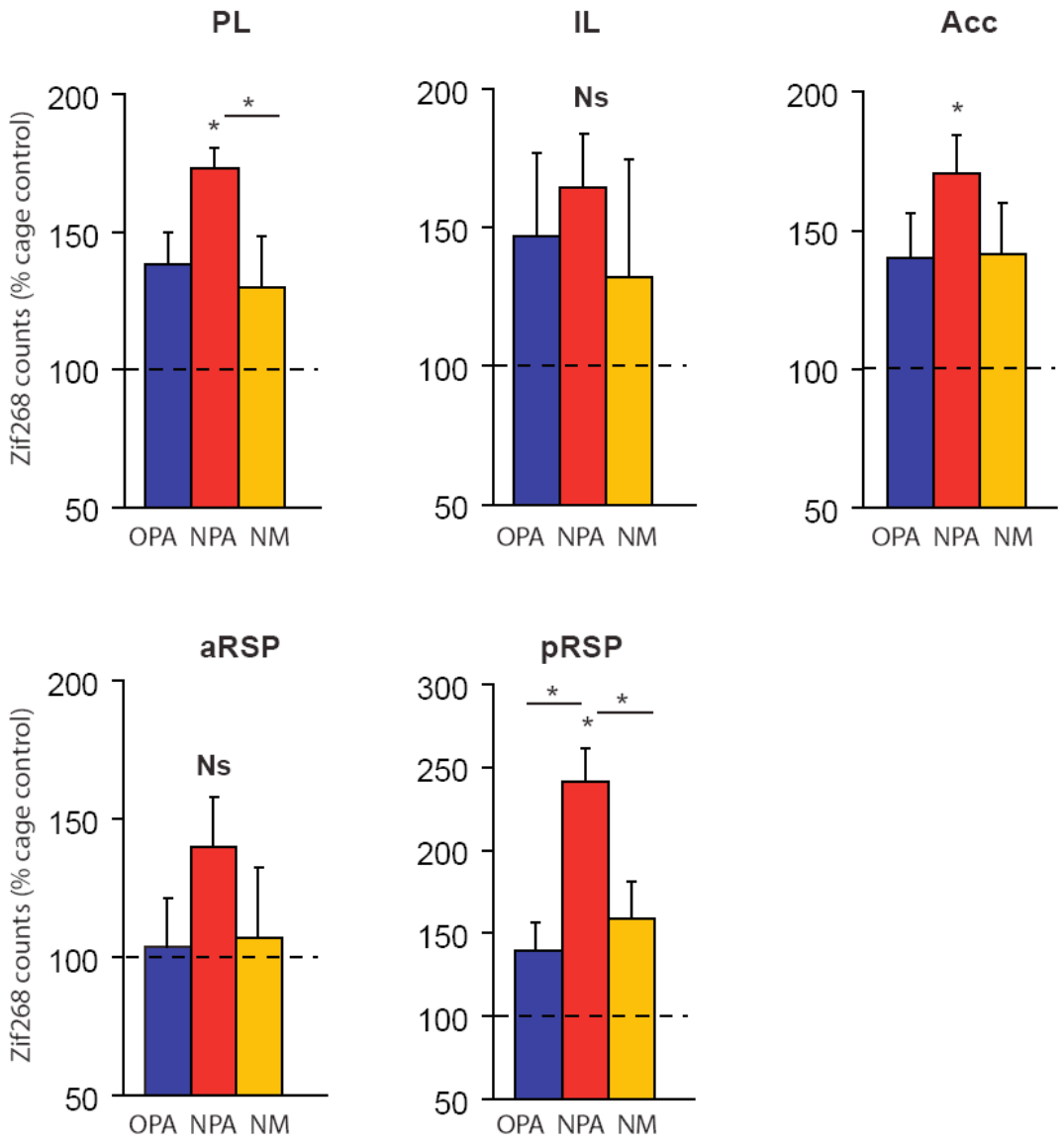

Figure 6.12 Normalised counts of Zif268 positive nuclei in the PL, IL, ACC, aRSP and pRSP. Ns, nonsignificant. *$p < 0.05$

Primary and secondary sensory regions

Figure 6.13 represents the normalised Zif268 counts in Orb, Ins and Ssp.

In Orb, a one way ANOVA revealed a significant difference in the normalised Zif268 counts between the OPA, NPA, NM and CC groups [$F(3,20) = 4.24$, $p < 0.05$]. The NPA group's Zif268 count was significantly higher than CC's Zif268 count ($p < 0.05$ each, REGWR) but not higher than the OPA and NM group counts.

In Ins and Ssp; there were no significant differences in the normalised Zif268 counts between the OPA, NPA, NM and CC groups [Ins: $F(3,20) = 0.22$, $p > 0.05$; Ssp: $F(3,20) = 0.33$, $p > 0.05$] .

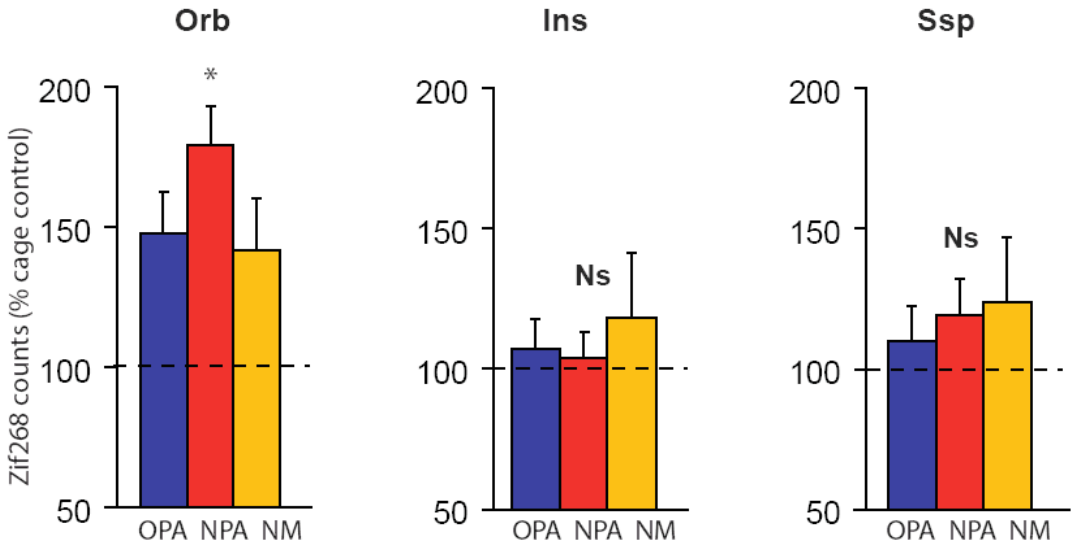

Figure 6.13 Normalised counts of Zif268 positive nuclei in the Orb, Ins and Ssp.
Ns, nonsignificant. *$p < 0.05$

Hippocampus and entorhinal cortex

As shown in Figure 6.14, in CA1, there was a trend for the NPA and NM groups having higher Zif268 counts than the OPA group; however, this did not reach statistical significance [$F(3,18) = 2.12$, $p > 0.05$].

In CA3, DG and LEnt (Figure 6.13); there were no significant differences in the normalised Zif268 counts between the OPA, NPA, NM and CC groups [CA3: $F(3,20) = 0.15$, $p > 0.05$; DG: $F(3,20) = 0.15$, $p > 0.05$; DG: $F(3,20) = 1.59$, $p > 0.05$]. A feature of these data is the substantially greater variability observed in area CA3.

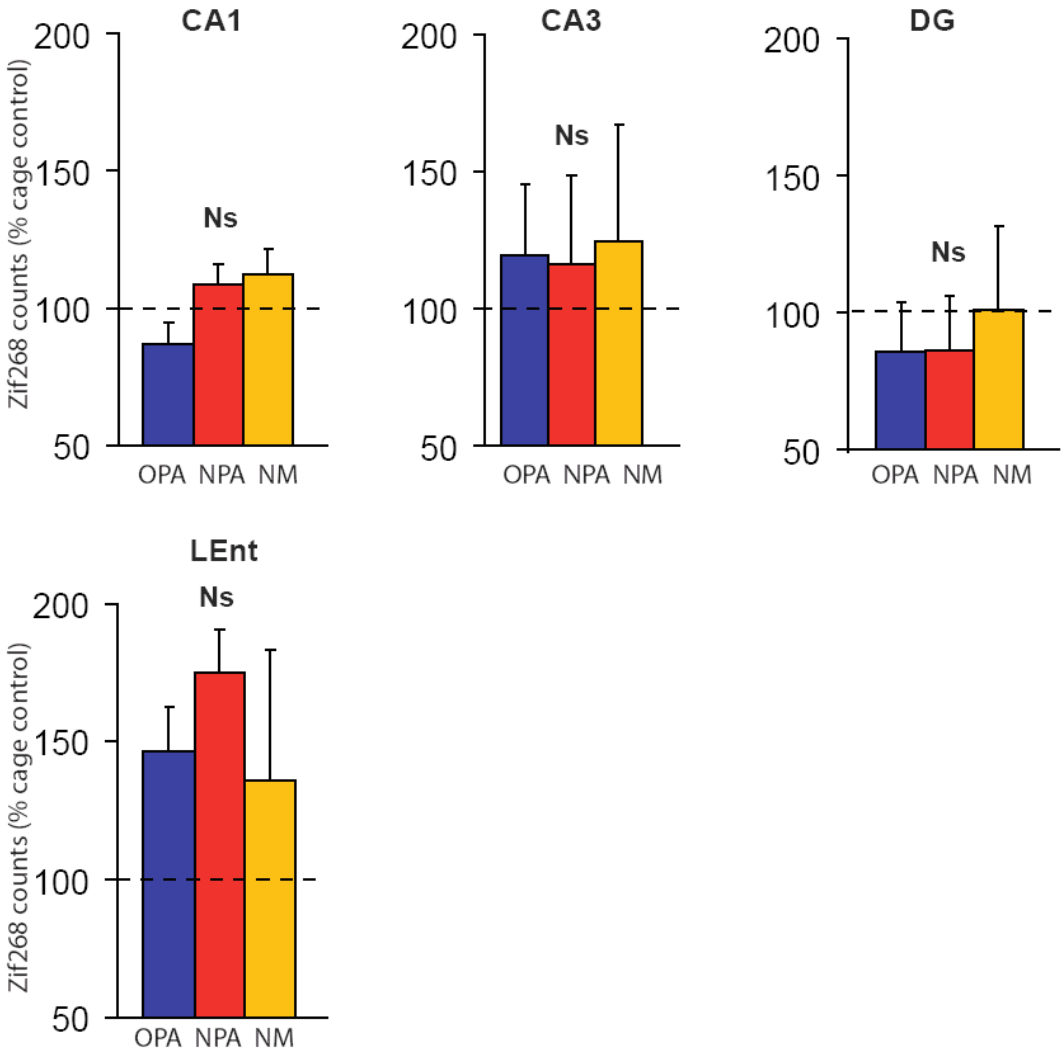

Figure 6.14 Normalised counts of Zif268 positive nuclei in the CA1, CA3, DG and LEnt. Ns, nonsignificant

Figure 6.15 shows representative sections of Zif268 immunoreactivity in the OPA, NPA, NM and CC groups in prelimbic cortex.

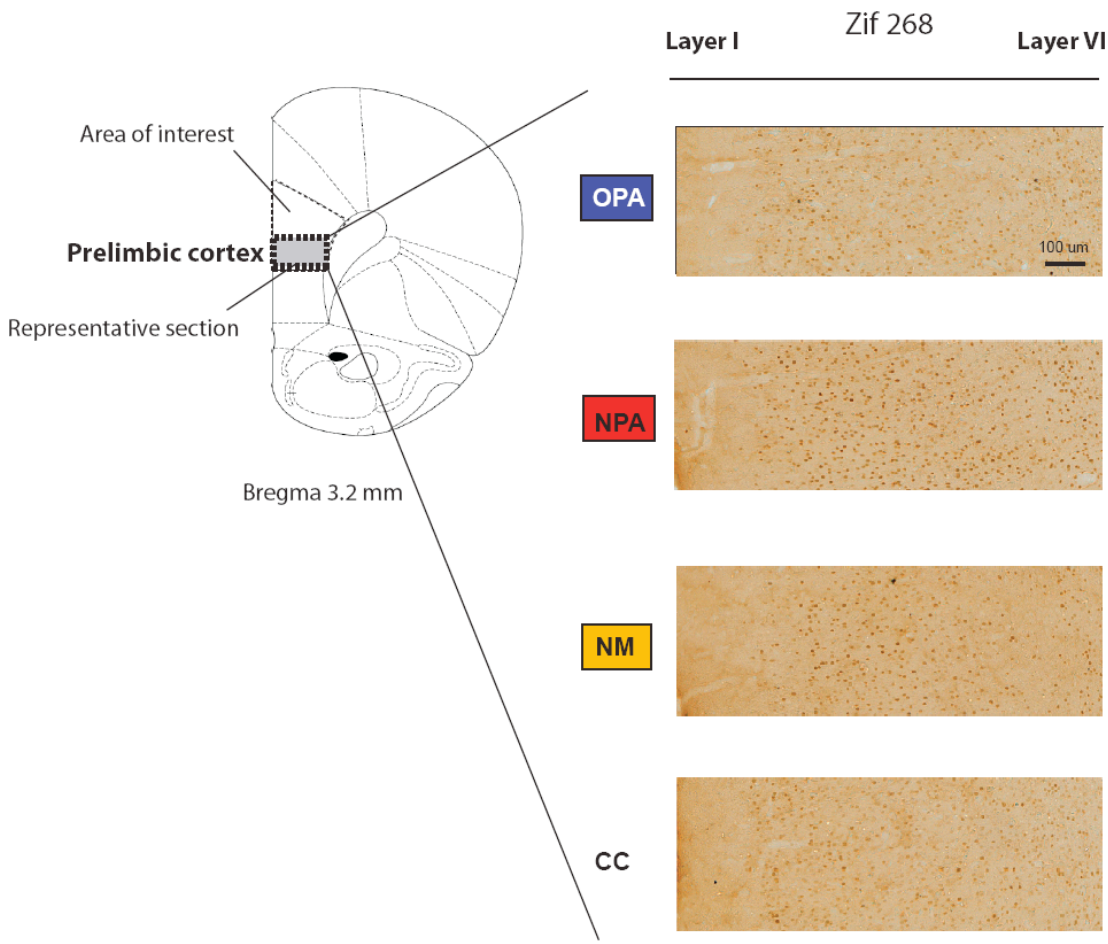

Figure 6.15 Representative sections showing Zif268 immunoreactivity in the OPA, NPA, NM and CC groups in prelimbic cortex.

6.3.2.1 *Arc* expression in hippocampus and various cortices

Various brain regions

Figure 6.16A represents the normalised Arc counts across all the brain regions. These regions contain of PL, ACC, aRSP, Ssp and HPC. Cell counts of Arc expression in other remaining brain regions (IL, pRSP, Orb, Ins and LEnt) are still underway.

In various brain regions, an ANOVA revealed a significant difference in the normalised *arc* counts between the OPA, NPA, NM and CC groups [$F(3,24) = 12.9$, $p < 0.001$]. The NPA and NM group's Arc count was significantly higher than OPA, and CC's Arc count ($p < 0.05$ each, REGWR).

However, it may be the case that brain regions play differential roles within the different groups and that this could be highlighted by the IEG cell count. Therefore, a regional breakdown of the Arc counts was performed.

Different Arc expressions patterns in different regions

In PL, ACC and aRSP (Figure 6.16B), a one way ANOVA revealed a significant difference in the normalised Arc counts in prelimbic cortex between the OPA, NPA, NM and CC groups [$F(3,24) = 17.1$, $p < 0.001$]. The NPA group's Arc count was significantly higher than OPA, NM and CC's Arc count ($p < 0.05$ each, REGWR). When comparing these regions to the hippocampal formation (CA1, CA3 & DG), a two way ANOVA revealed that there was an interaction between region and group [$F(3,24) = 12.10$, $p < 0.001$]. This shows that the expression pattern observed in these cortical regions is significantly different than the expression pattern observed in CA1, CA3 & DG. This is similar to the Zif268 expression pattern.

In the hippocampal formation (Figure 6.16B), an ANOVA revealed a significant difference in the normalised *Arc* counts between the OPA, NPA, NM and CC groups

[F(3,24) = 12.91, $p < 0.001$]. The NPA and NM group's Arc count was significantly higher than OPA, and CC's Arc count ($p < 0.05$ each, REGWR).

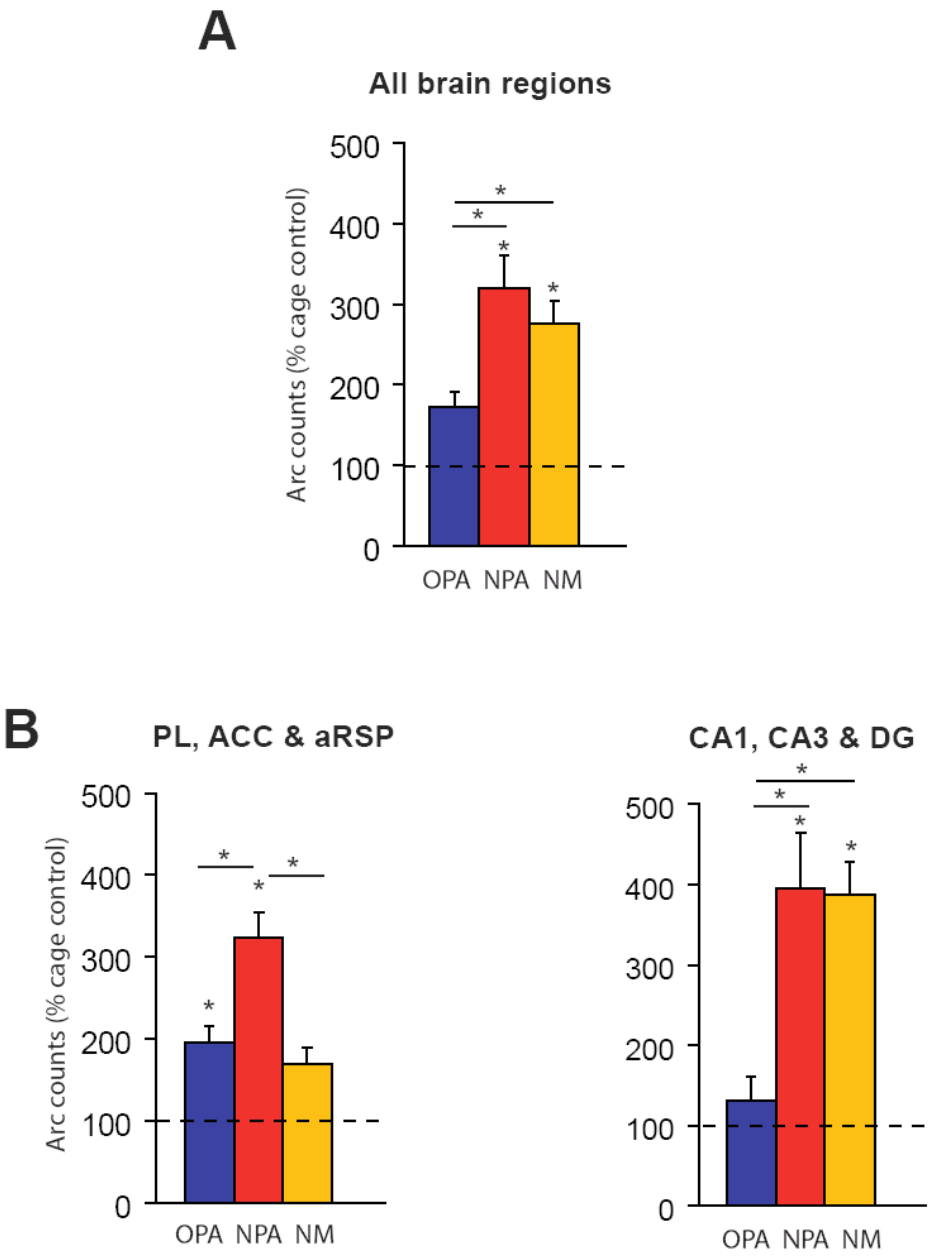

Figure 6.16 (A) Normalised counts of Arc positive nuclei in all brain regions. (B) Normalised counts of Arc positive nuclei in different brain regions.
*$p < 0.05$

In prefrontal and anterior retrosplenial cortices, Arc expression had a similar pattern to that of Zif268 expression

Figure 6.17 represents the normalised Arc counts in PL, ACC and aRSP. A one way ANOVA revealed a significant difference in the normalised Arc counts in prelimbic cortex between the OPA, NPA, NM and CC groups [$F(3,24) = 14.13$, $p < 0.001$]. The NPA group's Arc count was significantly higher than OPA, NM and CC's Arc count ($p < 0.05$ each, REGWR). This is similar to the Zif268 expression in prelimbic cortex.

Similarly, in ACC, a one-way ANOVA revealed a significant difference in the normalised Arc counts between the OPA, NPA, NM and CC groups [$F(3,24) = 14.00$, $p < 0.001$]. The NPA group's Arc count was significantly higher than NM and CC's Arc count ($p < 0.05$ each, REGWR) but not OPA's Arc count.

In aRSP, a one way ANOVA revealed a significant difference in the normalised *arc* counts between the OPA, NPA, NM and CC groups [$F(3,24) = 12.69$, $p < 0.001$]. The NPA group's Arc count was significantly higher than OPA, NM and CC's Arc count ($p < 0.05$ each, REGWR). This is similar to the Zif268 expression in aRSP.

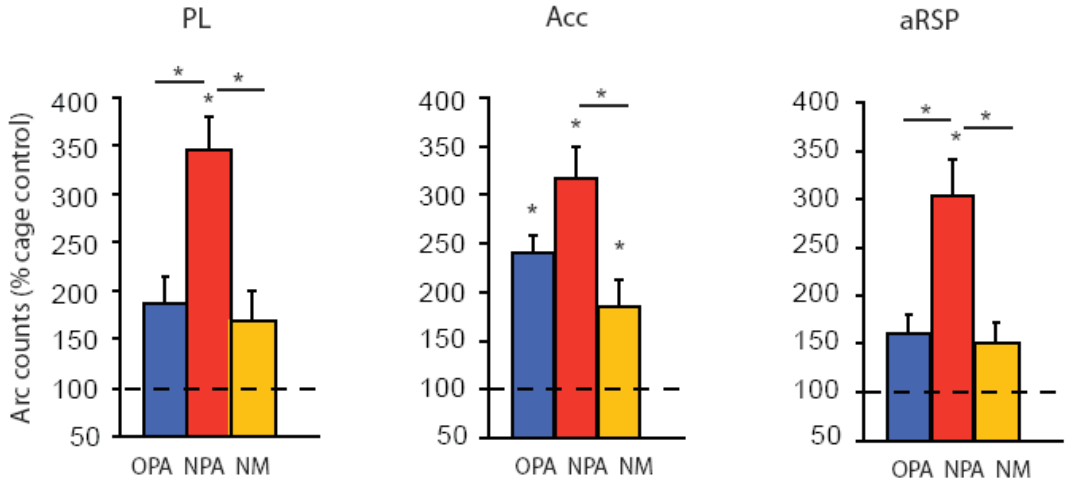

Figure 6.17 Normalised Arc counts in prelimbic, anterior cingulate and anterior retrosplenial cortices. *$p < 0.05$

Sensory region

Figure 6.18 represents the normalised Arc counts in Ssp. In Ssp, a one-way ANOVA revealed a significant difference in the normalised Arc counts between the OPA, NPA, NM and CC groups [$F(3,24) = 22.35$, $p < 0.001$]. The OPA and NM group's Arc count was significantly higher than NPA and CC's Arc count ($p < 0.05$ each, REGWR). This result is surprising because this expression pattern is completely different to that of the Zif268 counts for this region. In Zif268, there is no significant difference in the normalised Zif268 counts between the OPA, NPA, NM groups.

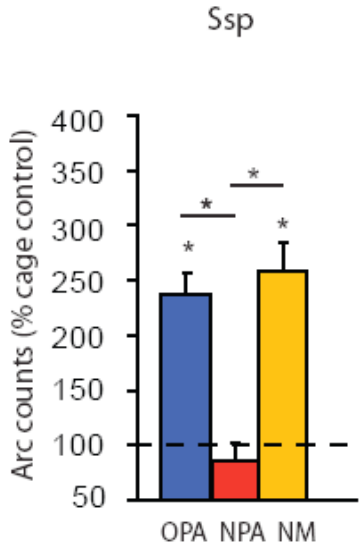

Figure 6.18 Normalised Arc counts in somatosensory cortex. *$p < 0.05$

Hippocampus

In CA1, Arc expression had a similar pattern to that of Zif268 expression

In CA1 (Figure 6.19), a one way ANOVA revealed a significant difference in the normalised *arc* counts between the OPA, NPA, NM and CC groups [$F(3,24) = 19.51$, $p < 0.001$]. The NPA and NM group's Arc count was significantly higher than OPA, and CC's Arc count ($p < 0.05$ each, REGWR). This is probably due to the novelty effect in NPA and NM as both groups were presented with new flavours and locations.

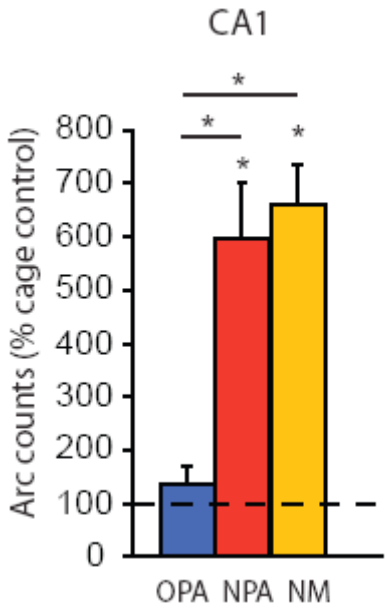

Figure 6.19 Normalised Arc counts in HPC. *$p < 0.05$

Figure 6.20 tabulates the **normalised Zif268 and Arc** cell counts in each group.

	Zif268						Arc					
Area	OPA	*n*	NPA	*n*	NM	*n*	CC	OPA	*n*	NPA	*n*	NM	*n*	CC	*n*
PrL	137.9 ± 11.6	6	172.8 ± 8.0$^{**,\#}$	6	129.6 ± 18.2	6	100.0 ± 6.7	186.5 ± 27.6	7	345.3 ± 35.5$^{***,\dagger\dagger\dagger,\#\#\#}$	7	168.8 ± 30.7	7	100.0 ± 9.4	7
IL	146.6 ± 30.7	6	165.0 ± 18.5	6	132.5 ± 41.9	6	100.0 ± 7.1	112.4 ± 12.1	7	122.4 ± 10.5	7	99.0 ± 16.4	7	100.0 ± 6.5	7
Orb	148.0 ± 14.3	6	179.0 ± 14.0*	6	141.9 ± 18.4	6	100.0 ± 16.4	100.0 ± 10.1	7	115.7 ± 10.8	7	100.9 ± 16.0	7	100.0 ± 8.0	7
Ins	107.2 ± 10.4	6	103.6 ± 9.3	6	118.3 ± 23.4	6	100.0 ± 21.8	109.0 ± 13.2	7	110.5 ± 14.5	7	105.0 ± 15.8	7	100.0 ± 11.2	7
ACC	139.7 ± 16.8	6	171.0 ± 13.3*	6	141.7 ± 18.2	6	100.0 ± 9.9	239.2 ± 18.2**	7	317.2 ± 32.3***,‡‡	7	185.0 ± 27.8*	7	100.0 ± 15.5	7
Ssp	109.9 ± 12.3	6	119.0 ± 13.5	6	123.9 ± 23.3	6	100.0 ± 21.4	236.6 ± 20.0***	7	85.3 ± 17.1$^{\dagger\dagger\dagger,\#\#\#}$	7	259.3 ± 24.9***	7	100.0 ± 12.2	7
aRSC	103.9 ± 17.7	6	139.8 ± 17.6	5	106.6 ± 25.6	5	100.0 ± 16.7	161.7 ± 19.6	7	303.2 ± 37.4$^{***,\dagger\dagger\dagger,\#\#\#}$	7	151.2 ± 20.9	7	100.0 ± 12.9	7
CA1	86.7 ± 7.8	6	108.3 ± 8.1	5	112.3 ± 9.0	5	100.0 ± 6.7	134.5 ± 32.8	7	594.6 ± 106.1***,†††	7	660.3 ± 73.4***,†††	7	100.0 ± 16.3	7
CA3	119.6 ± 25.6	6	116.3 ± 31.9	5	124.7 ± 41.9	5	100.0 ± 23.2	130.1 ± 32.5	7	291.2 ± 51.9**,††	7	298.3 ± 31.0**,††	7	100.0 ± 16.4	7
DG	85.3 ± 18.2	6	86.1 ± 19.9	5	100.8 ± 30.4	5	100.0 ± 26.9	126.4 ± 30.1	7	301.1 ± 56.7**,††	7	206.7 ± 37.5	7	100.0 ± 15.2	7
pRSC	139.7 ± 16.2	6	241.3 ± 20.6$^{**,\dagger,\#}$	6	159.4 ± 38.4	5	100.0 ± 21.5	106.6 ± 9.8	7	118.2 ± 8.8	7	106.1 ± 17.3	7	100.0 ± 8.2	7
LEnt	146.6 ± 15.9	6	174.6 ± 15.9	5	135.8 ± 47.5	5	100.0 ± 21.7	108.4 ± 18.0	7	127.6 ± 13.7	7	108.1 ± 12.5	7	100.0 ± 8.0	7

Data were expressed as mean ± SEM. *n*, numbers of animal. Significantly different from Group CC (Post hoc REGWR test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).
Significantly different from Group OPA ($^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$). Significantly different from Group NM ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$).

Figure 6.20 Table to show normalised Zif268 and Arc cell counts in cortices and hippocampus in OPA, NPA, NM and CC groups (± standard error).

Figure 6.21 tabulates the **raw Zif268 and Arc** cell counts in each group.

	Zif268							Arc						
Area	OPA	*n*	NPA	*n*	NM	*n*	CC	*n*	OPA	*n*	NPA	*n*	NM	*n*	CC	*n*
PrL	204.9 ± 17.5	6	254.9 ± 13.8**	6	190.9 ± 26.8	6	148.5 ± 10.2	6	83.4 ± 13.2*	7	146.1 ± 11.1$^{***,\dagger\dagger\dagger,\#\#\#}$	7	72.8 ± 12.9	7	44.6 ± 4.4	7
IL	79.7 ± 12.3	6	83.3 ± 5.5	6	66.5 ± 17.9	6	60.6 ± 9.0	6	15.5 ± 1.7	7	16.5 ± 1.3	7	13.4 ± 2.2	7	13.8 ± 0.9	7
Orb	222.1 ± 20.8	6	264.3 ± 25.0*	6	211.6 ± 31.9	6	151.0 ± 26.5	6	66.9 ± 6.8	7	76.4 ± 6.8	7	66.9 ± 10.5	7	66.9 ± 5.2	7
Ins	143.1 ± 11.0	6	143.0 ± 9.1	6	159.7 ± 28.0	6	135.4 ± 28.4	6	45.0 ± 5.9	7	45.5 ± 5.6	7	43.2 ± 6.1	7	41.0 ± 4.5	7
ACC	272.8 ± 27.4	6	317.1 ± 29.5	6	268.4 ± 41.6	6	202.2 ± 28.6	6	70.5 ± 5.9***	7	90.8 ± 8.2$^{***,\#\#}$	7	52.9 ± 7.8*	7	29.4 ± 4.8	7
Ssp	210.3 ± 22.6	6	229.2 ± 23.6	6	237.1 ± 41.6	6	191.8 ± 40.2	6	85.7 ± 7.2***	7	28.3 ± 4.6$^{\dagger\dagger\dagger,\#\#\#}$	7	90.2 ± 9.2***	7	36.5 ± 5.2	7
aRSC	107.5 ± 20.8	6	139.1 ± 20.1	5	109.7 ± 29.3	5	100.2 ± 18.1	6	86.0 ± 11.1	7	150.4 ± 13.4$^{***,\dagger\dagger\dagger,\#\#\#}$	7	77.2 ± 10.9	7	53.3 ± 7.9	7
CA1	222.7 ± 20.6	6	285.6 ± 26.6	5	294.4 ± 27.3	5	259.2 ± 20.5	6	20.8 ± 4.9	7	89.9 ± 15.6***,†††	7	101.3 ± 11.0***,†††	7	15.6 ± 2.7	7
CA3	16.2 ± 5.4	6	16.3 ± 4.3	5	13.9 ± 3.7	5	18.0 ± 9.6	6	16.4 ± 4.0	7	36.1 ± 6.3**,††	7	37.4 ± 3.9**,††	7	12.7 ± 2.2	7
DG	16.5 ± 4.4	6	17.0 ± 4.8	5	19.7 ± 7.0	5	18.4 ± 5.1	6	8.7 ± 2.1	7	21.1 ± 4.1**,†	7	14.4 ± 2.7	7	6.9 ± 1.0	7
pRSC	68.7 ± 9.8	6	111.7 ± 18.2*	6	80.2 ± 22.2	5	49.0 ± 12.1	6	64.2 ± 6.9	7	68.1 ± 3.6	7	61.7 ± 9.9	7	60.0 ± 5.2	7
LEnt	71.4 ± 15.3	6	78.5 ± 16.9	5	71.1 ± 29.6	5	46.2 ± 10.6	6	49.4 ± 8.2	7	57.8 ± 6.0	7	49.0 ± 5.8	7	45.7 ± 3.6	7

Data were expressed as mean raw counts (per 1 mm^2) ± SEM. *n*, numbers of animal. Significantly different from Group CC (Post hoc REGWR test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Significantly different from Group OPA ($^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$). Significantly different from Group NM ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$).

Figure 6.21 Table to show raw Zif268 and Arc cell counts in cortices and hippocampus in OPA, NPA, NM and CC groups (± standard error).

Figure 6.22 tabulates the comparison between the **normalised Zif268 and Arc** cell counts in OPA, NPA and NM groups.

	Zif268			Arc		
Area	OPA	NPA	NM	OPA	NPA	NM
PL	–	Significant different from NM	–	–	Significant different from OPA, NM	–
IL	–	–	–	–	–	–
Orb	–	–	–	–	–	–
Ins	–	–	–	–	–	–
ACC	–	–	–	–	Significant different from NM	–
Ssp	–	–	–	–	Significant different from OPA, NM	–
aRSP	–	–	–	–	Significant different from OPA, NM	–
CA1	–	–	–	–	Significant different from OPA	–
CA3	–	–	–	–	Significant different from OPA	–
DG	–	–	–	–	Significant different from OPA	–
pRSP	–	Significant different from OPA, NM	–	–	–	–
LEnt	–	–	–	–	–	–

Figure 6.22 Table to compare the normalised Zif268 and Arc cell counts in cortices and hippocampus in OPA, NPA, NM groups.

6.4 Discussion

This is the first study to use the event arena to study the activation of IEGs during memory encoding and retrieval; these findings are necessarily original. The key observations are:

1. IEG activation occurs differentially as a function of brain area;
2. Hippocampal structures appear to show a pattern that reflects PA novelty;
3. Certain prefrontal and retrosplenial structures, notably prelimbic and posterior retrosplenial cortices, show an activation pattern that reflects some interaction between novelty and prior schema knowledge.

6.4.1 Brain regions play differential roles within different groups

In this IEG study, different brain regions display a different expression pattern. In prefrontal and retrosplenial regions, the IEG counts of the NPA group were significantly higher than OPA, NM and CC groups counts. In the hippocampus, the NPA and NM groups had a higher IEGs expression than the OPA group. In the sensory cortices analysed, there were no significant differences in the normalised Zif268 counts between the OPA, NPA, NM groups.

These differential patterns were expected because IEG expression is correlated with neuronal activity. Numerous studies have shown differential patterns in different brain regions as a function of what an animal was doing. For example, Maviel et al. (2004) showed that in rats, prefrontal, anterior cingulate, and retrosplenial cortices become increasingly activated, as measured by Zif268, during retrieval of 5-arm maze remote memory tested at 30 days. In contrast, hippocampal and entorhinal areas show declining Zif268 activation over the period. Similarly, Frankland et al. (2004) showed increases in Zif268 and c-fos activation in the anterior cingulate, prefrontal and

temporal cortices and decreases in CA1 activation during retrieval of contextual fear memory tested at 36 days. In addition, Ross and Eichenbaum (2006) suggested that orbitofrontal, anterior piriform and lateral entorhinal cortices were involved in retrieval of a remote non spatial (social transmission of a food preference) memory. The findings are tabulated in Figure 6.23.

Studies	**IEGs**	**Behavioural tasks**	**Type of memory**	**Phase of memory**	**Results**
Maviel et al. (2004)	*Zif268*	5-arm maze	Spatial	Retrieval	Prefrontal, anterior cingulate and retrosplenial cortices were involved in remote memory
Frankland et al. (2004)	*Zif268* *c-fos*	Contextual fear conditioning	Non-spatial	Retrieval	Prefrontal, anterior cingulate and temporal cortices were involved in remote memory
Ross & Eichenbaum (2006)	*c-fos*	Social transmission of a food preference	Non-spatial	Retrieval	Orbitofrontal, anterior piriform and lateral entorhinal cortices were involved in remote memory
Present study (E4)	*Zif268* *Arc*	PAs schema task in event arena	Spatial	Encoding/ retrieval	Prefrontal, retrosplenial cortices are involved in encoding of new PAs in rapid schema memory

Figure 6.23 Table to show different IEGs studies that have investigated memory.

Ross and Eichenbaum (2006) suggested that perhaps different cortical areas may be responsible for the long term storage of different types of memory and this appears to be the case (Figure 6.23).

6.4.2 Hippocampal structures appear to show a pattern that reflects PA novelty

Novelty detection has been shown to induce the expression of IEGs. When exposing rats to a novel environment for brief periods of exploration, IEG expression in the HPC increases (Papa et al., 1993; Guzowski et al., 2006). In this study, both Zif268 and Arc expression displayed a similar pattern across the 3 groups in the HPC; both

NPA and NM groups had a higher Arc expression than OPA group. This is likely due to the fact that both of the NPA and NM groups had novel flavours and locations, whereas the OPA group did not.

Although both Zif268 and Arc expression displayed a similar pattern across the 3 groups in the HPC, there was no significant difference in the Zif268 counts between the NPA, NM groups with the OPA group, while in Arc expression, there was a significant difference between the NPA, NM groups with the OPA group.

One explanation for these differences may be that different IEGs have different properties. IEGs can be conceptually divided into 2 functional classes. One class encodes regulatory transcription factors, which may indirectly influence cellular physiology by increasing expression of specific downstream genes (Herdegen and Leah, 1998). Zif268 is such a regulatory gene. The second class encodes a diverse range of biological effector proteins, which have more defined and direct effects on cellular function than regulatory transcription factors (Lanahan and Worley, 1998). Arc is such an effector gene. In addition, Zif268, is known to have a much higher basal level than other IEGs in most of the brain areas apart from dentate gyrus (Milbrandt, 1987). Furthermore, Guzowski and colleagues (2001) suggested that, although the expressions of Arc and Zif268 have many similarities, Arc was more responsive to differences in behavioural task demands than Zif268 in hippocampus. It seems a possibility, therefore, that the differential IEG expression patterns in CA1 may be because Zif268 is less sensitive than Arc to changes in behavioural task demands.

6.4.3 Prefrontal and retrosplenial structures show an activation pattern that reflects some interaction between novelty and prior schema knowledge

In this study, in the prefrontal and retrosplenial regions, NPA group IEG counts were significantly higher than OPA, NM and CC groups counts. This indicates that these regions may play a role in the encoding of new PAs, specifically the PL and pRSP. Previous studies that have investigated memory using IEGs expression have suggested that prefrontal and anterior cingulate cortices play a general role in the storage of long-term memories, and that these areas integrate the cortical representations necessary for long-term memory (Maviel et al., 2004; Frankland et al., 2004).

One thing to note is that, in these previous studies, only retrieval was investigated, whereas in this present study, *encoding* of new PAs and *retrieval* of original PAs were under investigation (see Figure 6.23).

6.4.4 Future studies

This IEGs study investigated the *encoding* of the *new* PAs and the *retrieval* of *original* PAs in a PA schema. However, it would also be valuable to investigate the *retrieval* of the *new* PAs of a schema to compare with the IEG expression found during *retrieval* of the *original* PAs. One may speculate that the new PAs information may be initially processed by the relevant primary and associative cortical areas. These regions will need some kind of intra-cortical connectivity and this is perhaps provided by the prefrontal and retrosplenial cortices. The original schema information may be stored in these cortices.

In addition, as IEG imaging data are merely correlative, the next sensible step was to use another approach - pharmacological manipulations - to ask more directly whether these prefrontal and retrosplenial cortices are involved in encoding of new PAs in the PAs schema paradigm task. This could involve a variety of techniques, including temporary inactivation of either AMPA receptors or NMDA receptors during encoding of new PAs. This approach will be described in the following chapter.

Chapter 7 – Experiment 5

Contributions of prelimbic NMDA and AMPA receptors in novel paired-associates schema memory

7.1 Introduction

The immediate early genes (IEGs) data reported in the previous chapter (E4) revealed a network of cortical structures, extending from the prefrontal regions of the forebrain to the anterior cingulate and retrosplenial cortices, in which there is elevated *Zif268* and *Arc* expression during encoding of 2 new flavour-place paired-associates (PAs). This increase is significantly higher than that seen during reactivation of the original PAs or during the encoding of an entirely new set of PAs. The most significant elevation in both *Zif268* and *Arc* expression was observed in the prelimbic cortex, indicating that it may be critical for the rapid assimilation of new information into a pre-existing schema. However, as IEG imaging data are correlative, the next sensible step was to use another approach to investigate directly whether prelimbic cortex plays a causal role in the encoding of new PAs.

The approach taken was pharmacological intervention; specifically, the temporary inactivation of either AMPA receptors or NMDA receptors during encoding of new PAs. The aim of this study was to examine whether encoding of new PAs in the PAs schema paradigm task requires glutamate neurotransmission in prelimbic cortex. First, the contribution of AMPA receptor neurotransmission within the prelimbic cortex during encoding of the *new* PAs was examined using CNQX, an AMPA receptor antagonist. Second, the contribution of NMDA receptors in the prelimbic cortex during encoding of the *new* PAs was examined using D-AP5, an NMDA receptor

antagonist. Third, the study also investigated the contribution of prelimbic AMPA receptors and NMDA receptors to the retrieval of *new* and *original* PAs in the schema task.

While a study involving infusions of appropriate antisense oligodeoxynucleotides to disrupt *Zif268* or *Arc* may be considered a more direct follow-up, it should be recognised that these IEGs were merely being used as 'markers' to identify relevant cortical regions. Identifying the specific signal-transmission mechanisms may come later, but such a study is some way off.

7.1.1 Prelimbic cortex

The involvement of the hippocampus and the prefrontal cortex in learning and memory has been well documented over the years (Squire, 1992; Laroche et al., 2000). As mentioned in the introduction (chapter 2), the prefrontal cortex seems to play a privileged role in the processing of remote memory in systems memory consolidation (Frankland and Bontempi, 2005). Anatomically, the prefrontal cortex consists of several highly interconnected regions, including the prelimbic (which is the focus of the following experiment), infralimbic and anterior cingulate areas (Vertes, 2006). As these regions are reciprocally connected to sensory, motor and limbic cortices (Uylings et al., 2003), they may be ideally suited for integrating complex information from various sensory sources (Teixeira et al., 2006; Bontempi and Durkin, 2007). Experimental evidence for reorganisation of hippocampus-dependent memory indicates that the prefrontal cortex is involved in the consolidation and expression of remote memory in fear conditioning (Frankland et al., 2004; Maviel et al., 2004). In addition, Takehara et al. (2005) showed that the prefrontal cortex plays a critical role in the consolidation of remote memory for trace eyeblink

conditioning through NMDAR dependent processes and that their importance is during the first 2 weeks of the postlearning period. Together with the IEG data from previous chapter, one may speculate that NMDA receptors in the prelimbic cortex may have an important role during the encoding of the new PAs in the schema paradigm.

7.1.2 Choosing a suitable control region

The aim of the present experiment was to investigate the contributions of the prelimbic NMDA and AMPA receptors in assimilating novel PAs into a schema, or retrieving information from it. This involved infusing drugs into the prelimbic cortex. The use of a control region was essential to establish whether drug infusions into the cortex have a regionally-selective effect in this task. Somatosensory ('barrel') cortex was chosen for two reasons: *first,* it is unlikely that sensory processing in that region would contribute to detecting or discriminating tastes (e.g. in the start-box), or to retrieving information about the spatial location of a sand-well where more of that taste could be found; *second,* the Zif268 data for this region indicated no differential activation across trained groups or relative to the caged control.

7.1.3 Barrel cortex

7.1.3.1 The organisation of the whisker sensory system

It is well known that a rodent's ability to navigate depends, in part, on the use of its whiskers (Petersen, 2007; Diamond et al., 2008). A rodent has a highly sensitive array of whiskers on its snout. The whiskers on a rat's snout are arranged in a grid made up of 5 rows, designated A to E, and several numbered arcs, so that an individual whisker can be identified by its row and position within that row (Figure 7.1A). At the base of

each whisker, a follicle is surrounded by sensory nerve endings that detect whisker movements. The deflection of a whisker evokes action potentials in sensory neurons of the trigeminal nerve, which release glutamate into the brain stem. Brain stem neurons then send sensory information to the thalamus where a glutamatergic synapse excites thalamocortical neurons projecting to the primary somatosensory (barrel) cortex (Petersen, 2007; Diamond et al., 2008). The layout of the barrels in the somatosensory cortex replicates the layout of the whiskers on the snout (Figure 7.1B).

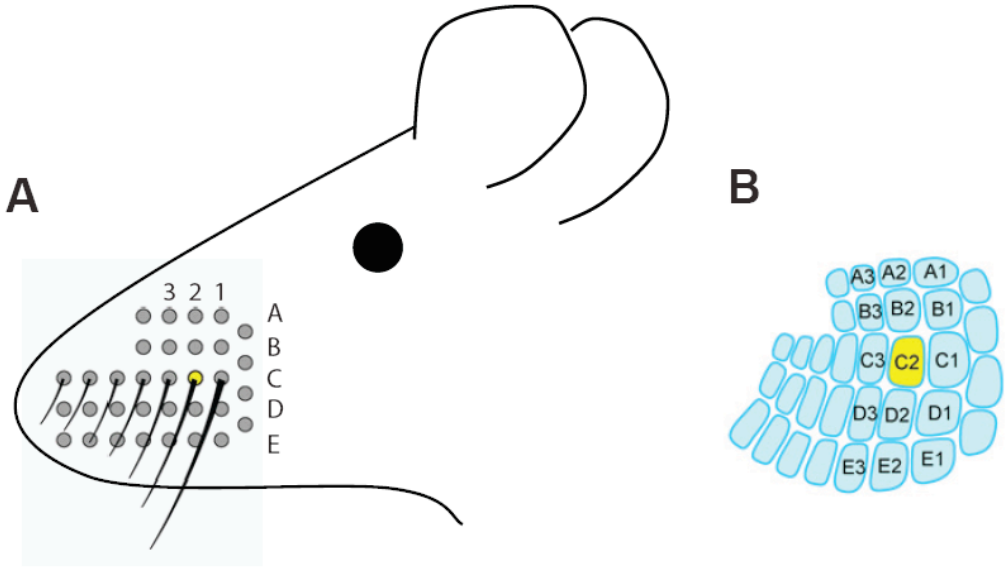

Figure 7.1 (A) The snout of the rodent is surrounded by arrays of whiskers (only C-row whiskers shown). At the base of each whisker the follicle is surrounded by sensory nerve endings that detect whisker movements. (B) The organisation of the somatosensory cortex (barrels in layer 4) matches the layout of the whiskers on the rodent snout. A standard nomenclature has been developed to describe both the patterns of whiskers and barrels which consist of the rows A–E and the arcs 1, 2, 3, etc. The C2 whisker follicle and the C2 barrel are highlighted in yellow. (Figure modified from Petersen, 2007).

7.1.3.2 Learned whisker-dependent behaviours

There are 2 main types of behavioural tasks used when investigating the rodent whisker sensorimotor system (Petersen, 2007). These are, the discrimination of textures and detecting the location of edges (Figure 7.2).

Rodents can use their whiskers to discriminate different textures (Figure 7.2A). Carvell and Simons, (1990) have trained rats to discriminate different textures using only whiskers; quantitative behavioural measurements indicate that the sensitivity of the rodent's whiskers is comparable to that of primate finger tips. This rodent ability is often used to investigate the rodent sensorimotor system (Petersen, 2007).

Another behavioural task is the detection of the location of edges. For example, a gap crossing task (Figure 7.2B). This task was first described by Hutson and Masterton (1986) and has been extensively studied since then. This task involves training a rodent to cross from one raised platform to another to receive a food reward. The rodent uses its whiskers to gauge the distance of a gap between the platforms, which is manipulated by the experimenter between trials. If the rodent locates the target platform with its whiskers, it will likely jump across to receive the food reward (Hutson and Masterton, 1986). As this is dependent upon whiskers, it may be a useful task for assessing possible behavioural effects when drugs are infused into the barrel cortex.

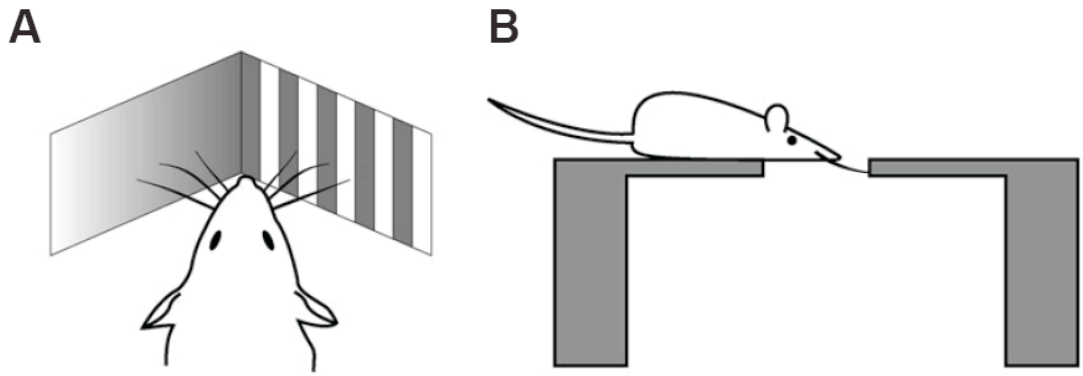

Figure 7.2 There are 2 main types of behavioural tasks used when investigating the rodent whisker sensorimotor system: (A) discrimination of textures, and (B) location of edges. (Figure reprinted from Petersen, 2007).

In the present experiment, a gap-crossing task was used as it was thought to be the more appropriate because it is relatively simple to train the rats on the task and was thus deemed the more time efficient of the 2 task types.

7.2 Material and methods

7.2.1 Subjects

Eleven adult male Lister-hooded rats (Charles River, UK) were used in this experiment as described in General Methods.

7.2.2 Surgery

Rats were implanted bilaterally with infusion guide cannulae in the prelimbic cortex and barrel cortex, as described in the general methods. After surgery, rats were given a recovery period of 7 days before the start of the behavioural procedures.

7.2.3 Drugs and microinfusions

The competitive NMDA receptor antagonist D-AP5 (Tocris, UK) was used at a concentration of 5.9 mg/ml (30 mM) and the competitive AMPA/kainate receptor antagonist CNQX (Tocris, UK) was used at a concentration of 0.89 mg/ml (3 mM). D-AP5, CNQX and NaCl (control infusion solution for D-AP5 and CNQX) (0.5 μl or 2.0 μl) were bilaterally infused at a rate of 0.4 μl/min over 2 to 5 min, after which the infusion cannulae were left in place for a further 1 min to ensure drug solution entered the brain. The infusion procedures are detailed described in General Methods.

PAs schema task

7.2.4 Apparatus

The event arena in which rats were trained to find flavoured food as described in General Methods.

7.2.5 Behavioural training

The time line of this experiment is shown in Figure 7.3.

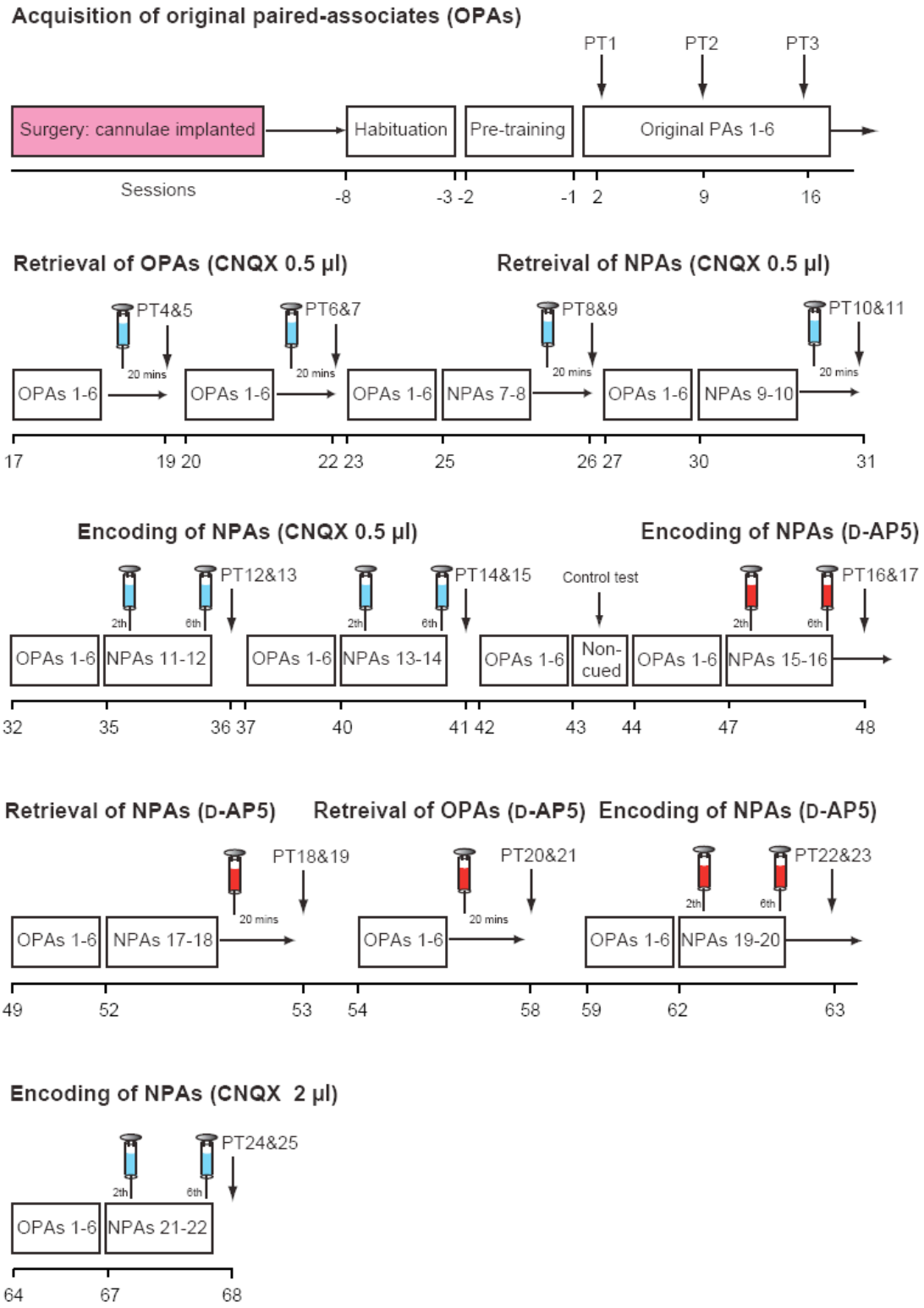

Figure 7.3 Timeline showing the design of Experiment 5. The acquisition of original paired-associates (OPAs), retrieval of OPAs and new paired associates (NPAs), and encoding of NPAs with CNQX and D-AP5. The syringe cartoons indicate the bilateral microinfusions into the prelimbic cortex or barrel cortex (no i.p. injections were actually used). The blue syringes indicate the infusion of CNQX while the red syringes indicate the infusion of D-AP5.

7.2.5.1 Habituation and pre-training

Rats were habituated and pre-trained in the event arena as described in General methods.

7.2.5.2 Training of paired-associates

The experiment was a within-subject, repeated-measures design, consisting of a series of initial training sessions, new PAs learning, cued recall of original PAs and new PAs, and drug infusions sessions (Figure 7.3).

7.2.5.3 Training of original paired-associates schema (Sessions 1-17)

The original arrangement of flavour place paired associates (PAs 1 to 6) is shown in Figure 7.4. Each PA was presented for 1 trial/session [PA1 = flavour 1 (F1) (strawberry) at location 1 (L1), PA2 = F2 (pina colada) at L2, PA3 = F3 (chocolate) at L3, PA4 = F4 (very berry) at L4, PA5 = F5 (marshmallow) at L5, PA6 = F6 (bacon) at L6]. The flavours used in the original schema are shown in Figure 7.5.

During the experiment, rats were trained on alternate days in 2 cohorts of up to 6 rats with each cohort receiving 3 training sessions per week (cohort 1: Monday, Wednesday and Friday; cohort 2: Tuesday, Thursday and Saturday).

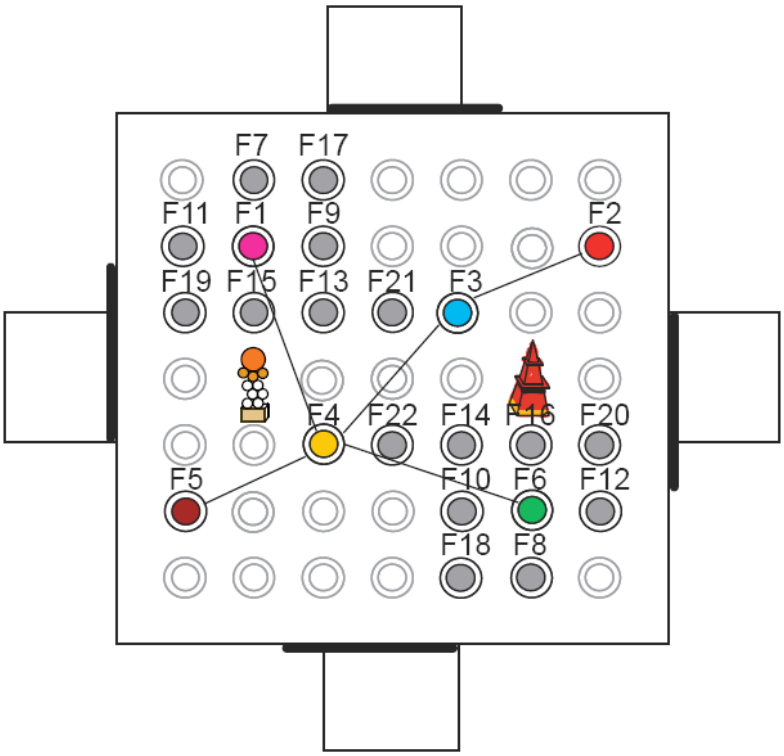

Figure 7.4 Spatial arrangement of the PAs in a schema. Colour circles (Flavours, F1 to F6) are original PAs (a schema) and grey circles (F7 to F22) are new PAs.

PAs	Flavours	Locations
PA1	F1 (strawberry)	L1
PA2	F2 (pina colada)	L2
PA3	F3 (chocolate)	L3
PA4	F4 (very berry)	L4
PA5	F5 (marshmallow)	L5
PA6	F6 (bacon)	L6

Figure 7.5 The flavours (F) used for the original schema (F1 to F6).

7.2.5.4 Non-rewarded probe tests (Sessions 2, 9, 16)

To examine cued-recall memory, several non-rewarded probe tests (PTs 1 to 3) were scheduled. During these tests, all 6 sand-wells were opened as usual and the rats were free to dig in any of them although none contained any food reward. The animals

were cued with a single flavour (as usual), and then allowed into the arena for a total of 120 s. Digging time was measured and used as an index of memory strength. As noted in the general methods, these probe tests also served as a further control for artefacts and uncontrolled factors.

7.2.5.5 Training and probe tests (Sessions 17-41): Impact of CNQX (0.5 µl) on retrieval of original and new PAs and encoding of new PAs in prelimbic and barrel cortices

Once the rats had learned 6 PAs in the schema paradigm, the next step was to investigate the impact of prelimbic or barrel cortex infusion of the AMPA receptors antagonist CNQX on the retrieval of original PAs (PTs 4 to 7), new PAs (PTs 8 to 11) and on the encoding of new PAs (Sessions 35 & 40). The experiment was a within-subject, repeated measures study.

Retrieval of original PAs (Sessions 17-22)

The basic procedure for the retrieval of original PAs is shown in Figure 7.6.

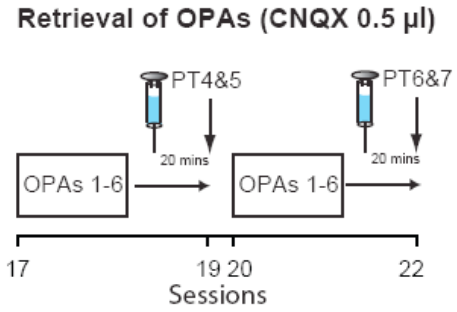

Figure 7.6 Schedule of retrieval of original paired-associates (OPAs).

The rats were presented with 2 sessions (17 and 18) of original PAs. Then, 24 hr later (session 19), CNQX (0.5 µl) or NaCl (0.5 µl) was infused 20 min before probe trials (4 and 5) in one brain region (either prelimbic or barrel cortex). These probe tests (4

& 5) were 3 hours apart within the same session. After 2 further sessions (20, 21) of original training, probe trials (6 and 7) were repeated to counterbalance the different conditions across the full set of 9 rats (Figure 7.7).

Regions	Prelimbic (PL)		Barrel cortex (Ssp)	
Drugs	NaCl	CNQX	NaCl	CNQX
Probe trials	PT4	PT5	PT6	PT7

Figure 7.7 One arrangement of the different conditions to examine memory retrieval of original paired associates. Other counterbalanced arrangements included barrel cortex being tested before prelimbic, and a differenbt order for CNQX and NaCl.

Training of new paired-associates

The new flavour place paired associates (PAs 7 to 22) arrangement is shown in Figure 7.3. The flavours used in the original schema are shown in Figure 7.8.

PAs	Flavours	Locations
PA7	F7 (apple)	L7
PA8	F8 (paprika)	L8
PA9	F9 (almond)	L9
PA10	F10 (cinnamon)	L10
PA11	F11 (cherry)	L11
PA12	F12 (ginger)	L12
PA13	F13 (rum)	L13
PA14	F14 (butter)	L14
PA15	F15 (grape)	L15
PA16	F16 (banana & cherry)	L16
PA17	F17 (orange)	L17
PA18	F18 (chocolate & apple)	L18
PA19	F19 (banana)	L19
PA20	F20 (apple & cinnamon)	L20
PA21	F21 (lemon)	L21
PA22	F22 (vanilla & brandy)	L22

Figure 7.8 The list of flavours (F) used in new PAs, F7-F22.

Retrieval of new PAs (sessions 23-31)

The basic procedure for the retrieval of new PAs is shown in Figure 7.9.

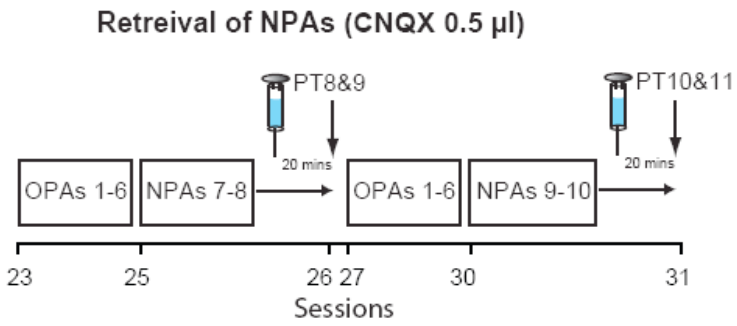

Figure 7.9 Retrieval of new paired-associates (NPAs).

The rats were then given further training on the original PAs for 2 sessions (23-24). Then, on session 25, the sand-wells for PA1 and PA6 were closed, and replaced by another 2 containing 2 novel flavours at neighbouring locations: PA7 (apple) and PA8 (paprika) (Figure 7.4). Rats were trained for a total of 6 trials: 1 trial for each of the 2 novel flavours and the 4 trials for the remaining PAs of the original schema (i.e. PAs 2-5). Twenty four hours later, on session 26, drugs (CNQX, 0.5 µl or NaCl, 0.5 µl) were infused 20 min before the probe trials (8 and 9). These probe trials were designed to test the rat's memory for the new PAs. Three further sessions (27-29) of original training were given to the rats, followed by 2 further novel PAs (PA9 = Almond, PA10 = Cinnamon) training (Figure 7.3). Probe trials (PTs 10-11) were scheduled 24 hr after the new PAs training sessions.

Encoding of new PAs (sessions 32- 41)

The basic procedure for the encoding of new PAs is shown in Figure 7.10.

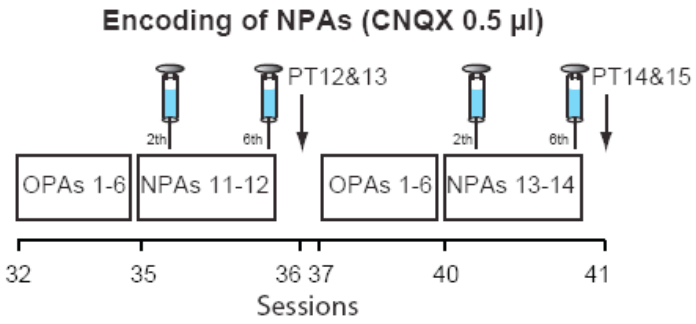

Figure 7.10 Encoding of new paired-associates (NPAs).

The next critical test examined the impact of CNQX on in encoding of new PAs in both prelimbic and barrel cortex. The rats were given further training on the original PAs for 3 sessions (32-34). On session 35, the sand wells for PA1 and PA6 were replaced by another 2 novel flavours at neighbouring locations: PA11 (cherry) and PA12 (ginger) (Figure 7.4). Rats were trained for a total of 6 trials - 1 trial for each of the 2 novel flavours and the 4 trials for the remaining PAs of the original schema (i.e. PAs 2-5). Drugs (CNQX, 0.5 µl or NaCl, 0.5 µl) were infused 20 min before trial 2 and again before trial 6 in one brain region (either prelimbic or barrel cortex). Twenty four hours later, on session 36, two probe trials (12 and 13) were scheduled. Three further sessions (37-39) of original training were given, and then 2 further novel PAs (PA13 = rum, PA14 = butter) training (Figure 7.4). Probe trials (14-15) were then scheduled 24 hr after the new PAs training sessions.

7.2.5.6 Non-cued control test

Another control test, session 43, was given to test whether the cue flavour given in the start box actually did guide the rats' search strategy rather than any potential cryptic olfactory cue at the goal. A standard session of 6 daily rewarded trials were conducted

in which the retrieval cues – the use of 6 separate flavoured pellets in the start box on 6 successive trials were absent. The performance index calculated for retrieval of the first cue pellet should fall to chance if this start box cue is critical for memory retrieval.

7.2.5.7 Training and probe tests (session 44-63): Impact of D-AP5 (0.5 μl) on retrieval of original and new PAs and encoding of new PAs in the prelimbic cortex

In these sessions, 44-63, D-AP5 or saline was infused into the prelimbic cortex and not the barrel cortex. This is because when AMPARs in the barrel cortex were inactivated (see results in 7.3.2), rats could still perform well in retrieval of both original and new PAs, and also in encoding of new PAs. Thus, further barrel cortex infusion would not serve a purpose.

Encoding of new PAs (sessions 44-48 and 59-63)

The next sessions were a critical test of prelimbic dependence of new PAs learning by examining the impact of the NMDA receptor antagonist D-AP5. The basic procedure for the encoding of new PAs is shown in Figure 7.11.

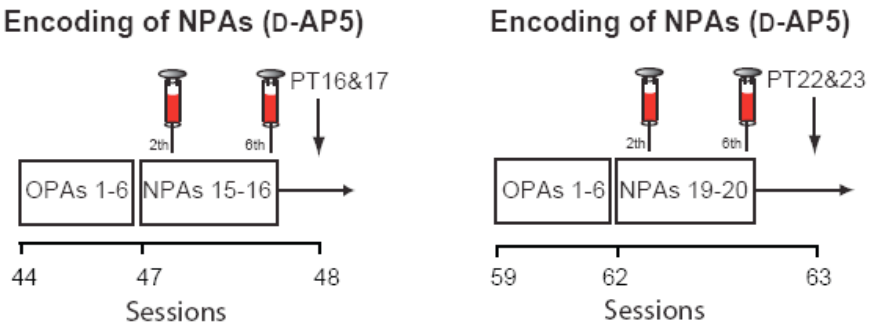

Figure 7.11 Encoding of new paired-associates (NPAs).

On sessions 44-46, rats were given training on the original PAs. On session 47, 2 novel flavours were introduced: PA15 (grape) and PA16 (banana and cherry) (Figure 7.4). Rats were trained for a total of 6 trials: 1 trial for each of the 2 novel flavours and 4 trials for the remaining PAs of the original schema (i.e. PAs 2-5). Drugs (D-AP5, 0.5 μl or NaCl, 0.5 μl) were infused 20 min before trial 2 and again before trial 6 in the prelimbic cortex. Twenty four hours later, on session 48, two probe trials (16 and 17) were conducted. This sequence was repeated again during sessions 59-63. Three further sessions (59-61) of original training were given to the rats, and then 2 further novel PAs (PA19 = banana, PA20 = apple and cinnamon) training (Figure 7.4). Probe trials (22-23) were scheduled 24 hr after the new PAs training sessions.

Retrieval of new PAs (sessions 49-53)

The basic procedure for the retrieval of new PAs is shown in Figure 7.12.

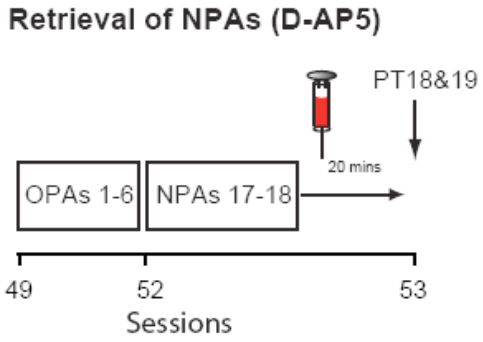

Figure 7.12 Retrieval of new paired-associates (NPAs).

On sessions 49-51, rats were given training on the original PAs. On session 52, two novel flavours were introduced: PA17 (orange) and PA18 (chocolate and apple) (Figure 7.4). Twenty four hours later, on session 53, drugs (D-AP5, 0.5 μl or NaCl, 0.5 μl) were infused into the prelimbic cortex 20 min before the probe trials (18 and 19).

Retrieval of original PAs (sessions 54-58)

The basic procedure for the retrieval of original PAs is shown in Figure 7.13.

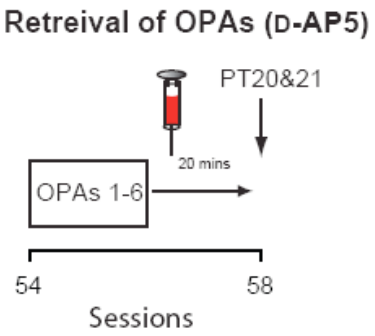

Figure 7.13 Retrieval of original paired-associates (OPAs).

On sessions 54-57, rats were given training on the original PAs. Twenty four hours later, on session 58, drugs (D-AP5, 0.5 µl or NaCl, 0.5 µl) were infused into the prelimbic cortex 20 min before the probe trials (20 and 21).

7.2.5.8 Training and probe tests (session 64-68): Impact of CNQX (2 µl) on the encoding of new PAs in the barrel cortex

The basic procedure for the encoding of PAs is shown in Figure 7.14.

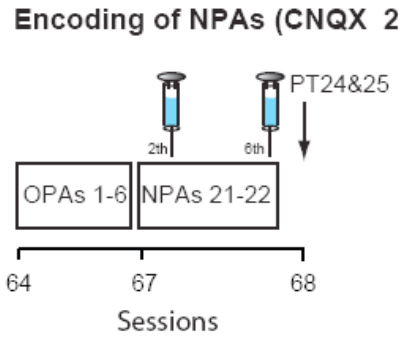

Figure 7.14 Encoding of new paired-associates (NPAs).

To examine whether a larger volume of CNQX infused into the barrel cortex would affect the encoding of new PAs, on sessions 64-66, rats were given training on the original PAs. On session 67, two novel flavours were introduced: PA21 (lemon) and PA22 (vanilla and brandy) (Figure 7.14). Twenty four hours later, on session 68,

drugs (CNQX, 2 μl or NaCl, 2 μl) were infused 20 min into the barrel cortex before the probe trials (24 and 25). The use of this larger volume - 2 μl compared to 0.5 μl – was guided by the results of the gap-crossing control protocol described below.

Gap crossing task

7.2.6 Apparatus

The apparatus was a modified version of that used by Harris et al., (1999) and Celikel and Sakmann (2007). The apparatus consisted of two individually moveable identical platforms (starting platform and the goal platform) made of transparent plexiglas (Figure 7.15). The two platforms (11 cm wide x 30 cm long) were elevated by 34 cm and 3 of the walls had 10 cm high walls. All the walls were covered in black adhesive cardboard. Velcro tape (3M, UK) was placed at the edges of the platforms to indicate the platform edge to the rats. A 50 cm long ruler was used to measure the distance between the platforms in each trial. A cup containing approximately 10 g of Weetos (Weetabix, UK) was available as reward at the goal platform. Each weeto in the cup was cut into half and each half weighed approximately 0.15 g.

A camera connected to a DVD recorder was placed above the gap to observe and record the rats' movements. To prevent the use of visual information, experiments were conducted under dim red light (wavelength 620–750 nm), invisible to the rats (Harris et al., 1999).

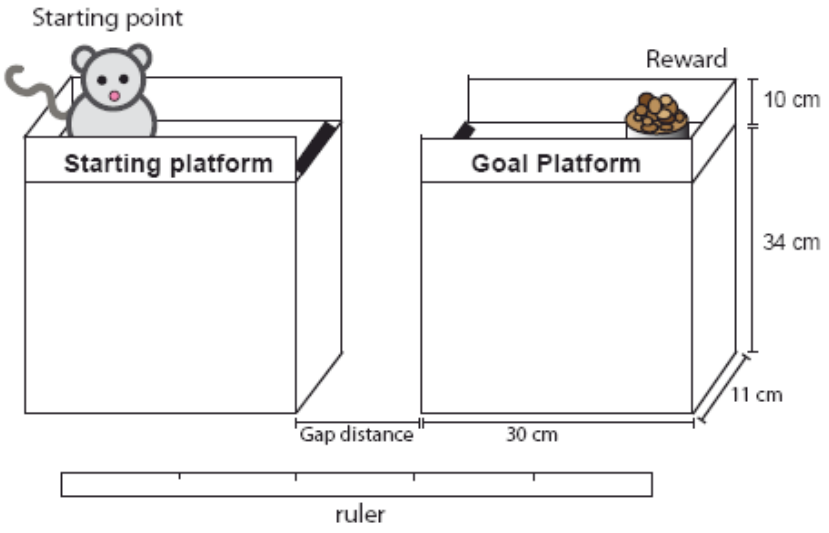

Figure 7.15 Gap crossing apparatus set-up.

7.2.7 Behavioural training

The protocol used in the experiment was adapted from that used by Dr Andreas Frick's laboratory (personal communication). The aim was to secure reliable data with a relatively short training protocol.

7.2.7.1 Habituation

The purpose of the habituation sessions were to allow the rats to become accustomed to exploring the platforms and getting rewards after they successfully move from the starting platform to the goal platform. Normal illumination was present in both habituation sessions. On habituation session 1 and 2, rats were placed on the starting platform and the gap distance between the 2 platforms was 0 cm. Rats were free to explore both platforms. A cup of Weetos was available as a reward at the end of the goal platform. Rats were allowed 10 s to eat the Weetos. The cup was kept full so that rats readily returned to reward platform. After 10 s, rats were picked up and carried to their home cage. Each rat was given 3 trials to obtain rewards from the goal platform.

Between trials, the platforms were wiped with ethanol to remove odours, which may have affected performance.

7.2.7.2 Main training

On training session 1, a 2 cm gap was created between the platforms for the first trial of the session. Gap length was increased in 2 cm increments following 3 successful trials of each distance, from 2 cm to 6 cm. This was carried out under normal lighting conditions. On session 2, a 6 cm gap was created between the platforms for the first trial of the session. Gap length was increased in 2 cm increments following 3 successful trials of each distance, from 6 cm to 10 cm. From this session on, the task was carried out under red illumination. On session 3, a 10 cm gap was created between the platforms for the first trial of the session. Gap length was increased in 2 cm increments following 3 successful trials of each distance, from 10 cm to 16 cm.

On sessions 4 and 5, distances of between 12 to 25 cm were imposed in pseudo random order. This was designed to determine the maximum distance that each individual rat would cross. To ensure that rats were obtaining information using only their whiskers, a 30 cm gap trial (a catch trial in which the gap was too wide to cross) was introduced pseudo randomly at least three times per session. By the end of session 5, the maximum gap distance that each rat would cross was determined. On session 6, 4 gap distances were introduced pseudo randomly at 4 times per session: 8 cm, 14 cm, the maximum distance for individual rat (typically 17-18 cm) and 30 cm ("a gap-too-far").

7.2.7.3 Impact of CNQX (0.5 μl) during the gap crossing task

On sessions 7 and 8, 20 mins before the gap crossing task, rats were injected with CNQX (0.5 μl) or NaCl (0.5 μl) in the barrel cortex. Four gap distances were introduced pseudo randomly at 4 times per session: 8 cm, 14 cm, maximum distance (16-18 cm) for individual rat and 30 cm.

7.2.7.4 Impact of CNQX (2 μl) during the gap crossing task

As the barrel cortex is a relatively large brain region (A.P relative to bregma: - 0.26 mm to - 4.16 mm), it is possible that 0.5 μl CNQX may not inactivate the whole region. Hence, a larger volume was infused into the barrel cortex to address this concern. On sessions 9 and 10, 20 min before the gap crossing task, rats were injected with CNQX (2 μl) or NaCl (2 μl) in the barrel cortex. 4 gap distances were introduced pseudo randomly at 4 times per session: 8 cm, 14 cm, maximum distance for individual rat and 30 cm.

7.2.7.5 Performance measures

During each trial of the gap crossing experiment, the latency for each rat to cross the gap between 2 platforms was measured. First, the time when a rat placed its front paws on the edge (the Velcro) of the starting platform was recorded. Second, the time when the rat placed its front paws on the edge (the Velcro) of the goal platform was recorded. The latency for a rat to cross the gap was calculated by taking the difference between these 2 time measurements.

7.2.8 Histology and perfusion

Detailed methods of perfusion and histology were described in general methods. Inspection of the location of the tips of the cannulae in coronal sections of the brain of the rats revealed that these were located in the prelimbic (Figure 7.16A & C) and the barrel cortices (Figure 7.16B & D).

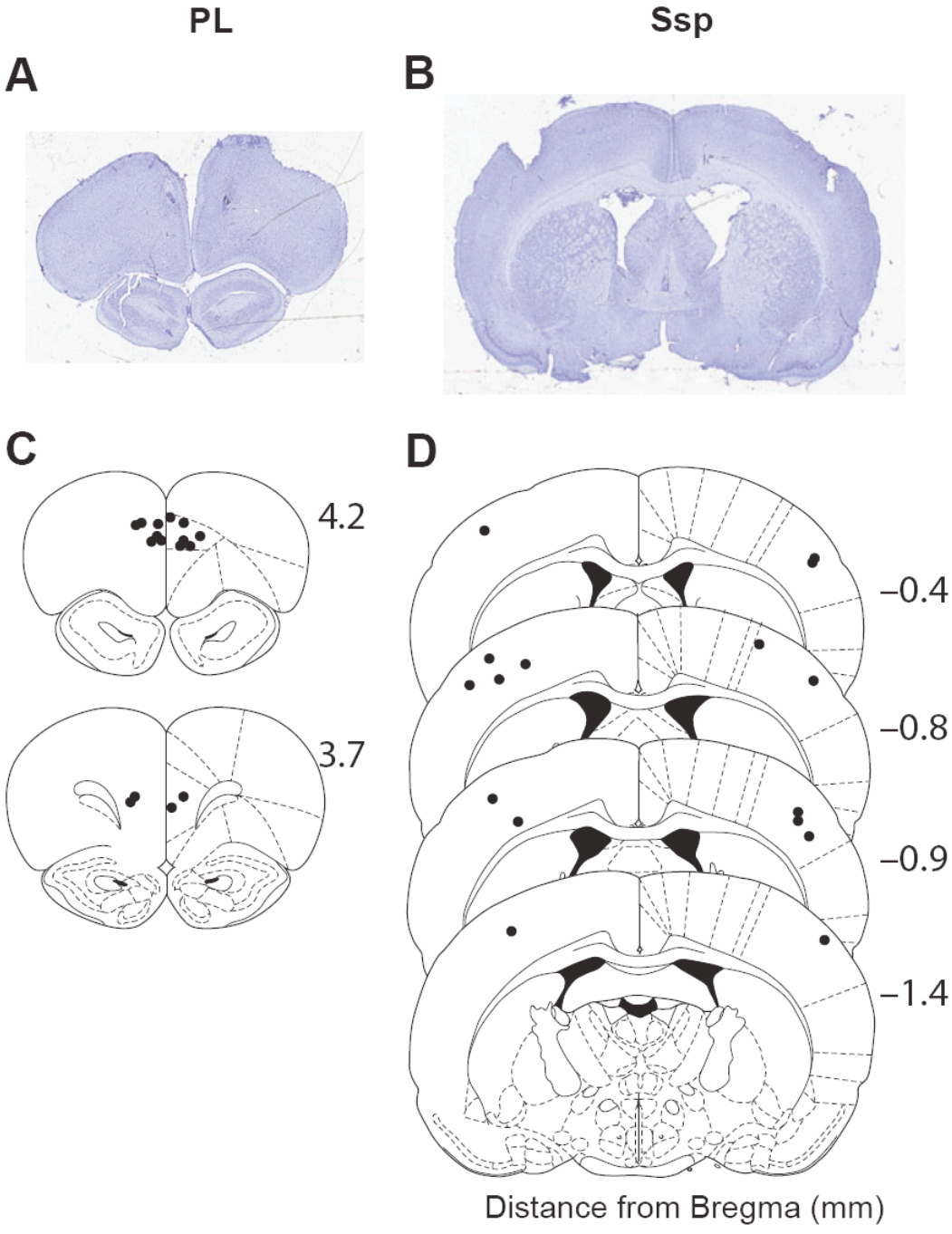

Figure 7.16 Histology of cannula positions.

7.3 Results

7.3.1 Initial training, probe tests and non-cued test (Sessions 1-16, 43)

Rats were trained on a set of 6 original PAs from session 1 to 17. Figure 7.17 shows the acquisition curve of this original PAs memory. The performance index scores for all the rats showed that performance improved across all training sessions and a repeated measures ANOVA showed that this was significant (F = 10.83, df = 6.08/42.54, p <0.001). Performance index score was computed as 100-[100×(error/5)]. From sessions 10 onwards, the performance index score was between 70% and 80%, and which is significantly difference from chance (ts > 6.42, df = 7, p < 0.001).

During the training phase, 3 non-rewarded probe trials (PTs 1-3) were scheduled as shown in Figure 7.17 to examine acquisition of PAs memory. Figure 7.18 represents the percentage of dig time at the cued location (striped bars) relative to that at the non-cued locations (white bars). These probe trials revealed, as expected, a graded learning of the original PAs from sessions 1-17 (F = 7.96, df = 1.78/19.56, p < 0.01). T-test results indicated that digging at the cued location was above chance in PT3 (t = 4.45, df = 11, p < 0.01).

To exclude the possibility that olfactory cues in the correct sand-well guided the rats' choices on training days, a single session of 6 trials in which the daily protocol was unchanged, except that no cue flavours were offered in the start box, was carried out. PI score fell to 60% (Figure 7.17, session 43), returning to above chance on the next "normal" session (session 44). It follows that animals were likely *not* being guided or influenced to the correct sand-well by odours emanating from it, or scents left by previous rats, but instead by cued-recall.

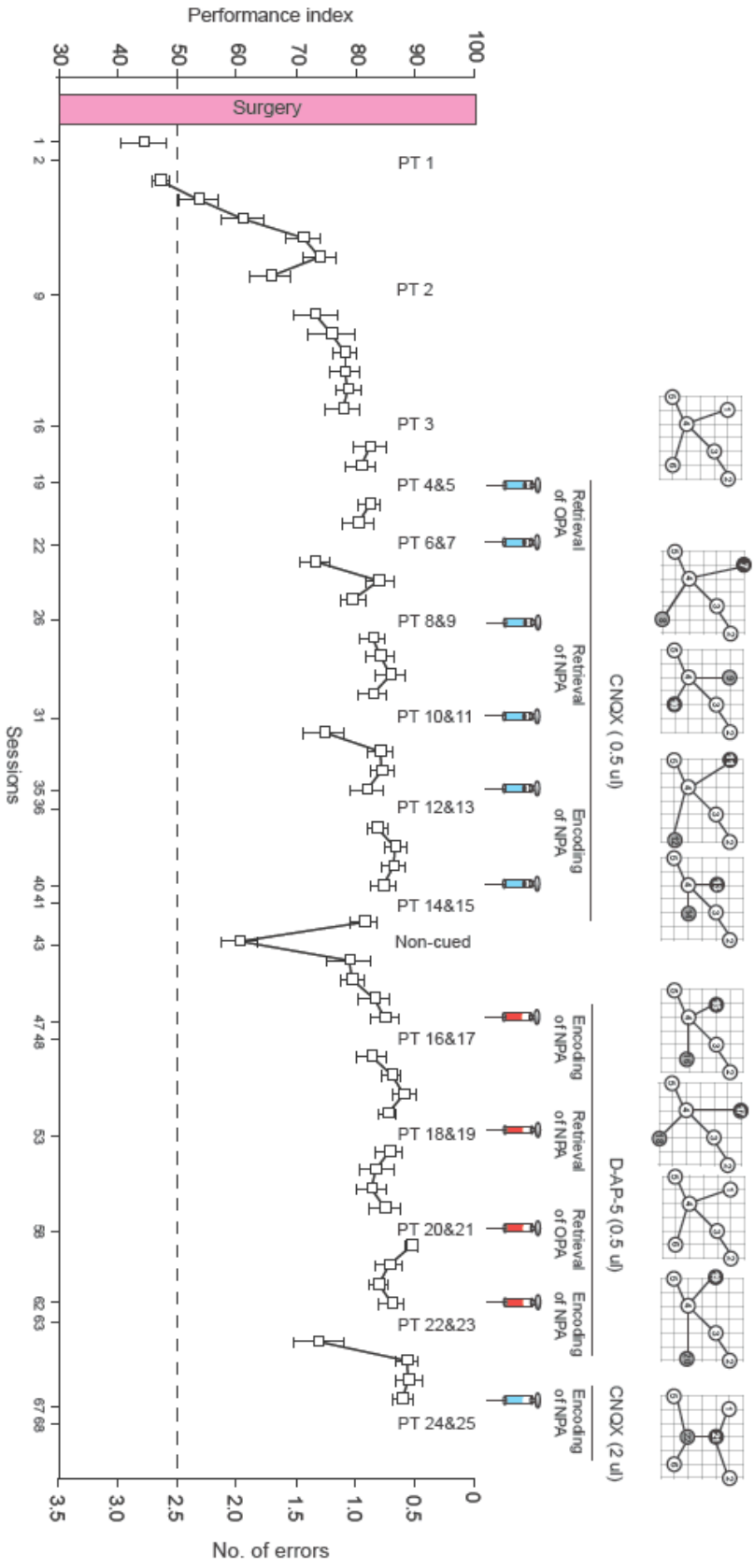

Figure 7.17 Acquisition of the original 6 paired-associates (sessions 1-16). Removing cue flavours from the start box on session 43 results in performance dropping to chance and then returning to 70 % correct on a succeeding normal session (session 43).

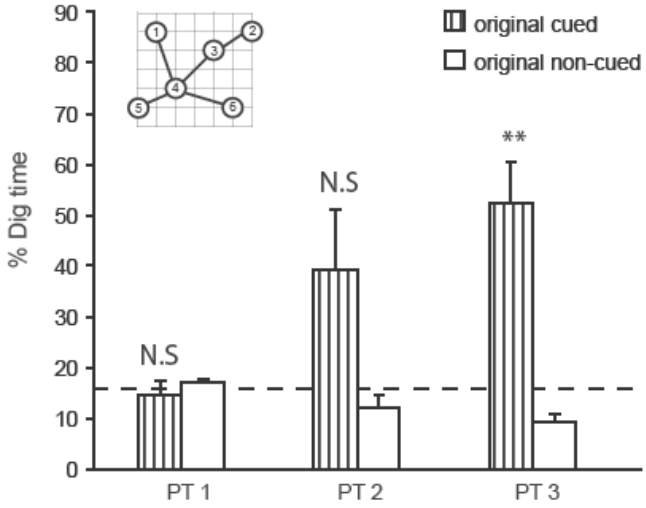

Figure 7.18 Three cued-recall probe trials for the acquisition of original schema. The graph represents percentage of dig time at the cued locations (striped bars) relative to that at the non-cued locations (white bars). N.s, nonsignificant. $^{**}p < 0.01$

7.3.2 Effects of CNQX and D-AP5 infusions in prelimbic and barrel cortices on the retrieval of original PAs and new PAs

The next step was to investigate whether AMPA receptors and NMDA receptors in the prelimbic cortex are involved in the retrieval of both original PAs and new PAs. This was achieved by infusing the AMPA receptor antagonist CNQX and the NMDA receptor antagonist D-AP5 in the prelimbic cortex prior to retrieval PAs in probe trials. Infusions were carried out 20 min before the start of probe trials.

AMPA dependency of retrieval of original PAs

Figure 7.19A presents percentage dig time data of probe tests 4-7. The ANOVA revealed an interaction between regions, drugs and digging locations (F = 14.28, df = 1/11, p <0.01). T tests that compared the proportion of time spent digging at the original cued location indicated above chance performance in both the control conditions (t > 10.22, df = 11, p < 0.001) and the CNQX condition (t = 4.23, df = 11, p < 0.001) in the barrel cortex but not the CNQX condition in the prelimbic cortex (t = 1.23, df = 11, NS).

AMPA dependency of retrieval of new PAs

Figure 7.19B presents percentage dig time data of probe tests 8-11. The ANOVA revealed an interaction between regions, drugs and digging locations (F = 6.16, df = 1.20/11.96, p <0.05). T tests that compared the proportion of time spent digging at the new cued location indicated above chance performance in both the control conditions (t > 5.67, df = 11, p < 0.001) and the CNQX condition (t = 4.10, df = 11, p < 0.01) in the barrel cortex but not the CNQX condition in the prelimbic cortex (t = 1.42, df = 11, NS).

Due to the fact that there was no effect on the retrieval memory when CNQX was infused in the barrel cortex, D-AP5 was only infused into the prelimbic cortex in the next stage of the experiment.

NMDA is not required in retrieval of original PAs

Figure 7.19C presents percentage dig time data of the probe tests 20-21. The ANOVA revealed no interaction between drugs and cue location ($F = 0.397$, df = 1/8, $p > 0.05$) and a significant difference in dig time at the cued and non-cued locations. T tests comparing the proportion of time spent digging at the original cued location indicated to chance were significant for both conditions (NaCl; $t = 3.64$, df = 9, $p < 0.01$; D-AP5; $t = 3.64$, df = 9, $p < 0.05$).

NMDA is not required in retrieval of new PAs

Figure 7.19D presents percentage dig time data of probe tests 18-19. The ANOVA revealed no interaction between drugs and cue location ($F = 4.31$, df = 1.17/9.33, $p > 0.05$) and a significant difference in dig time at the new cued and non-cued locations. T tests comparing the proportion of time spent digging at the new cued location indicated to chance were significant for both conditions (NaCl; $t = 6.0$, df = 8, $p < 0.001$; D-AP5; $t = 11.99$, df = 8, $p < 0.001$).

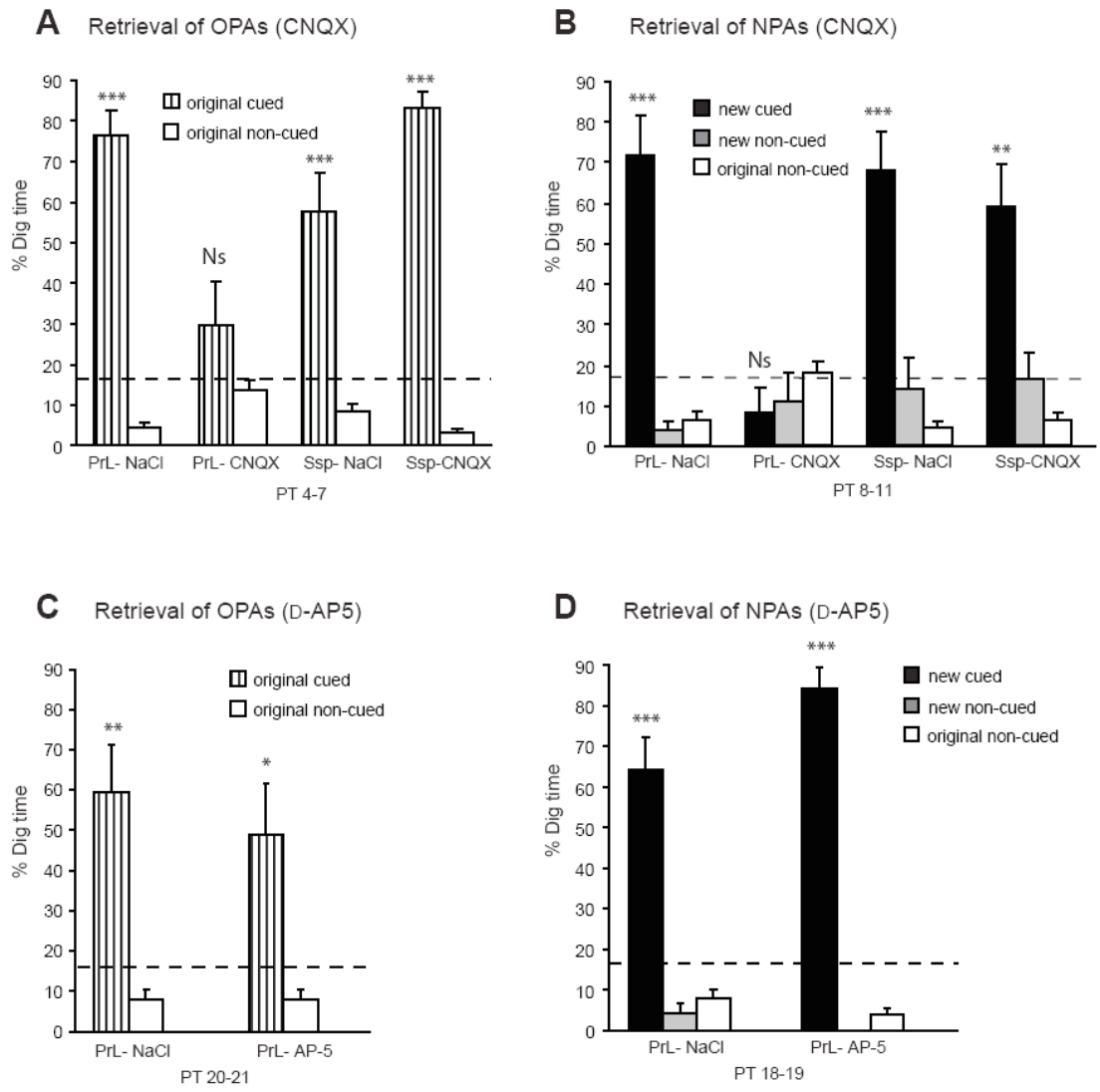

Figure 7.19 Effects of CNQX and D-AP5 infusions in prelimbic and barrel cortex on the retrieval of original and new PAs in cued recall probe trials as measured by percentage dig time. (A) CNQX infusions into PL prior to retrieval of original PAs significantly impaired memory but a similar infusion of CNQX into SSp had no such effect. (B) CNQX infusions into PL prior to retrieval of new PAs significantly impaired memory but a similar infusion of CNQX into Ssp had no such effect. (C) Infusion of D-AP5 into PL did not impair the retrieval of the original PAs. (D) Infusion of D-AP5 into PL did not impair the retrieval of the newly PAs.
Ns, nonsignificant. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Absolute dig time of various probe tests

The absolute dig time is shown for various probe tests in Figure 7.20. Using this measure, the differences in absolute dig time between different drug conditions in different regions during the 120 s probe tests was calculated.

Infusion of CNQX into prelimbic cortex resulted in a significantly lower absolute dig time than the rats infused with saline into prelimbic cortex in retrieval of *original* PAs (paired t-test; $t = 5.85$, df = 11, $p < 0.001$) (Figure 7.20A). Infusion of CNQX into prelimbic cortex resulted in a significantly lower absolute dig time than the rats infused with saline into prelimbic cortex in retrieval of *new* PAs (paired t-test; $t = 2.80$, df = 10, $p < 0.05$) (Figure 7.20B).

There were no differences in absolute dig time between the 2 other drug conditions (D-AP5 and NaCl) during retrieval of *original* PAs (Figure 7.20C) ($t < 1$, NS). There were no differences in absolute dig time between the 2 drug conditions (D-AP5 and NaCl) during retrieval of *new* PAs (Figure 7.20D) ($t < 1$, NS).

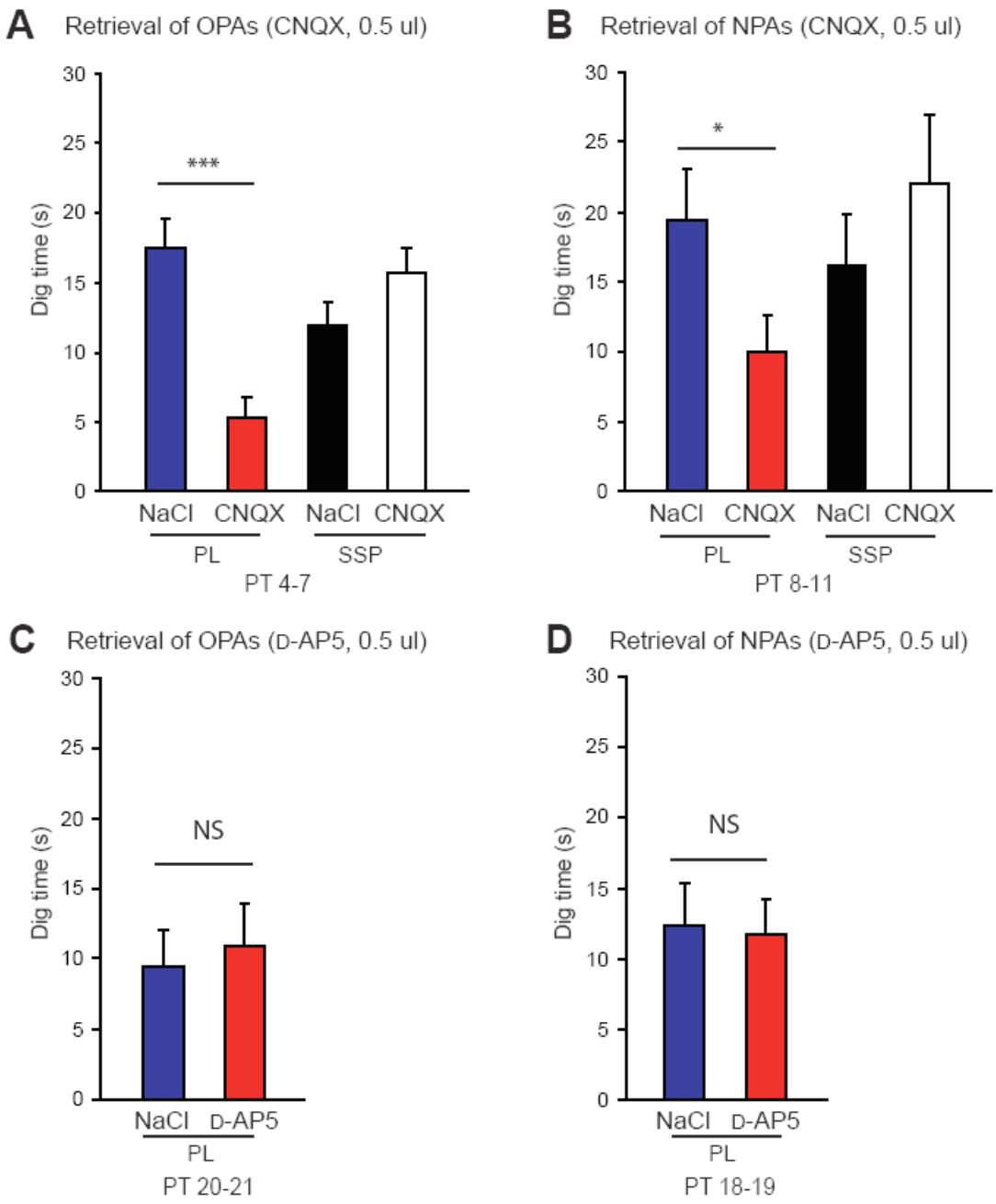

Figure 7.20 Effects of CNQX and D-AP5 infusions in prelimbic and barrel cortex prior to retrieval of original and new PAs on absolute dig time. (A) Infusion of CNQX into prelimbic cortex resulted in a significantly lower absolute dig time than the rats infused with saline into prelimbic cortex in retrieval of original PAs. (B) Infusion of CNQX into prelimbic cortex resulted in a significantly lower absolute dig time than the rats infused with saline into prelimbic cortex in retrieval of new PAs. (C) There were no differences in absolute dig time during retrieval of OPAs between D-AP5 and NaCl infusions into PL. (D) There were no differences in absolute dig time during retrieval of NPAs between D-AP5 and NaCl infusions into PL.
Ns, nonsignificant. *$p < 0.05$; ***$p < 0.001$

Control experiment

Infusion of CNQX into the prelimbic cortex resulted in a significantly lower absolute dig time than that of rats infused with saline into the prelimbic cortex on both the retrieval of the *original* PAs and the *new* PAs (Figure 7.20 A & B). This could have been due to the role normally played by prelimbic cortex in memory, or some sort of motor, motivational or procedural effect of the drug on the task.

A control experiment was devised that sought to distinguish these alternatives. This involved training rats to search and dig for control food pellets (non-flavoured) in the sand-well in the center location of the event arena for 1 trial. The rationale for this is that no spatial memory was required for this task – the animal had only to run into the arena to the centre and dig. Twenty-four hours later, drugs (CNQX or NaCl) were infused 20 min before the probe trial. No differences in absolute dig time between the 2 drug conditions (CNQX and NaCl) were observed during retrieval of food pellets ($t < 1$, NS) (Figure 7.21).

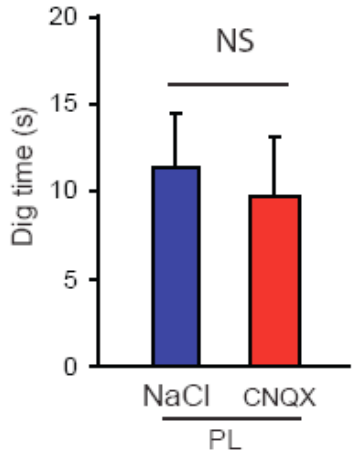

Figure 7.21 Effects of CNQX infusions in prelimbic cortex prior to retrieval of food pellet in the center of the event arena. There were no differences in absolute dig time between the 2 conditions. Ns, nonsignificant.

7.3.3 Effects of CNQX and D-AP5 infusions in the prelimbic and barrel cortices on the encoding of new PAs

The second aim of this study was to investigate whether prelimbic AMPARs and NMDARs are involved in the encoding of *new* PAs. This was achieved by infusing the AMPAR antagonist CNQX and the NMDAR antagonist D-AP5 in prelimbic cortex prior to encoding. Infusions were carried out 20 min before the start of encoding trials and memory was tested at 24 hr in probe trials.

AMPA dependency of novel PAs acquisition

Figure 7.22A presents percentage dig time data of probe tests 12-15. The ANOVA revealed an interaction between regions, drugs and digging locations (F = 13.14, df = 1.18/10.61, p <0.01). T tests that compared the proportion of time spent digging at the new cued location indicated above chance performance in both the control conditions (t > 5.67, df = 11, p < 0.001) and the CNQX condition (t = 4.10, df = 11, p < 0.01) in the barrel cortex but not the CNQX condition in the prelimbic cortex (t = 1.42, df = 11, NS).

NMDA dependency of novel PAs acquisition

Figure 7.22B presents percentage dig time data of probe tests 16-17 and 22-23. The ANOVA revealed an interaction between drugs and digging locations (F = 24.91, df = 1.63/13.04, p <0.001). T tests that compared the proportion of time spent digging at the new cued location indicated above chance performance in the control condition (t = 11.66, df = 8, p < 0.001) but not the D-AP5 condition in the prelimbic cortex (t = -1.26, df = 8, NS).

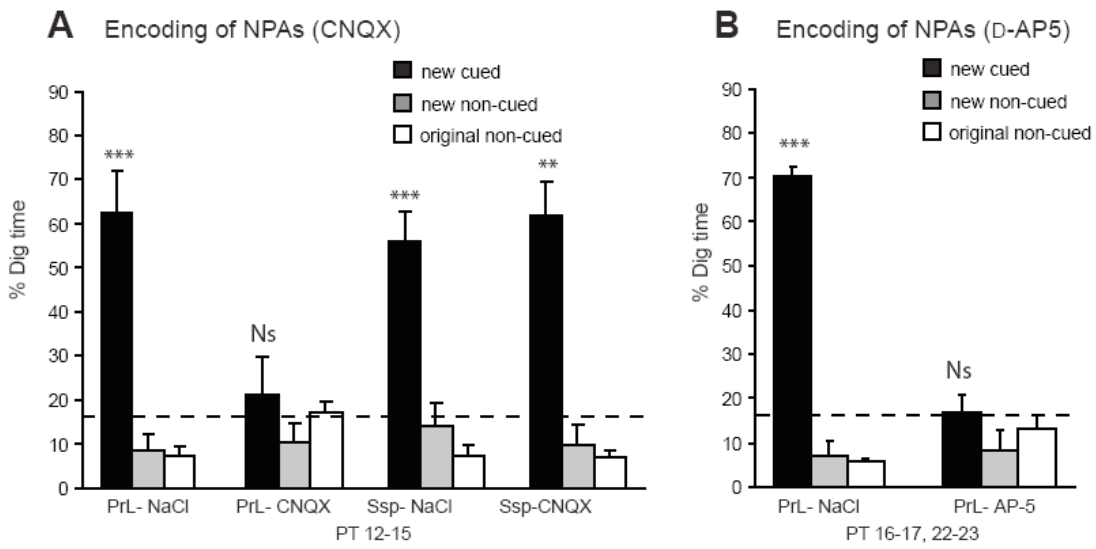

Figure 7.22 Effects of CNQX and D-AP5 infusions in prelimbic and barrel cortex prior to encoding of new PAs on percentage dig time. Memory was tested 24 hr later in cued recall probe trials. (A) CNQX infusions into prelimbic cortex prior to encoding significantly impaired the memory tested at 24 hr. (B) D-AP5 infusions into prelimbic cortex prior to encoding significantly impaired the memory tested at 24 hr. Ns, nonsignificant. $^{**}p < 0.01$; $^{***}p < 0.001$

7.3.4 Effects of infusing larger volumes of CNQX (2 μl) in the barrel cortex during the encoding of new PAs

When CNQX (0.5 μl) was infused into the prelimbic cortex at the time of (1) encoding of new PAs, (2) retrieval of new PAs and (3) retrieval of original PAs, memory was impaired, but not when CNQX was infused into the barrel cortex made under the same conditions (Figure 7.19 A & B, 7.22 A). This phenomenon could be the result of regional dissociations or it could be explained trivially, for example, by an insufficient volume of CNQX being infused into the barrel cortex to affect much of this larger structure and so have any behavioural effect. A positive control experiment was vital to check whether an identical drug infusion into the barrel cortex has a behavioural effect in a somatosensory task. The whisker dependent behavioural gap crossing task tested this possibility.

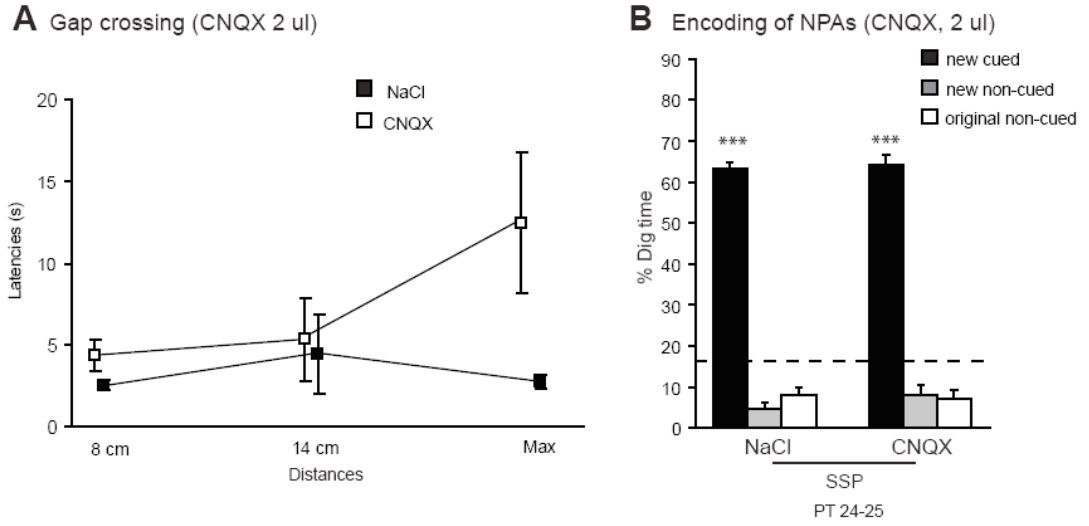

Figure 7.23 (A) Latencies for gap crossing. When CNQX was infused into the barrel cortex, there was a large significant increase in latency for crossing the maximum gap length. (B) CNQX infusions into barrel cortex (Ssp) prior to encoding of new PAs did not impair the memory tested at 24 hr. ***$p < 0.001$

An ANOVA of these latencies revealed no overall interaction between groups and distances. However, pairwise comparisons, with Bonferroni's corrections for multiple comparisons and focusing exclusively on the maximum distance, showed significant

longer latencies to cross the maximum distance when the rats were treated with CNQX than saline ($p < 0.05$).

After the gap crossing task, the rats were brought back to perform the PAs schema task. To examine if a larger volume of CNQX infused into the barrel cortex would affect the encoding of new PAs, rats were given training on the original PAs for 3 sessions. Then on next session, 2 novel PAs were introduced. Twenty-four hours later, a probe trial was performed.

Figure 7.23B presents percentage dig time data of probe tests 24-25 in the event arena. The ANOVA revealed there is no interaction between drugs and digging locations ($F = 0.125$, df = 1.13/7.94, $p > 0.05$). T tests that compared the proportion of time spent digging at the new cued location indicated above chance performance in the control condition ($t = 5.35$, df = 7, $p < 0.001$) and in the CNQX condition in the barrel cortex ($t = 5.53$, df = 7, $p < 0.001$).

These results show that the larger volume of CNQX had an effect on the whisker dependent task whilst the same volume had no measurable effect on learning NPAs in the event arena.

7.4 Discussion

The present study sought to elucidate the contributions of prelimbic NMDA receptors and AMPA receptors in PAs schema memory. The main findings of this chapter are as follows: (1) The encoding, but not retrieval, of new PAs in a PAs schema memory requires NMDA receptor-dependent mechanisms in the prelimbic cortex, possibly synaptic plasticity; (2) The retrieval of both the original PAs and new PAs depends on AMPA receptor-mediated mechanisms, such as fast excitatory prelimbic transmission. The implications of these findings are considerable.

7.4.1 Parallel encoding in the prelimbic cortex and the hippocampus of new PAs in a PAs schema

Perhaps the most striking implication is that these findings reveal, for the first time, parallel encoding of paired-associate in HPC and cortex that, against the background of a previously learned mental schema, leads to rapid memory consolidation. Both our hippocampal lesion data (Tse, Langston et al., 2007) and the pharmacological data of E3 in this thesis (inactivation NMDA receptors with D-AP5) suggest that the integrity of the hippocampus and of NMDA receptor dependent neurotransmission is necessary for encoding of new PAs. The present inactivation study suggested that NMDA receptor-dependent plasticity in the prelimbic cortex is also necessary for encoding of the new PAs. That is, this hippocampal-dependent learning task requires parallel glutamatergic activation in the prelimbic cortex – well outside the sensory areas likely to be involved in taste discrimination or cortical storage of spatial memory.

7.4.2 The role of the medial prefrontal cortex in rapid memory consolidation

Previous studies have highlighted the importance of the prefrontal cortex in memory. Prefrontal cortex involvement in the processing of remote memory in systems memory consolidation was highlighted by Frankland and Bontempi (2005). Experimental evidence indicates that the prefrontal cortex is involved in the consolidation and expression of remote memory in fear conditioning (Maviel et al., 2004) and important in the consolidation of remote memory through NMDA receptor dependent processes during the first 2 weeks after trace eyeblink conditioning (Takehara-Nishiuchi et al., 2005). Together with the IEG data from the previous chapter and the pharmacological study (inactivation of AMPA receptors and NMDA receptors) reported in this chapter, these studies suggest that NMDA receptor dependent plasticity in the prelimbic cortex is involved during encoding of new PAs. This implies that the prelimbic cortex along with HPC may both be equally important in rapid memory consolidation.

These findings (IEG and pharmacological intervention data in this thesis) complement the recent observations on cortical tagging by Lesburgueres et al. (2011) and, together with similar data concerning hippocampal-mPFC-other cortex interactions secured in human brain-imaging studies (van Kesteren 2010a,b), challenge certain models of systems memory consolidation.

Chapter 8

General discussion

8.1 Summary of findings

As outlined in the introduction, according to the so-called 'standard' model of systems memory consolidation, memory traces are rapidly encoded in hippocampal networks but it takes time for them to be embedded in cortical networks and so become independent of the hippocampus (Squire and Alvarez, 1995). The hippocampus seems to be a fast learner whereas the cortex is a slow learner (Norman and O'Reilly, 2003). In addition, rapid acquisition of new memories appears to be disadvantageous in the neocortex because this might cause disruption of existing stored information (McClelland et al., 1995).

However, the central premise of this thesis is that something is missing in this model. Specifically, it has nothing to say about whether the process of consolidation would be affected by prior stored knowledge. To the contrary, the data presented here and in previous publications indicates that, if there is a relevant and activated schema available, the neocortex appears to be able to incorporate and store new information rapidly (Tse, Langston et al., 2007). Building upon this rapid cortical memory consolidation concept, this thesis has explored several aspects of the neurobiology of schemas in animals.

The first main experiment (E1) of the thesis examined the causal significance of having a relevant schema with respect to new information processing. Rats were trained, in distinct environments, in both a consistent and an inconsistent schema. In the consistent schema room, rats were observed to learn new PAs in a single trial; in the inconsistent schema room, the animals failed to remember new PAs for as short an

interval as 24 h. The interpretation offered is that in the consistent schema room, the animals had an activated schema to facilitate rapid memory consolidation of new PAs; in the other room, they did not. This within-subjects protocol indicates that the mere possession of a schema is insufficient for rapid consolidation; it has also to be activated and that activation can be context-specific.

The second main experiment (E3) of the thesis investigated the role of hippocampal NMDA receptors and dopamine receptors during the encoding of new PAs. Bilateral hippocampal infusion of either the NMDA receptor antagonist D-AP5 or the D_1/D_5 dopamine receptor antagonist SCH23390 before encoding of new PAs resulted in impaired memory tested at 24 hr. This result suggests that the encoding of new PAs is dependent upon NMDA receptors in the hippocampus and also that dopamine is involved in some aspect of persistence of memory in new PAs.

The third main experiment of the thesis (E4) identified extrahippocampal regions in which the encoding of new PAs is associated with the activation of immediate early genes (*Zif268* and *Arc*). In a group of cortical structures, including the prelimbic cortex, there was significantly higher *Zif268* and *Arc* expression when encoding 2 new PAs compared to the reactivation of previously learned PAs or the encoding of 6 new PAs in the absence of a schema. The findings were unexpected but point to some kind of role for these connected cortical structures in the rapid assimilation of new information into a pre-existing schema.

Guided by these observations, the final experiment (E5) investigated the contributions of prelimbic NMDA and AMPA receptors to the encoding and retrieval of new PAs. Bilateral infusions of CNQX (an AMPA receptor antagonist) and D-AP5 (a NMDA receptor antagonist) resulted in poor learning of the new PAs in the schema task. This

finding was also unexpected as it pointed to hitherto unexpected parallel encoding of new PAs in the prelimbic cortex alongside what is happening in hippocampus.

The experimental results presented in this thesis suggest that the prelimbic cortex in particular plays a crucial role along with the hippocampus, during parallel encoding of new information in rapid memory formation.

8.2 A model of schema – dynamics of hippocampal-cortical interactions

In this section, a coherent schema model outlining the hippocampal and cortical interactions will be presented, integrating the main findings of the thesis.

The immediate early genes study (E4) revealed a network of cortical structures, extending from the prefrontal regions of the forebrain to the anterior cingulate and retrosplenial cortices, in which there is elevated Zif268 and Arc expression during encoding of new PAs. Amongst these regions, the prelimbic cortex had the most significant elevation in both Zif268 and Arc expression. And in the pharmacological intervention study (E5), encoding of new PAs into a schema required an NMDA receptor dependent mechanism in the prelimbic cortex. Although other cortical regions (e.g. anterior cingulate and retrosplenial cortices) may be involved in the rapid memory consolidation, the following discussion of the schema model will be focused on prelimbic cortex because its casual role is known.

The encoding, consolidation and retrieval phases of "schema facilitated memory" will be discussed. Before looking at these memory processes, the further process of retrieval of well-learned original paired-associates will be discussed. Retrieval of the elements of an existing schema is a vital component of memory processing.

8.2.1 Retrieval of well learned original paired associates (schema)

The hippocampal lesion data suggests that after the rats acquire a schema, retrieval of the original PAs is hippocampal independent (Tse, Langston et al., 2007). The hippocampal inactivation study (E5) from the present thesis suggested that retrieval of original PAs is dependent on AMPA receptors in the prelimbic cortex, but not NMDA receptor-dependent (Figure 8.1).

Events	Regions	Results
Inactivate AMPA receptors with CNQX *(E5 from present thesis)*	PL	Impaired
Inactivate NMDA receptors with D-AP5 *(E5 from present thesis)*	PL	Intact
HPC lesion *(Tse, Langston et al., 2007)*	HPC	Intact

Figure 8.1 Summary of experimental results in the retrieval of well learned original schema.

A model of schema in retrieval of original paired-associates

At this stage of our knowledge of what might be happening with respect to the storage and consolidation of memory traces in cortex, much is necessarily speculative. This would be a serious problem if it were not possible to translate such ideas into tractable experiments – but the schema protocols described in this thesis provide a vehicle for exactly that. That is, speculations can be tested by future experiments.

Thus, one may speculate that flavour and location information is initially processed by the relevant primary and associative cortical areas. These regions will need some kind of intracortical connectivity and it is perhaps this that the prelimbic and

associated regions provide. Indeed, the resent results raise the possibility, but are hardly definitive, that schema information is partly stored in the prelimbic cortex (Figure 8.2).

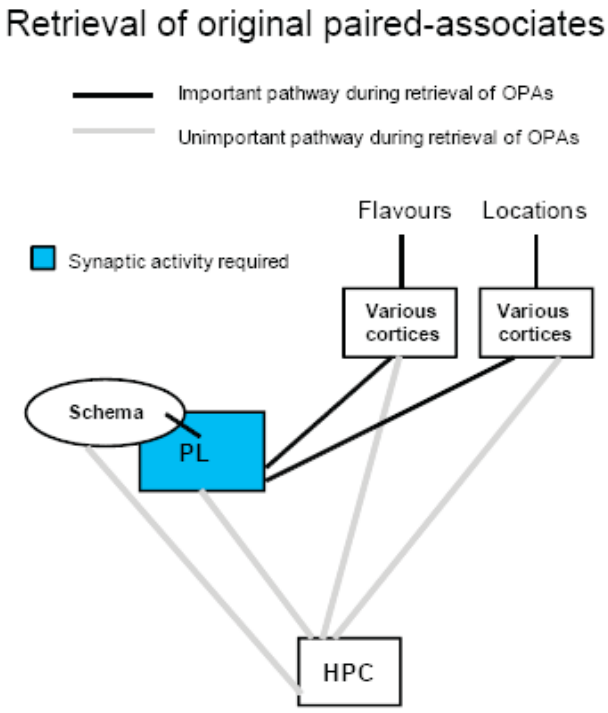

Figure 8.2 A model of schema in retrieval of original PAs. The retrieval of the original PAs involved AMPARs in prelimbic cortex (PL) but not in HPC.

8.2.2 Encoding of new paired-associates

Both the hippocampal lesion data (Tse, Langston et al., 2007) and the pharmacological data (inactivation NMDA receptors with D-AP5 in E3 from present thesis) suggested that NMDA receptor-dependent plasticity in hippocampus is always necessary for new PA encoding. The surprising result of the pharmacological study in this thesis (inactivation AMPA receptors and NMDA receptors in E5) is that NMDA receptor-dependent plasticity in prelimbic cortex is also necessary for new PAs encoding. The findings are tabulated in Figure 8.3.

Events	Regions	Results (probe trials at 24 h)
Inactivate AMPA receptors with CNQX *(E5 from present thesis)*	PL	Impaired
Inactivate NMDA receptors with D-AP5 *(E5 from present thesis)*	PL	Impaired
HPC lesion *(Tse, Langston et al., 2007)*	HPC	Impaired
Inactivate NMDA receptors with D-AP5 *(E3 from present thesis)*	HPC	Impaired

Figure 8.3 Summary of experimental results in encoding of new PAs.

A model of schema in encoding of new paired associates

A further speculation is that the HPC integrates the various features of the new PAs experience and fuses them rapidly into a coherent memory trace. The assumption is that LTP-like mechanism occurs in HPC when flavour and location information converge on the same neurons. This new information may also be processed in the prelimbic cortex. Hence, parallel encoding of new PAs occurs (Figure 8.4).

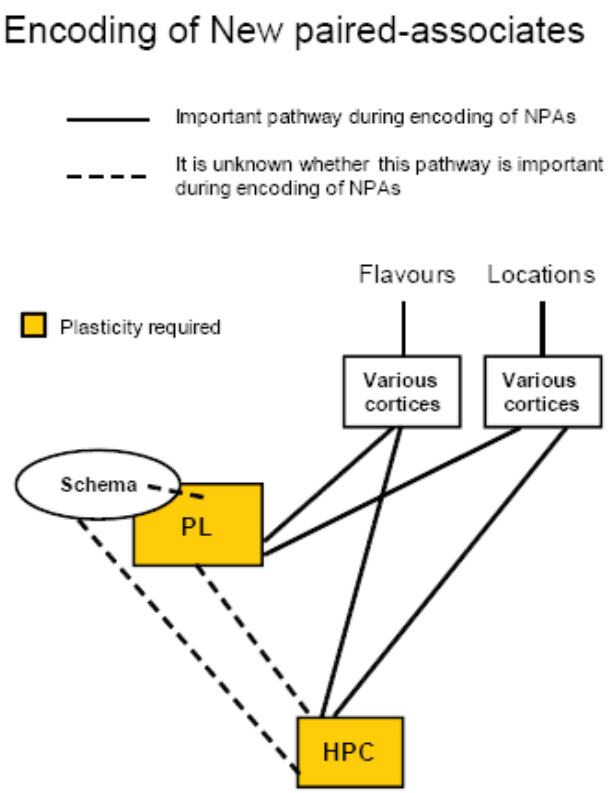

Figure 8.4 A model of schema in encoding of new paired-associates. Flavours, locations information are initially processed by several specialized primary and associative cortical areas. The hippocampus integrates the various features of the new PAs experience and fuses them rapidly into a coherent memory trace. This new information also goes into a cortical area (e.g. PL). Hence, parallel encoding of new PAs occurred.

8.2.3 Consolidation of new PAs

The hippocampal lesion data suggested that after the rats have acquired a schema, systems memory consolidation of new PAs happens between 3 to 48 hr after encoding (Tse, Langston et al., 2007). The hippocampal inactivation study (E3 from present thesis and Bethus et al., 2010) suggested that intrahippocampal dopamine modulates the persistence of new PAs over time in hippocampus (Figure 8.5).

Events	Regions	Behavioural results
HPC lesions made 3 h and 48 h after encoding new PAs *(Tse, Langston et al., 2007)*	HPC	48 h HPC lesioned group had memory but not 3 h HPC lesioned group
Inactivate D1/D5 receptors with SCH23390 *(Bethus et al., 2010)* *(E3 from present thesis)*	HPC	Test in 30 min after encoding: Intact Test in 24 h after encoding: Impaired

Figure 8.5 Summary of experimental results in consolidation of new PAs.

A model of schema in consolidation of new paired associates

One may speculate that during consolidation of new PAs, the changes in the strength of the connections between the hippocampal system and the prelimbic cortex are rapid and transient, whereas changes in the connections between prelimbic cortex and other cortical areas are also rapid and perhaps long lasting (Figure 8.6).

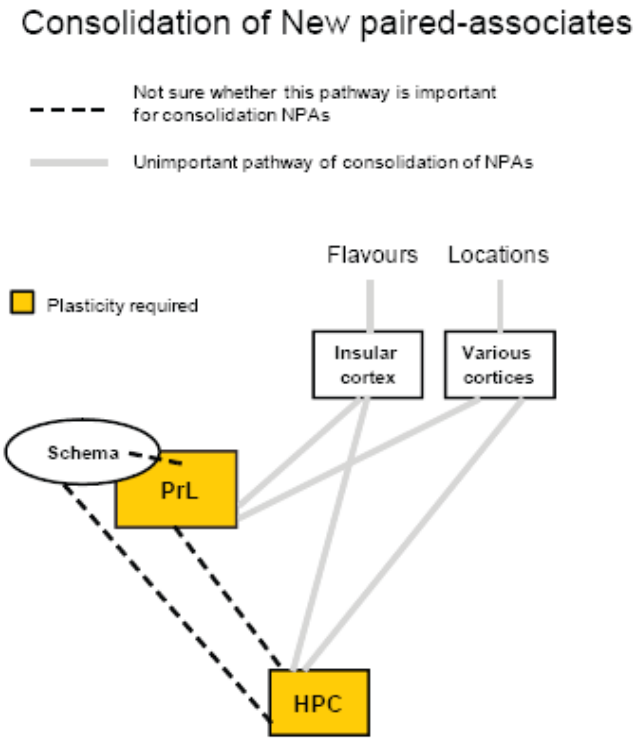

Figure 8.6 A model of schema in consolidation of new PAs.

8.2.4 Retrieval of new PAs

The hippocampal lesion data suggests that after the rats acquired a schema, retrieval of new PAs is hippocampal independent within 48 hrs (Tse, Langston et al., 2007). The hippocampal inactivation study (E5) from present thesis suggested that retrieval of new PAs is prelimbic AMPA receptors dependent but not NMDA receptors dependent (Figure 8.7).

Events	Regions	Behavioural results
Inactivate AMPA receptors with CNQX *(E5 from present thesis)*	PL	Impaired
Inactivate NMDA receptors with D-AP5 *(E5 from present thesis)*	PL	Intact
HPC lesion *(Tse, Langston et al., 2007)*	HPC	Intact

Figure 8.7 Summary of experimental results in retrieval of new PAs.

A model of schema in retrieval of new paired associates

Similar to the retrieval of original paired associates, one may speculate that flavours and locations information are initially processed by several specialised primary and associative cortical areas. These regions will need some kind of intracortical connectivity and it is perhaps this that the prelimbic and associated regions provide. The present results raise the possibility that schema information is partly stored in the prelimbic cortex (Figure 8.8).

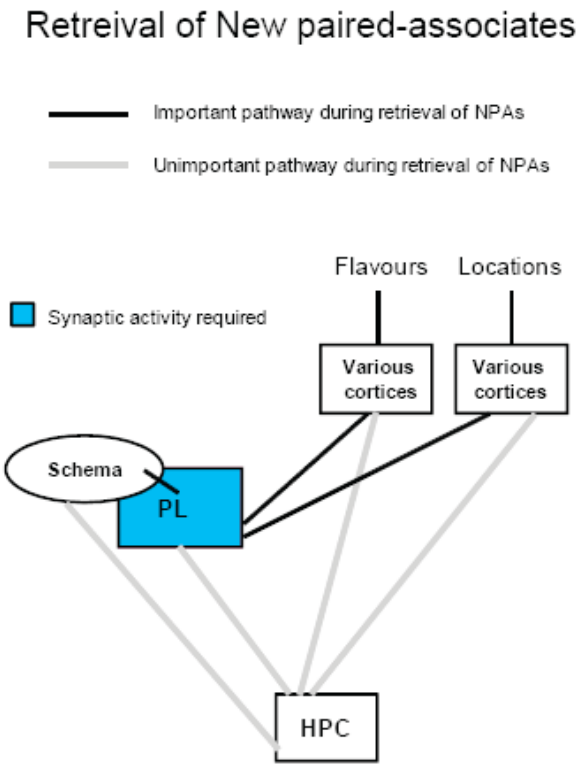

Figure 8.8 A model of schema in retrieval of new paired-associates. The retrieval of the new PAs involved AMPA receptors in PL but not in HPC.

8.2.5 Conclusion

In the schema rapid memory consolidation, as outlined above, during the encoding of new PAs, both the prelimbic cortex and the HPC are involved. And one may speculate that in consolidation of new PAs, the prelimbic cortex is also involved. One should bear in mind that other cortical regions (e.g. anterior cingulate, retrosplenial cortices) may also play an important role in the encoding and perhaps consolidation of new PAs. Future experiments should test this.

8.3 Directions of future research

A neurobiological theory of hippocampal and neocortical memory formation (Morris, 2006; Wang and Morris, 2010) asserts several propositions. First, when encoding new information, the system may be able to automatically capture all information online; however, most of the memories are lost. The memory traces in hippocampal formation are likely "indices" of locations in the neocortex where more detailed sensory/perceptual features of information are stored, and it is these that are normally activated during recall. Second, this automaticity is accompanied by the rapid decay of hippocampal formation memory traces, a decay that helps to avoid the saturation of distributed associative storage, with cellular consolidation acting as a kind of filter to decide what information is worth keeping. The index traces in hippocampal formation can persist for longer if encoding happens around the time of synthesis, distribution and synaptic capture of plasticity-related proteins at tagged synapses. Third, after the cellular consolidation process has determined what is to be kept, the separate process of systems memory consolidation gets underway to determine what memory traces last. These hippocampal traces enable a systems consolidation process that builds connections between relevant modules in cortex. While this may sometimes be slow, it can also occur very rapidly if there is an activated associative schema previously stored in the neocortical networks.

Although this thesis has attempted to discover aspects of the neurobiology of rapid cortical consolidation, there are still a lot of questions that have not been answered. It would be valuable to complement various approaches (cellular and molecular, pharmacological, physiology and computational etc.) to explore these unanswered questions. Some of the unanswered questions are as follows.

8.3.1 HPC-prelimbic cortex relationship in schema

As noted above, perhaps the most striking implication is that the findings in this thesis reveal, for the first time, parallel encoding of PAs in the HPC and the prelimbic cortex that, against the background of a previously learned mental schema, leads to rapid memory consolidation. However, the relationship between the prelimbic cortex and the HPC in encoding and consolidation of new PAs in PAs schema memory has not been addressed. The ventral CA1/subiculum regions of the HPC projects directly to the prelimbic cortex (Jay and Witter, 1991). One may speculate that this direct projection is important in sending information to facilitate memory consolidation. Inspired by a study (Floresco et al., 1997) on the role of hippocampal and prefrontal cortex in radial arm maze tasks with or without a delay, and a study (Barker and Warburton, 2008) on the NMDA receptor plasticity in the perirhinal and prefrontal cortex in the acquisition of long term object in place associative memory, a valuable experiment may be to investigate this direct pathway from HPC to the prelimbic cortex in facilitating rapid schema memory consolidation.

In this experiment, aimed to examine this HPC and prelimbic cortex relationship, rats could receive unilateral inactivation of the HPC combined with a unilateral inactivation of the prelimbic cortex during encoding of new PAs. This asymmetric disconnection procedure has shown itself to be particularly useful in defining the route of serial information transfer between different brain regions in rats (Everitt et al., 1991; Floresco et al., 1997). In a contralateral group, the HPC and the prelimbic cortex could be inactivated in opposite hemispheres, while in an ipsilateral group, unilateral HPC and prelimbic cortex could be inactivated in the same hemisphere. The rational for this study is that if the 2 areas are functionally interacting, contralateral inactivation of the HPC and the prelimbic cortex would be expected to impair

performance in encoding of new PAs compared to ipsilateral inactivation. One could then use this paradigm to investigate the rapid memory consolidation associated with one trial learning of PAs in the schema paradigm.

8.3.2 Dopamine modulation in the prelimbic cortex in schema

As mentioned in the introduction (chapter 2), it is known that there is reciprocal connectivity between the HPC and the mPFC (Jay and Witter, 1991). In addition, the relationship between the HPC and the mPFC in learning and memory has been demonstrated (Laroche et al., 2000; Simons and Spiers, 2003; Takehara-Nishiuchi et al., 2003; Kesner and Rogers, 2004; Wiltgen et al., 2004). Hippocampal dopamine (DA) projections to the mPFC play an important role in modulation of HPC-mPFC interactions during memory processes (Seamans et al., 1998; Goto and Grace, 2008).

It is known from Bethus et al. (2010) that hippocampal DA is involved in the persistence of memory of new PAs and it is now known from the inactivation study in this thesis (E5) that parallel encoding of new PAs in the prelimbic cortex and hippocampus occurs. This evidence leads to some interesting questions in relation to rapid memory consolidation. For example, does DA in prelimbic cortex modulate the encoding or the persistence of the memory for the new PAs when an appropriate schema is activated?

8.3.3 Neural representation of cued recall in schema

To date, no one has looked into the neural representation of cued recall in schema using ensemble single unit recording. It would be valuable to investigate whether a cue flavour presented in a start box could reflect the neural representation of the associative location in the event arena. One could investigate the hippocampal neural

activity when rats (1) are acquiring original PAs schema, (2) are acquiring new PAs and (3) have such an original schema are recalling both original and new PAs. It would be interesting if any differences between these 3 conditions were found.

In relation to condition 1, it would be interesting to see whether there is differential place field firing during the learning of original PAs. In a recent study (Dupret et al., 2010), they found that CA1 place fields (but not CA3 place fields) tended to move towards the food reward locations over the course of a 40 trials session in which rats had to locate 3 food rewards on a cheeseboard maze, and that this correlated with memory of the locations tested after 24 hr. Bearing this in mind, it would be interesting to see whether there is a similar trend in learning the original PAs in the event arena using the PAs schema task. One expectation, based on this study, may be that place fields are over represented at PA locations.

8.3.4 Sleep mechanism in relation to prelimbic and HPC in schema

In a recent ensemble recording study, Peyrache and colleagues (2009) found that during slow-wave sleep, replay of rat mPFC neuronal firing patterns that occurred during learning of a behavioural rule coincided with hippocampal sharp-wave ripples (SWRs) (Peyrache et al., 2009). This synchrony between HPC and mPFC was dependent on the rats learning the rule in the behavioural task. In addition, a study in which hippocampal SWRs were selectively blocked during post learning sleep has shown that this leads to memory deficits on a hippocampal dependent radial arm maze task (Girardeau et al., 2009; Ego-Stengel and Wilson, 2010).

This evidence is interesting as it suggests that one possible reason that, in the seminal PAs schema study (Tse and Langston et al., 2007), the group of rats that received HPC lesions after only 3 hr did not remember the new PAs was because they did not

have enough/any sleep between encoding and surgery. The group of rats that had a 48 hr period between learning and lesion surgery presumably had multiple periods of SWS.

In another recent recording study, Dupret et al. (2010) demonstrated that, in a behavioural task in which rats had to learn the locations of 3 food rewards on a "cheeseboard" maze, hippocampal SWRs that occurred *during* the task near to goal locations were correlated with memory of the locations in a subsequent probe test. Additionally, they found that SWRs during a subsequent sleep period were also correlated with memory of the task. Bearing this in mind, it would be interesting to look at the interplay between hippocampal SWRs and mPFC in a PAs schema study in which one trial learning of PAs is encoded by the HPC and the prelimbic cortex in *parallel*, and look in particular at periods of time following encoding.

8.4 Conclusion

The experiments described in this thesis set out to explore several aspects of the neurobiology of schemas in animals. The imaging studies (E4), coupled to the inactivation studies (E5) suggest that there is parallel encoding of new information in the prelimbic cortex during what is ostensibly a hippocampal-dependent flavour place paired-associate learning task. In addition, the schema models (chapter 8) highlight that the prefrontal cortex may not only have a privileged role in 'slow' consolidation (Frankland and Bontempi, 2005), but also a role in rapid cortical memory consolidation or assimilation into schema.

Over the years, considerable progress has been made in unravelling the neurobiological bases of memory consolidation. However, despite these considerable advances, it seems that it is still far from understanding all the precise cellular and molecular mechanisms underlying memory reorganisation within cortical network during the course of system consolidation (Bontempi and Durkin, 2007). Numerous questions remain to be addressed. In addition, how do these questions relate to the schema models?

Answers to these questions will likely involve various integrative approaches that involve cross species analyses and combine several levels of analyses ranging from innovative cognitive paradigms to cellular, molecular, imaging, electrophysiological and computational modelling techniques to enabling one to understand the functional involvement of single neurons and also the functioning of complex neuronal ensembles.

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Appendix 1

The macro used for cell counting in chapter 6 is listed below.

```
Sub zif_cell_counting()

ret = IpMacroStop("please draw your 10 AOIs.", 0)

ret = IpBlbSetAttr(BLOB_OUTLINEMODE,1)

ret = IpBlbFromAoi(1)
        ret = IpBlbSaveOutline("C:\data.scl")
ret = IpMacroStop("Select the image to trasfer the AOI's to", 0)
        ret = IpBlbLoadOutline("C:\data.scl")

        ReDim blbpts(1000) As POINTAPI

Dim numpoints As Integer, numobj As Integer
Dim status As Integer, i As Integer

' get the total number of objects, in-range and out-of-range,
' hidden and visible.

ret = IpBlbGet(GETNUMOBJ, 0, 0, numobj)

For i = 0 To numobj - 1

ret = IpBlbGet(GETSTATUS, i, 0, status)
        Debug.Print ret ' (status)
        ' if object in-range and visible...
        If status >= 0 Then
        '       get the outline of the object
                numpoints = IpBlbGet(GETPOINTS, i, 1000, blbpts(0))
                If numpoints > 0 Then
                        ' create AOI out of the object outline and XOR it.
                        ret = IpAoiCreateIrregular(blbpts(0), numpoints)
                        ret = IpAoiMultShow(1)
            ret = IpAoiMultAppend(1)
                        'ret = IpOpNumberLogic(0, OPL_NOT, 0)
                End If
End If
Next i
ret = IpBlbDelete()
ret = IpAppSelectDoc(DOCSEL_PREVID)
        ret = IpBlbDelete()
        ret = IpAppSelectDoc(DOCSEL_NEXTID)

Dim DensStatsArray(10) As Single
Dim SDevStatsArray(10) As Single
Dim ThresholdValue As Double

ret = IpBlbLoadSetting("D:\Documents and Settings\s0453346\Desktop\new
settings_feb09_2.env")
```

```
ret = IpBlbDelete()
ret = IpBlbFromAoi(0)
ret = IpBlbShowStatistics(1)

ret = IpBlbShow(1)
        ret = IpBlbFromAoi(1)
        ret = IpTemplateMode(1)
        ret = IpBlbSaveOutline("C:\sd.scl")
        ret = IpTemplateMode(0)

'collect mean density and standard deviation for optical density
ret = IpBlbGet(GETSTATS, 0, BLBM_DENSITY, DensStatsArray(0))
ret = IpBlbGet(GETSTATS, 0, BLBM_DENSDEV, SDevStatsArray(0))
Debug.Print DensStatsArray(0)
Debug.Print SDevStatsArray(0)
' calculate threshold value and apply
ThresholdValue = 10^((Log(256) / Log(10)) - (DensStatsArray(0) + (SDevStatsArray(0) *
2.75)))
Debug.Print ThresholdValue
ret = IpBlbShow(1)
ret = IpBlbSetRange(0, ThresholdValue)
        ret = IpAoiMultAppend(0)
ret = IpTemplateMode(1)
ret = IpAoiCreateIrregular(Pts(0), 7)
ret = IpTemplateMode(0)
ret = IpBlbCount()
ret = IpBlbUpdate(0)
ret = IpBlbSplitObjects(1)
ret = IpMacroStop("save AOI.", 0)
ret = IpBlbShow(1)
        ret = IpBlbFromAoi(1)
        ret = IpTemplateMode(1)
        ret = IpBlbSaveOutline("C:\sd.scl")
        ret = IpTemplateMode(0)
ret = IpMacroStop("AOI to object.", 0)
ret = IpBlbFromAoi(1)
ret = IpBlbShowStatistics(1)

End Sub
```

Appendix 2

Behavioral/Systems/Cognitive

Dopamine and Memory: Modulation of the Persistence of Memory for Novel Hippocampal NMDA Receptor-Dependent Paired Associates

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Three experiments investigated the role in memory processing of dopamine (DA) afferents to the hippocampus (HPC) that arise from the ventral tegmental area. One hypothesis is that D_1/D_5 receptor activation in HPC is necessary for the encoding of novel, episodic-like information; the other is that DA activation ensures the greater temporal persistence of transient hippocampal memory traces. Rats ($n = 35$) were trained, in separate experiments using an episodic-like memory task, to learn six paired associates (PAs) in an "event arena" involving a repeated association between specific flavors of food and locations in space. After 6 weeks of training, rats had learned a "schema" such that two new paired associates could be acquired in a single trial in one session (episodic-like memory). We show that encoding of novel PAs is sensitive to intrahippocampal microinfusion of the NMDA antagonist D-AP-5. Experiment 1 established that intrahippocampal infusion of the D_1/D_5 dopaminergic antagonist SCH23390 [*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride] before encoding of new PAs caused impaired memory 24 h later but that SCH23390 had no effect on the later memory of previously established PAs. Experiment 2 established that SCH23390 modulated the persistence of new memories over time (30 min vs 24 h) rather than affecting initial encoding. Experiment 3 revealed that the impact of SCH23390 was not mediated by state dependence nor had an effect on memory retrieval. These findings support the second hypothesis and establish that persistent, long-term memory of rapid, hippocampal-mediated acquisition of new paired associates requires activation of D_1/D_5 receptors in HPC at or around the time of encoding.

Introduction

What function(s) does dopamine serve in the hippocampus (HPC) during learning? The aim of these experiments was to contrast two alternatives: (1) it is critical for encoding, or (2) it is critical for the persistence of memory traces at or around the time of memory encoding. Both possibilities are consistent with the hypothesis of Lisman and Grace (2005) of novelty modulation of hippocampal memory processing.

The dopaminergic mesocorticolimbic pathway arises from the ventral tegmental area (VTA) and is activated by novelty (Steinfels et al., 1983), but the functional impact of this dopamine (DA) neurotransmission may depend on the neural system to which it is afferent. For example, although triggered in common by novelty, its impact in the HPC may differ from the error-detection role that DA serves in the striatum (Schultz et al., 1992; Schultz and Dickinson, 2000). Anatomical, cell-biological, and neuropharmacological data have indicated a VTA projection to the HPC, the presence of DA in the dorsal HPC, and the presence of metabotropic D_1 receptors whose activation can lead to transcriptional regulation of plasticity-related genes such as the cAMP response element-binding protein CREB (Dahlström and Fuxe, 1964; Swanson, 1982; Gasbarri et al., 1994, 1997; Lazarov et al., 1998; Jay, 2003). Electrophysiological studies *in vivo* and *in vitro* point to a specific role for DA in the temporal persistence of long-term potentiation (LTP) (Frey et al., 1990, 1991; Huang and Kandel, 1995; Swanson-Park et al., 1999; O'Carroll and Morris, 2004).

If the impact of novelty within HPC is, via VTA activation, to extend the "persistence" of synaptic potentiation (Morris, 2006), it follows that a memory task that requires activity-dependent synaptic plasticity within HPC at encoding should also show delay-dependent sensitivity to local D_1 receptor blockade. Memory traces may be successfully encoded and stored as a spatial distribution of changes in synaptic weights (Martin et al., 2000), but such changes may not persist. Previous work has examined the DA dependence of learning for step-down inhibitory avoidance, the radial maze, and water maze (Packard and White, 1991; Gasbarri et al., 1996; Bernabeu et al., 1997; O'Carroll et al., 2006) but not yet the memory delay dependency of such tasks. We therefore used a new task in which rats learn flavor–place paired associates (PAs) (Day et al., 2003) in a protocol that begins with the "schema" training of six of these PAs across several weeks (Tse et al., 2007). We then introduced novel PAs for a single trial of training and tested memory for these after short or long delays. Using this hippocampal-dependent associative recall paradigm, which en-

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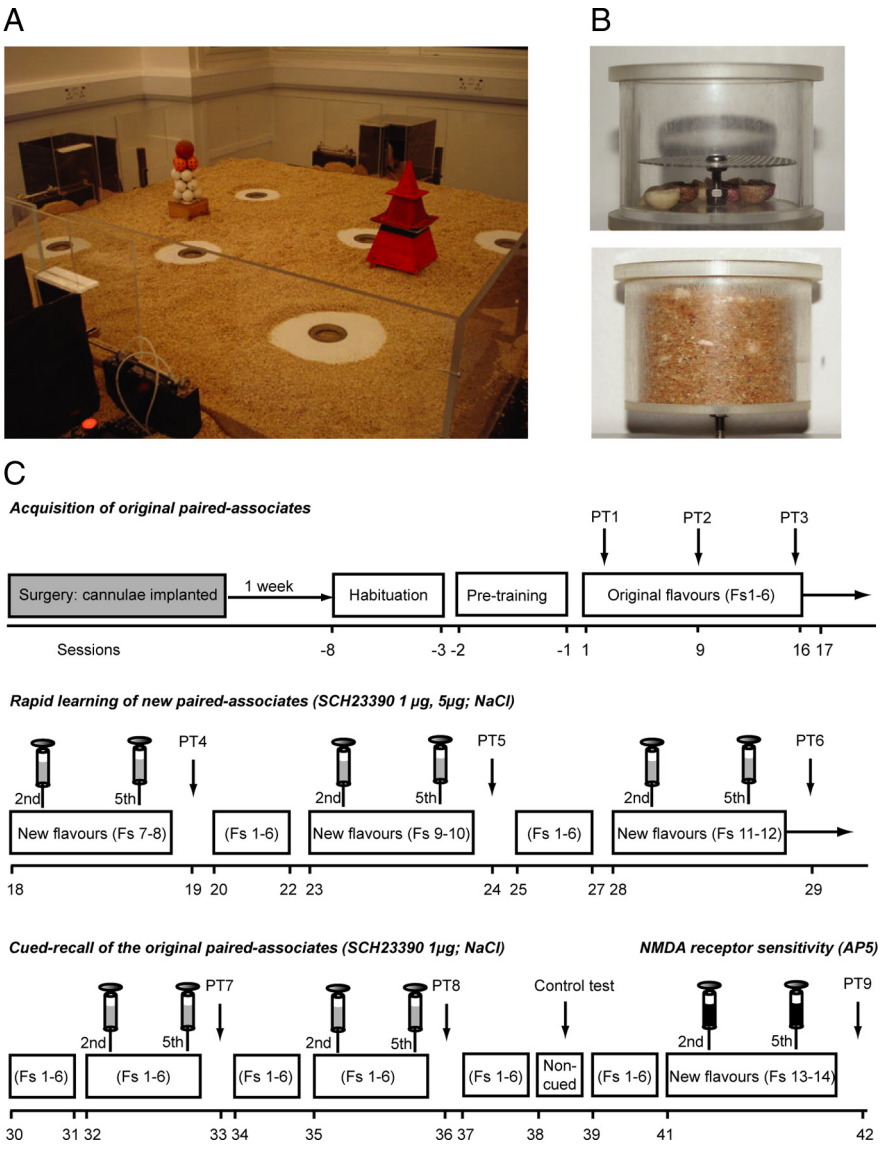

Figure 1. Experimental apparatus and design. ***A***, Layout of the event-arena apparatus (1.6 × 1.6 m) with the four start boxes and the six sand wells available to which the animals can run to collect food. ***B***, Top, Example of an empty sand well with an inner diameter of 6 cm and a total depth of 5 cm. The sand well contains a grid 3.5 cm from the top under which 1 g of each of the six flavors pellets were placed to provide comparable olfactory cues at each sand well. ***B***, Bottom, An example of a "full" sand well with the food pellets hidden, including any accessible reward pellets of a specific flavor. C, Schema of training and new paired associates. Timeline showing the design of experiment 1 (Fs, flavors). The syringes indicate the time of the bilateral iHPC infusions (before the beginning of the second and fifth trials on the training day of the new flavors).

ables rapid learning against the background of a learned schema, we examined the impact of intrahippocampal (iHPC) infusion of the D_1/D_5 receptor antagonist SCH23390 [*R*(+)-7-chloro-8- hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride]. We also checked that the recall of memory acquired in the presence of SCH23390 is not state dependent (i.e., only effective if the drug is also present during recall). A novel within-subjects protocol enabled successive, single-trial encoding experiences of novel PAs across days (episodic-like memory) and thus the possibility of a definitive comparison of the performance of individual subjects in each of three separate experiments.

Materials and Methods

The detailed methods sufficient for independent replication are described in the supplemental data (available at www.jneurosci.org as supplemental material), with information given here sufficient for understanding the training protocols and their rationale.

Animals and surgery. The subjects (experiment 1, $n = 11$; experiment 2, $n = 12$; experiment 3, $n = 12$) were group-housed adult male Lister-hooded rats (Charles River), maintained at 85% of their free-feeding weight. Standard surgical procedures, under isoflurane anesthesia, were used to implant 26 gauge cannulae for drug infusions targeted at the dorsal hippocampus bilaterally. The rats had a 7 d recovery period before the start of behavioral procedures.

Apparatus. The "event arena" (Fig. 1*A*) was made of clear Plexiglas (1.6 × 1.6 m), with the floor containing a 7 × 7 grid of 49 circular holes (6 cm diameter, 20 cm spacing, with plastic lids level to the floor) and covered by 3 cm of sawdust. Two distinctive intra-arena landmarks, a glued stack of golf balls and a pyramid, were placed in two locations: row 4, column 2 and row 4, column 6 of the 7 × 7 grid. Access to the arena was from any of four Plexiglas start boxes (25 × 25 × 25 cm), covered with black paper to make them dark inside, that were located centrally in each sidewall. These had computer-controlled sliding doors for arena access. Plastic sand wells could be inserted into the circular holes in the floors of the arena, and food rewards (0.5 g pellets manufactured in various flavors, all with equal nutritional value) could be placed inside them (Fig. 1*B*). The sand was mixed with ground-up food pellets of all flavors used in an ~90:10 ratio. A key design feature of these sand wells was a false floor with a lower compartment containing masking odors and flavors (Fig. 1*B*, contrast top and bottom pictures), these being obscured by the added sand mixture.

Drugs and drug infusions. The D_1/D_5 antagonist SCH23390 was used at concentrations of 5 µg/µl (15.4 mM) and 1 µg/µl (3 mM) and was made up as described in supplemental Methods (available at www.jneurosci.org as supplemental material). The competitive NMDA antagonist D-AP-5 was used at a concentration of 5.9 µg/µl (30 mM). The pH of the drug solutions was adjusted to 7.2 by the addition of 1 M NaOH solution. Standard procedures were used for intracerebral drug infusions at a rate of 0.2 µl/min over 5 min, after which the infusion cannulae were left in place for an additional 2 min to avoid backflow and stylets were replaced into the guide cannulae.

Behavioral training. Within-subjects, repeated-measures designs were used in three experiments that each consisted of a series of initial training sessions (Fig. 1*C*). The original arrangement of flavors (F1–F6) is shown in supplemental Figure S1, *A* and *B* (available at www.jneurosci.org as supplemental material). New PAs (F7–F14) (supplemental Fig. S1*A*, available at www.jneurosci.org as supplemental material) were trained later, with and without drug infusions, followed by interposed memory probe tests (PTs). The primary focus of these additional tests was to examine the impact of blockade of hippocampal D_1/D_5 receptors on short-term and long-term (LTM) memory and state dependence. We also scheduled various essential control conditions. Sessions were numbered from the first paired-associate training session (experiment 1) (Fig. 1*C*). During the main part of each experiment, the animals were trained on alternate days in two cohorts of up to six rats with each cohort receiv-

ing three training sessions per week (cohort 1: Monday, Wednesday, and Friday; cohort 2: Tuesday, Thursday, and Saturday).

The initial training sessions consisted of the following: (1) habituation (sessions −8 −3); (2) acquisition of original schema of six flavor paired associates (sessions 1–17); and (3) nonrewarded PTs (sessions 2, 9, 16).

For performance measures, several parameters were measured during training trials: (1) the number of incorrect sand wells at which digging occurred before choosing the correct sand well [primary error measure: chance, 2.5 errors; conversion to a performance index = 100 − 100 * (errors/5)]; (2) latency to dig at the correct sand well (seconds); (3) a "choice" was recorded only when a rat placed its front paw on or into a sand well. Rats running past or sniffing around one were not considered as making a choice, because running around a nonchosen well was an inevitable feature of the arena design. In rare cases, it was difficult to tell from the video monitors whether or not the rats had made a choice as so defined. In this case, when the experimenters entered the room at the end of a trial, they checked carefully whether there were any traces of digging, i.e., whether the sand had been displaced around the sand well(s).

To calculate the primary measure of memory recall, in probe tests, the time spent digging at each of the six sand wells over the 120 s test (none of which contained reward during a probe test). The primary measure of memory recall, calculated as the proportion of time spent at the cued location relative to the average time at the noncued locations, including the noncued location of the other of each of two novel flavors introduced during a training day. Although not rewarded during the 120 s probe test time, the rats were given three half pellets (correct flavor) in the correct location at the end of probe tests to limit extinction.

Experiment 1: does D_1/D_5 receptor activation in HPC contribute to the memory of novel paired associates? The primary focus of experiment 1 was to investigate the impact of blocking D_1 receptors in the hippocampus on new and old paired associates. After this, we conducted an important control test and checked the hippocampal dependence of the learning of new paired associates by investigating its sensitivity to intrahippocampal NMDA receptor blockade: (1) training and probe tests (Fig. 1*C*, sessions 18–29), impact of SCH23390 on new learning; (2) training and probe tests (Fig. 1*C*, sessions 30–36), impact of SCH23390 on previously trained flavors; (3) noncued control test; and (4) training and probe tests (Fig. 1*C*, sessions 41–42), impact of D-AP-5 during learning.

Experiment 2: does D_1/D_5 receptor activation play a role in memory encoding or the long-term persistence of novel paired associates? The purpose of the second experiment was to investigate the effect of blocking D_1/D_5 receptors in HPC on the short-term or long-term memory of the new paired associates. The aim was to establish whether SCH23390 affected memory encoding or the temporal persistence of newly formed memories. Sessions 25 onward focused on short-term versus long-term memory (design in supplemental Fig. S2, available at www.jneurosci.org as supplemental material). A new batch of animals was used ($n = 12$).

Experiment 3: can the impact of a D_1/D_5 antagonist memory be explained in terms of state dependency, memory retrieval, or the persistence of newly encoded information? The aim of the third experiment was to test whether (1) the results of experiment 2 could be interpreted in terms of state dependency and (2) whether intra-HPC SCH23390 affects memory retrieval. State-dependent learning is a phenomenon in which information learned in one state of the organism is retrieved best if a similar state is reinstated at the time of the testing (Dudai, 2002). Sessions 18 onward focused on specific manipulations to investigate state dependency and retrieval (design in Fig. S3, available at www.jneurosci.org as supplemental material). A new batch of animals was trained ($n = 12$).

Perfusion and histology. All rats were terminally anesthetized with Euthatal (Rhône Mérieux) and then perfused intracardially with 0.9% saline, followed by 4% Formalin. The brains were removed and stored in 4% Formalin for a minimum of 24 h. Coronal 30 μm sections were cut using a cryostat with one in every five sections recovered for histological analysis. These sections were mounted on slides, stained with cresyl violet, and coverslipped using DPX (mixture of distyrene, tricresyl phosphate, and xylene). The sections were examined with a light microscope under 20-fold magnification to verify cannulae placements. For each brain, the infusion site was plotted by determining the deepest point at which tissue damage was evident and marking this location on the appropriate coronal section taken from the atlas of Paxinos and Watson (1998) (see Fig. 3*C*).

Statistical analyses. All numerical data are presented as mean ± SEM. For all experiments, the number of errors was assessed in the main training sessions and time spent digging in each sand well during the probe tests. The latency to dig at the correct well was also routinely analyzed, but these data never showed significant differences but are presented for experiment 1 only in the supplemental data (available at www.jneurosci.org as supplemental material). Statistical significance was determined by repeated-measures ANOVAs and, when appropriate, one-sample *t* tests. The Greenhouse–Geisser correction for repeated measures was applied (with appropriate alteration of degrees of freedom). In experiments 1 and 2, after the ANOVA, pairwise comparisons were conducted with a Bonferroni's correction for multiple comparisons. In experiment 3, a set of appropriate orthogonal comparisons was created to analyze the impact of SCH23390 on persistence, state dependency, and/or memory retrieval.

Results

Before turning to the main results, it is important to convey qualitatively how event-arena experiments of this kind proceed. After habituation and pretraining, the animals readily eat the "cue" food in the start box and then, after exploratory head movements at the entrance to the arena (Johnson and Redish, 2007), enter it and run between the sand wells in search of the available reward of the same flavor. They dig through the sand at these wells and, at the correct one, soon find the 0.5 g food pellets that they then carry (one by one) back to the start boxes to eat. This natural carrying behavior is typical of rats (Maaswinkel and Whishaw, 1999), and we made no attempt to prevent it; in fact, unpublished observations in our laboratory indicate that having the animals eat the retrieved food in the start box adds to their ability to use this flavor as a recall cue on later sessions, presumably because this cue is given at a start-box location.

Experiment 1: gradual acquisition of paired-associate memory, a schema, and then rapid drug-dependent acquisition of new paired associates (SCH23390 and D-AP-5)

As shown in Figure 2*A*, the animals are initially at chance on the baseline task, but they improve across 15 sessions to a performance index (PI) score of between 70 and 80%. This is lower than the 90 or 95% demanded in standard discrimination-learning experiments; however, these typically consist of only two choices, whereas there are six choices here. The *z*-scores for the PI relative to chance were, at asymptote, around or greater than $z = 5$, and this is indicative of highly significant choice performance. Once this level is reached, the main part of each study commences with the within-subjects introduction of new PAs in the presence or absence of drugs.

After the training phase (Fig. 2*A*, sessions 1–17, white background) in which the animals were trained on alternate days to learn concurrently six paired associates (each flavor associated to a distinct location; see supplemental data, available at www.jneurosci.org as supplemental material), we tested the effect of SCH23390 on (1) the rapid acquisition of the new PAs (intra-HPC infusions given bilaterally 20 min before new PAs exposure) and (2) the memory of the previously acquired six PAs.

During the training phase, three initial nonrewarded probe tests (PT1–PT3) were scheduled as shown in Figure 1*C* (top row) to examine acquisition of paired-associate memory. Figure 2*B* represents percentage of dig time at the cued location (black bars) relative to that at the noncued locations (white bars). These probe tests revealed, as expected, a graded learning of the original PAs from sessions 1–16 ($F = 23.16$, df = 1.75/17.51, $p < 0.001$). *t* tests

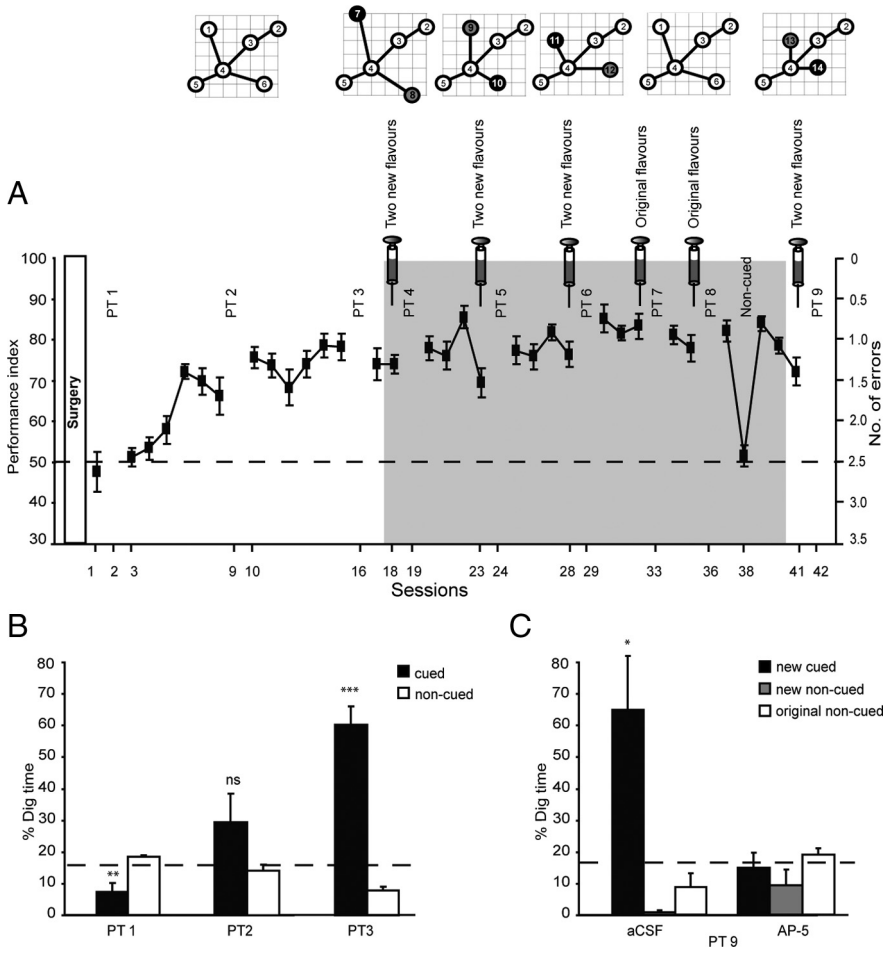

Figure 2. Acquisition of the schema, new paired associates, and NMDA dependency. ***A***, Performance index of acquisition of the original six paired associates (sessions 1–17; white background). Single days of training of new paired associates alternated with 3 d of the original schema (sessions 18–42; gray background). Performance rose to a stable level of 80% with minimal variability. Note that performance fell to chance on session 38 when the start-box flavor cues were absent. ***B***, Three cued-recall probe trials for the acquisition of the original schema. The graph represents percentage of dig time at the cued location (black bars) relative to that at the noncued locations (white bars). ***C***, Hippocampal NMDA receptor dependence of the acquisition of the new paired associates. The graph represents percentage dig time at the new cued location (black bars) and the new noncued location (gray bars), as well as the average of the four original paired associates (white bars) for probe test 9 in the control (aCSF) and drug (AP-5) conditions. ns, Nonsignificant. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ for *t* tests comparing the proportion of digging for the cued paired associates relative to chance level of 16.7%.

comparing the proportion of digging for the cued PA were above chance in PT3 ($t = 7.76$, df = 10, $p < 0.001$).

At the end of the study (sessions 41–42), we sought to extend our finding of the hippocampal dependence of PA acquisition that had been established using lesions (Tse et al., 2007). A next step was to examine the possible role of hippocampal plasticity, via iHPC infusion of the NMDA antagonist D-AP-5 at the time of novel PA encoding. Because the primary focus of this experiment was on the impact of SCH23390 on the memory of novel and familiar PAs, collection of the D-AP-5 data was not until PT9 (Fig. 1*C*, bottom row). Figure 2*C* presents dig time data of this probe test in session 42. Because the drug was given on session 41, 20 min before the trials for each of two new PAs that were trained in one session, the graph includes the relative digging during the probe test 24 h later at both the new cued PA and the new noncued PA, as well as the average of the four original PAs. Of the 10 animals that completed training to this stage of the study (from an initial $n = 11$), half were give D-AP-5 and the others vehicle. The ANOVA revealed an interaction between drug (a between-subjects factor) and paired associates ($F = 10.04$, df = 1.14/9.15, $p < 0.01$). The pairwise comparisons analysis with Bonferroni's corrections for multiple comparisons showed a significant difference between the control group [artificial CSF (aCSF)] and the D-AP-5 group only on digging at the location of new cued paired associates (Fig. 2*C*, black bars) ($p < 0.05$). Additional *t* tests compared the proportion of digging for the new cued PA relative to chance, revealing that digging was above chance for the control group (aCSF, $t = 3.14$, $p < 0.035$) but not for the D-AP-5 group (D-AP-5, $t < 1$, NS). This finding confirms the HPC dependence of novel PA acquisition and indicates that it involves local NMDA receptor activation.

Revealing the hippocampal dependency of the task sets the stage for a focused analysis of the impact of iHPC SCH23390 on memory for PAs. Figure 3 shows a decline in memory at 24 h associated with SCH23390 present at new encoding.

SCH23390 and new encoding

The ANOVA revealed an interaction between drug and digging locations ($F = 8.5$, df = 2.49/24.9, $p < 0.001$) (Fig. 3*A*). Pairwise comparisons, with Bonferroni's corrections for multiple comparisons and focusing exclusively on digging at the new cued location across drug conditions (i.e., black bars), showed significantly less digging when the animals were treated with SCH23390 (1 μg, $p < 0.05$; 5 μg, $p < 0.01$) than with saline. Similarly, *t* tests that compared the proportion of digging at the cued location indicated above chance performance in the control condition ($t = 3.42$, df = 10, $p < 0.01$) but not for the SCH22390 1 μg condition ($t = 1.02$, df = 10, NS). Unexpectedly, there was less digging than expected by chance in the SCH23390 5 μg condition ($t = 9.80$, df 10, $p < 0.05$). Given this, and considering that our lower concentration of SCH23390 was sufficient to impair memory at 24 h, we used only the lower concentration in later experiments.

SCH23390 and previously trained memories

Figure 3*B* shows the lack of an effect of SCH23390 on memory of previously trained PAs. An overall ANOVA revealed a difference in dig time at the cued and noncued locations ($F = 44.80$, df = 1/10, $p < 0.001$) but no significant interaction between drug and cued location ($F < 1$). *t* tests comparing the proportion of digging for the cued PA relative to chance were significant for both conditions (NaCl: $t = 4.12$, df = 10, $p < 0.002$; SCH23390 at 1 μg: $t = 9.88$, df = 10, $p < 0.001$).

It should be noted that the mean time spent digging at the cued location for the original trained PAs (57.5 s in Fig. 3*B*) was higher than that for the new PAs in the NaCl group (43.5 s in Fig. 3*A*). This difference is to be expected given that the original PAs had, by this point, 21 training trials (sessions 1–32), whereas new

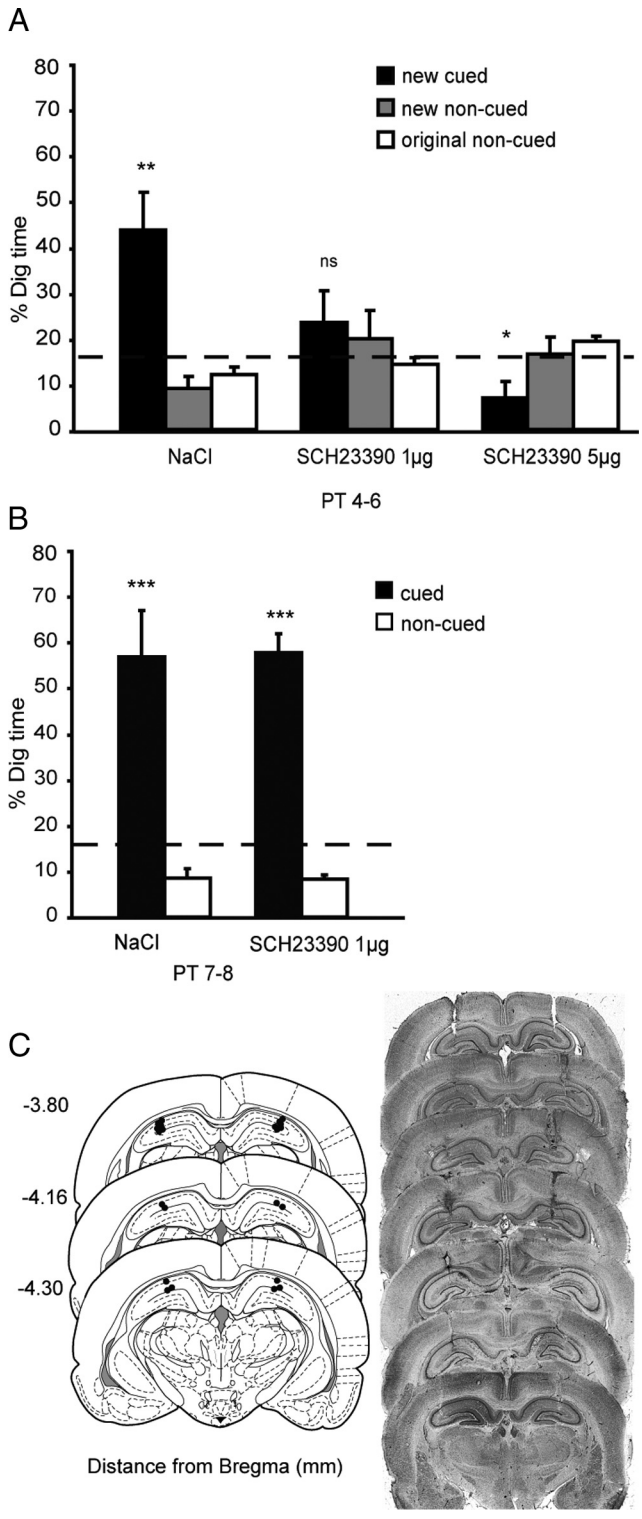

Figure 3. Experiment 1: ***A***, Dopamine dependency of encoding of new paired associates. Percentage dig time in all three conditions (NaCl, SCH23390 1 μg, and SCH23390 5 μg) across the three counterbalanced probe tests (PT4–PT6). ***B***, SCH23390 and previously trained memories. Percentage dig time for the original paired associates in two conditions (NaCl and SCH23390 1 μg). ***C***, The left shows plots of the locations of cannulae tips ($n = 11$ per HPC; experiment 1). Infusion sites are marked on the appropriate section of a stereotaxic brain atlas (Paxinos and Watson, 1998). The right shows Nissl-stained sections showing examples of representative cannulae tracks in the dorsal hippocampus in each hemisphere of the brain. ns, Nonsignificant. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

PAs only ever have a single training trial. Nonetheless, it is striking that the probe test score for a new PA trained in the presence of saline is as good as it is and well above chance. That is, against the background of a trained schema, new PA learning can occur in one trial.

Latency to dig at correct sand well on the day of drug infusions

We also examined whether SCH23390 had any impact on the latency with which the animals completed a trial (on sessions 18, 23, 28 and sessions 32, 35). On the first three of these, we could make a within-session comparison of new PA trials (in the presence or absence of the drug) with the latency on the remaining original PA trials without the drug. Latencies on the new PA trials averaged 38.5 s and varied between 15 and 50 s depending, in part, on the proximity of the sand well to the start box and the number of errors made (supplemental Fig. S4*A*, available at www.jneurosci.org as supplemental material). An ANOVA of these latency scores revealed no overall interaction between drug and trial ($F = 2.5$, df $= 1.72/17.2$, $p > 0.1$), although the graph showed a nonsignificant increase in latency for new PA trials in the presence of SCH23390. These data are discussed in the supplemental data (available at www.jneurosci.org as supplemental material). In the last two sessions examined (sessions 32 and 35), we could make a within-session comparison of the impact of the drug on the latency to complete original PA trials (supplemental Fig. S4*B*, available at www.jneurosci.org as supplemental material). The animals were typically faster, taking between 15 and 22 s to complete a trial, with the ANOVA also revealing no interaction between drug and trial ($F = 3.8$, df 1/10, $p > 0.05$). These findings indicate that, 20 min after an SCH23390 injection, there was no significant locomotor impairment that might have affected memory encoding in an indirect manner.

Cannula placement

Inspection of the location of the tips of the cannulae in coronal sections of the brain of all tested animals revealed these to be located in dorsal hippocampus, in the CA1 region of the HPC (Fig. 3*C*).

Experiment 2: D_1/D_5 receptors are critical for the memory persistence but not the encoding of new paired associates

The aim of experiment 2 was to compare the impact of SCH23390 on short- and long-term memory. To test this hypothesis, a new batch of animals ($n = 12$) were trained to learn the six paired-associates schema, followed by the introduction and training new PAs preceded 20 min before by intra-HPC drug or vehicle infusions. The memory probe tests proceeded after a short (30 min) or a long (24 h) memory delay.

Impact on short-term versus long-term memory

Figure 4 shows the percentage dig time in the series of four counterbalanced probe tests conducted 30 min or 24 h after the encoding of new paired associates. An overall ANOVA revealed a triple interaction between memory delay, drug, and paired associate ($F = 18.16$, df $= 1.94/21.38$, $p < 0.001$). Pairwise comparisons, again with Bonferroni's corrections for multiple comparisons, focused on digging at the new cued paired associates (black bars). This showed no significant difference between the NaCl and SCH22390 conditions at a 30 min delay ($F = 2.1$, $p > 0.18$) but a significant decline in memory in the SCH23390 condition at 24 h ($F = 5.6$, $p < 0.04$). Separate comparisons across time intervals revealed no difference between the two probe tests at 30 min and 24 h with NaCl ($F < 1$) but a highly significant delay-dependent decrease across the two SCH23390

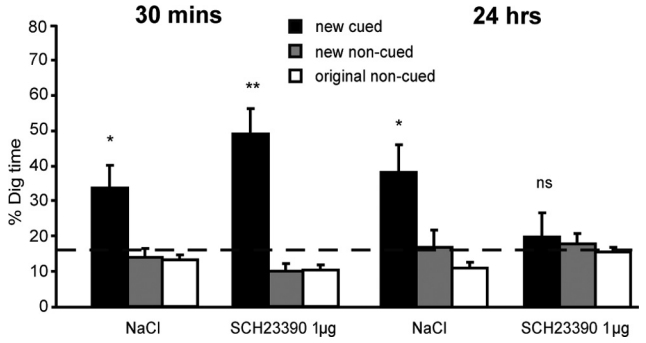

Figure 4. Experiment 2: delay dependency. Impact of the SCH23390 on short-term (30 min) and long-term (24 h) memory. Percentage dig time at both delays (30 min or 24 h) in two drug conditions. Note good performance in the presence of the drug at 30 min but not at 24 h. ns, Nonsignificant. $*p < 0.05$; $**p < 0.01$.

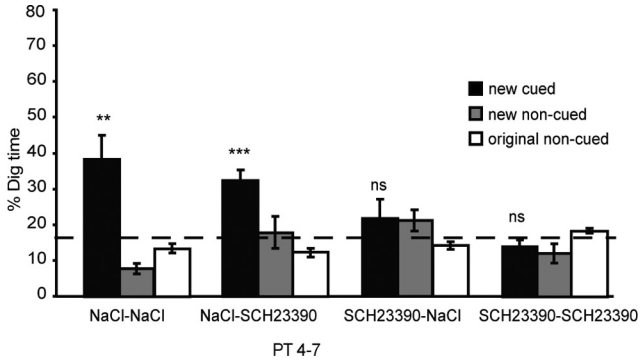

Figure 5. Experiment 3: state dependency and memory retrieval. Percentage dig time across the four counterbalanced probe tests (PT4–PT7) in all four conditions (NaCl before encoding and before retrieval, NaCl before encoding and SCH23390 before retrieval, SCH23390 before encoding and NaCl before retrieval, and SCH23390 before encoding and retrieval). Note that memory was impaired only when the drug was present at the time of encoding. ns, Nonsignificant. $**p < 0.01$; $***p < 0.001$.

tests ($F = 16.9$, $p < 0.002$). Additional t tests compared the proportion of digging for the cued paired associates relative to chance. As shown in Figure 4, this revealed that digging was above chance at 30 min in both conditions (NaCl: $t = 2.67$, $p < 0.025$; SCH23390: $t = 4.78$, $p < 0.001$) but only for the NaCl condition at 24 h (NaCl: $t = 2.85$, $p < 0.02$; SCH23390: $t = 0.5$, NS). Thus, these data reveal a delay-dependent effect of a D_1/D_5 antagonist on the memory of novel PAs. These receptors must be activated at encoding for memory to persist.

Experiment 3: the state dependency hypothesis does not explain results obtained in experiments 1 and 2, and D_1/D_5 receptor blockade in HPC does not affect retrieval

The aim of experiment 3 was to check that the apparent delay dependency of experiment 2 is not an artifact or consequence of state dependency. The phenomenon of "state-dependent" memory, in which information learned in one state (e.g., presence of a drug) is retrieved best if a similar state is reinstated at the time of testing, could explain the apparent impairment of memory of new PAs at 24 h but not 30 min with SCH23390 because testing of the former but not the latter is in a different drug "state."

This possibility can be tested by performing memory retrieval probe tests similar or different drug conditions to those of exposure to new PAs. A full 2×2 design was deployed, enabling both an examination of an impact of the drug on memory retrieval and a replication of our findings with respect to memory persistence.

Figure 5 presents the percentage dig time across the four counterbalanced probe tests (PT4–PT7). The ANOVA comparing performance across drug conditions (SCH22390–SCH23390, NaCl–SCH23390, SCH23390–NaCl, and NaCl–NaCl) and all dig locations revealed an interaction ($F = 5.42$, df = 2.93/32.26, $p < 0.005$).

Inspection of the data suggests that memory for new PAs trained under NaCl are above chance at retrieval without regard to drug condition, whereas new cued PAs trained in the presence of SCH23390 are at chance. A second ANOVA, focusing on the new cued locations only (Fig. 5, black bars), also showed an interaction between drug and digging location ($F = 5.93$, df = 2.1/23.37, $p < 0.01$). This overall F value was partialed out using a set of three orthogonal comparisons that compare groups on the basis of a memory persistence, a state dependency, or a retrieval hypothesis of the impact of SCH23390. The first orthogonal comparison (persistence hypothesis) revealed a significant difference between drug conditions (NaCl–NaCl + NaCl–SCH23390 vs SCH23390–NaCl + SCH23390–SCH23390; $F = 10.9$, df = 1/23.4, $p < 0.005$), indicating that the drug impaired the memory persistence of new PAs if present at the time of encoding. The state-dependency hypothesis H2 (NaCl–NaCl + SCH23390–SCH23390 vs NaCl–SCH23390 + SCH23390–NaCl) was not significant ($F < 1$), and, similarly, the retrieval hypothesis was also nonsignificant ($F = 1.59$, NS). Additional t tests compared the time of digging at the new cued PA relative to chance, revealing that digging was above chance in the probe test for drug conditions NaCl–NaCl ($t = 3.27$, $p < 0.007$) and NaCl–SCH23390 ($t = 5.69$, $p < 0.001$) but not for drug conditions SCH23390–NaCl ($t < 1$, NS) or SCH23390–SCH23390 ($t = 1.37$, NS).

Discussion

The main findings are as follows. (1) Intrahippocampal infusion of the D_1/D_5 antagonist SCH23390 impaired memory for new PAs in an episodic-like memory task in which rats had previously acquired a flavor-place schema; the drug had no effect on the memory of previously established PAs. (2) SCH23390 modulated the persistence of new memories over time rather than affecting encoding per se. (3) This impact of SCH23390 was neither mediated by state dependence nor affected retrieval. We also established (4) the hippocampal dependency of this cued-recall task using intrahippocampal infusions of the selective NMDA receptor antagonist D-AP-5. These findings have implications for the role of D_1/D_5 receptors in HPC on the neuromodulation of memory processing.

The hippocampal dependence of the PA task

Our use of this relatively new paired-associate schema task makes it possible, for the first time, to examine the neurobiology of one-trial PA encoding of new information on a within-subjects basis. The task is sensitive to hippocampal lesions (Tse et al., 2007), and our new data establish further that bilateral iHPC infusions of D-AP-5 before the encoding of new PAs results in memory loss when tested 24 h later. Previous autoradiographic work has established that drug diffusion with this iHPC protocol is primarily restricted to the dorsal HPC (Steele and Morris, 1999). Thus, the task is not only "hippocampal dependent" in the classical sense of being impaired by lesions but is also dependent on NMDA receptor-dependent mechanisms within HPC at the

time of encoding, presumably involving associative synaptic potentiation (Bliss and Collingridge, 1993). In passing, the previous lesion study was subject to the criticism that ibotenic acid lesions might result in aberrant neocortical activity after the lesion (Rudy and Sutherland, 2008; Tse et al., 2008). The present data renders this concern even less likely because iHPC infusion of D-AP-5 is not known to have such an effect on neocortical activity. Thus, although the memory traces of multiple PAs are probably in neocortex (Bontempi et al., 1999; Maviel et al., 2004; Tse et al., 2007), the encoding of new PAs critically involves an NMDA receptor-dependent mechanism in HPC, followed by cellular and then rapid systems consolidation.

Functional role of D_1/D_5 receptors in the dorsal hippocampus

The key new finding is that activation of D_1/D_5 receptors in HPC is critical for the persistence of memory that depends on hippocampal processing at or around the time of encoding. Our new results point to a delay-dependent effect of blocking D_1/D_5 receptors on LTM.

A possible alternative account of the apparent delay-dependent memory impairment could be that of state dependency. According to this, poor memory at 24 h might be explained by the animal being in a different physiological state during memory retrieval in the absence of the drug than it had been at encoding in its presence (Overton, 1964). Good memory at 30 min could be because the animals are still under the effects of the drug. Experiment 3 tested this by comparing SCH23390 in three conditions: before encoding, before retrieval (24 h later), or before both encoding and retrieval. The results are inconsistent with state dependency because the animals had a similar impairment of memory when SCH23390 was infused only before encoding as when infused before both encoding and retrieval. They also exclude a potential role of HPC D_1/D_5 receptors at or around the time of retrieval, because their iHPC blockade 20 min before the recall test had no effect.

Although we cannot exclude the possibility that SCH23390 has agonist actions at 5-HT_{2A} and 5-HT_{2C} receptors at the concentration used (Millan et al., 2001), such actions should have resulted in a facilitation of hippocampus-dependent memory rather than an impairment (Buhot et al., 2000; Harvey, 2003). In addition, the lack of memory impairment in the recall test 30 min after encoding (50 min after drug infusion) and the absence of difference in latency to dig in the correct sand well between drug and nondrug trials rules out interpretations of our results in terms of hypokinesia (Fink and Smith, 1980; Taghzouti et al., 1985), a reduction of motivation, or an impairment of motor-skill learning, such as pellet retrieval (Smith-Roe and Kelley, 2000; Willuhn and Steiner, 2006).

After a long time during which many studies have focused on the role of DA in error-correcting reward processing in the striatum (Schultz, 2007), attention is now being focused on the role of mesohippocampal DA projections to HPC (Lisman and Grace, 2005). Previous studies have implicated DA in both learning and memory by blocking or activating hippocampal DA receptors before (Umegaki et al., 2001) or immediately after (Packard and White, 1991) training or via lesions of mesohippocampal DA projections before training (Gasbarri et al., 1996). However, these studies did not contrast encoding versus persistence. In keeping with the LTP data described in Introduction, O'Carroll et al. (2006) observed an impairment of the persistence of water-maze spatial memory with intra-HPC infusion of SCH23390 at the time of encoding that the present data support.

Recently, an elegant series of experiments (Rossato et al., 2009) has shown a contribution of DA to the maintenance of LTM storage through a mechanism involving D_1 receptor signaling in dorsal CA1 that occurs quite a long time (12 h) after acquisition. Previous findings of Bernabeu et al. (1997) are consistent with this new idea about DA involvement in memory consolidation, as is recent work by McNaughton's group (Valdes et al., 2008) who have shown that VTA cells can fire during periods of memory "replay." The possibility that there are actions of DA on memory consolidation at or around the time of encoding (our findings) and separately at later time points are not mutually exclusive. Indeed, the synaptic tagging and capture hypothesis of protein synthesis LTP (Frey and Morris, 1997, 1998) raises the possibility of novelty-associated upregulation of plasticity proteins at a time separate from encoding. These could help stabilize learning-associated synaptic change provided local synaptic tags are set at the time of learning. Data consistent with a role for such a mechanism in memory consolidation have been reported by Moncada and Viola (2007).

Toward better "episodic-like" memory tasks and the impact of novelty on memory persistence

We used a new PA learning task in which, in the same paradigm and with the same animals, the relative impact of a drug on well trained PAs across days can be distinguished from that on the encoding, storage, and/or retrieval of new PAs. These dissociations are not possible using inhibitory avoidance (Rossato et al., 2009) nor using the free-recall delayed matching-to-place water-maze task (O'Carroll et al., 2006) because, in the latter case, a change in the location of the escape platform entails both acquisition of a new location and extinction of a previous location. The use here of distinct recall cues (different foods) supersedes this limitation. Our new paradigm also confirms that new PAs can be integrated into a preexisting schema after a single training trial (Tse et al., 2007). Such encoding is "episodic-like" in that encoding of both object information (what food) and place information (where in the arena) occurs in a single trial, but it falls short of a "pure" definition of episodic-like memory that involves the triad of "what–where–when," i.e., memory of time as well (Clayton and Dickinson, 1998).

Some recent human imaging studies have also shown that midbrain DA neurons may also play a critical role in modulating HPC-dependent episodic memory (Lisman and Otmakhova, 2001; Schott et al., 2004; Wittmann et al., 2005; Adcock et al., 2006; Shohamy and Wagner, 2008). Shohamy and Wagner (2008) suggest that an HPC–midbrain network may not only provide a mechanism for the enhancement of LTM for individual episodes but also for cross-episode integration by enabling rapid behavioral generalization in the future. Integrating new episodes with past experiences is a key feature of our schema paradigm.

Our findings of a role for DA in modulating memory persistence are in line with the HPC–VTA loop model of Lisman and Grace (2005). When novelty is detected in the HPC, a neural signal is thought to pass first via the subiculum to the nucleus accumbens (NAcc). There, together with information from the prefrontal cortex, NAcc neurons stimulate the firing of the VTA. In keeping with this, Kentros et al. (2004) demonstrated that increased place field stability in CA1 is subject to neuromodulation by hippocampal DA released from terminals emanating from VTA neurons. They suggest that a higher-order cognitive process, such as attention, is mediated by one or more neuromodulatory inputs that switch short-term into long-term plasticity. In the presence of novelty, DA is released in the HPC, and

early LTP is transformed into late LTP. Cellular consolidation of the memory representation of new PAs in our training paradigm would favor their integration into the schema via systems consolidation. Additional examination of this distinctive role of DA in HPC, compared with its error-correcting role in the striatum, would be to conduct tract-tracing studies of VTA projections to striatum and HPC and to test whether lesions specific to DA cells from the VTA projecting to the HPC have a selective impact on the persistence of memory but are without effect on initial encoding.

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Appendix 3

Schemas and Memory Consolidation
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dissociative electrophilic alkylation of the double bond in IPP by the allylic cations generated from DMAPP or GPP (*23*). By analogy, for biosynthesis of irregular monoterpenes, we suggest that a related dissociative electrophilic alkylation of the double bond in DMAPP by the dimethylallyl cation results in a protonated cyclopropane intermediate. This species can be deprotonated to give CPP or rearrange to a tertiary cation, which can in turn be deprotonated to give LPP. Alternatively, the tertiary cation can cyclize to give a cyclobutylcarbinyl cation that can then be deprotonated to give MPP or LPP. Formation of any specific product would be controlled by the ability of the enzyme to stabilize a specific intermediate along the reaction coordinate through dipolar and electrostatic interactions and to facilitate the selective removal of protons. The stereochemistries of the products result from the conformations of the two bound substrate molecules before the reaction. Only minor changes in the relative positions of the substrates are required to accommodate the formation of the different products.

This scenario provides an attractive mechanism for the evolution of the isoprenoid pathway through gene duplication and random mutagenesis of the duplicate genes to give new proteins, one of which is constrained to retain its original function, whereas the other is free to acquire a new activity. The isoprenoid fold first seen in the *E*-selective chain-elongation enzyme avian FPPase (*12*) has also been found in the cyclopropanation enzyme squalene synthase (*13*) (sterol biosynthesis) and several different terpenoid cyclases (*14*) along with aspartate-rich motifs involved in binding allylic diphosphate substrates, indicating that the enzymes evolved from a common ancestor. Phylogenetic correlations suggest that the cyclopropanation enzyme phytoene synthase (carotenoid biosynthesis) also has an isoprenoid fold. Our discovery that chimeric enzymes from FPPase and CPPase catalyze branching and cyclobutanation reactions suggests that WT enzymes with these activities also share this common ancestor.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5821/73/DC1
Materials and Methods
Figs. S1 to S5
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Chemical Ionization (CI) Mass Spectra
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Schemas and Memory Consolidation

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Memory encoding occurs rapidly, but the consolidation of memory in the neocortex has long been held to be a more gradual process. We now report, however, that systems consolidation can occur extremely quickly if an associative "schema" into which new information is incorporated has previously been created. In experiments using a hippocampal-dependent paired-associate task for rats, the memory of flavor-place associations became persistent over time as a putative neocortical schema gradually developed. New traces, trained for only one trial, then became assimilated and rapidly hippocampal-independent. Schemas also played a causal role in the creation of lasting associative memory representations during one-trial learning. The concept of neocortical schemas may unite psychological accounts of knowledge structures with neurobiological theories of systems memory consolidation.

The concepts of "mental schema" and "mental models" as frameworks of knowledge are now well established (*1*, *2*), with implications for story recall, deductive inference, and education (*3*, *4*). For example, the memory of grammatically correct but semantically unusual prose passages is substantially better when subjects have an activated and relevant mental framework with which to understand them (*5*). An everyday experience for working scientists is remembering complex new information in an academic seminar. Our ability to do so depends as much on our possession of an appropriate mental schema as on the communicative skill of the speaker in logically conveying his or her message. In the absence of such mental frameworks, we are unable to follow scientific inferences in a talk and have the phenomenological experience of being functionally amnesic for its content a surprisingly short time later.

Curiously, this fundamental idea about memory has had little impact in neuroscience. Selective activation of a specific region within the posterior parietal cortex occurs in human subjects when, having been given relevant pictorial information earlier, they correctly interpret unusual textual information that would otherwise be incomprehensible (*6*). Animal studies have rarely considered the issue of what the animal itself brings in the way of knowledge to a learning situation, with the exception of studies of spatial and relational memory (*7*–*9*). This is partly because most experiments are conducted with "experimentally naïve" animals, and also because the creation of a mental schema is difficult to map precisely onto concrete neuroscience concepts such as anatomical connectivity or synaptic plasticity. The present experiments test the idea that the schema concept is directly relevant to the neural mechanisms of systems memory consolidation (*10*–*12*).

Experiments on schema learning. We trained rats to learn several flavor-place associations concurrently, using different flavors of food (flavor cues) and sand wells (place cues) located within a familiar testing environment called an "event arena" (*13*). The task was to learn which

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flavor was in which location such that, when cued with a specific flavor in start boxes at the side of the arena, the animals would be rewarded for going to the correct location by receiving more of that same food (i.e., "cued recall"). They should be able to recall that banana-flavored food is at one location, bacon-flavored food at another, and so on (Fig. 1, A and B). Such paired-associate learning is likely to be mediated by the hippocampus initially (*14*–*16*), with long-term storage of paired-associate memory traces eventually consolidated in the neocortex (*17*, *18*). This makes this paradigm ideal for looking at the temporal dynamics of systems memory consolidation (*10*, *12*, *19*, *20*), a process widely held to be quite slow. Additionally, the use of location as one member of each paired associate allowed the animals to learn each association as either an isolated declarative "fact," in which spatial information is generally considered as no different from other kinds of information (*10*), or as some kind of mapping of flavors to arena locations, resulting in the formation of a spatial or relational framework (*7*, *21*).

After habituation, the animals were started from one start box of the arena (at north, south, east, or west) on all six trials of a session. A different start box was used for each session. A trial began when the rat was given a cue flavor in the start box. Upon entering the arena, the animal was confronted by six sand wells (Fig. 1, A and B) of which only one contained flavored food—the same flavor given as a cue in the start box (*22*). The animals visited and sometimes dug at incorrect sand wells, which did not contain food on that particular trial, until they found the correct one. On each trial, the animals would retrieve the first of three buried food pellets, return to the start box to eat it, and then run back to the correct sand well to collect and transport the second and third pellets. One hour later, the second trial began with a different cue flavor in the start box and a different sand well baited. There were six trials per session, with the next session run 48 hours later (*23*).

We began by examining the impact of neurotoxic hippocampal lesions made before training (experiment 1). After 13 sessions, sham-lesioned animals were digging less frequently at incorrect sand wells before going to the correct one, whereas the hippocampal-lesioned animals did not improve. A single nonrewarded probe trial was then scheduled, which started with the provision of a cue flavor in the start box. The sham-lesioned animals spent significantly more time digging at the cued location than at the other five incorrect sand wells, whereas the hippocampal-lesioned animals were at chance (Fig. 1C; see tables S1 and S2 and figs. S1 to S3 for the lesions and full experimental design). The lesions were extensive, leaving minimal residual tissue throughout the longitudinal axis of the hippocampus (Fig. 1D).

To investigate the properties of paired-associate learning and its consolidation in more detail (experiment 2), we trained normal animals in a similar way. Probe tests, other controls, and novel context training were scheduled at various stages before and after making sham or hippocampal lesions (fig. S4). Using the same paired-associate layout as in experiment 1, we examined acquisition of sand-well choice behavior during training. A "performance index" was calculated, and this index improved monotonically across sessions (Fig. 2A). In nonrewarded probe trials, preferential digging at the correct location rather than the other five locations increased from chance levels at the outset of training to a highly significant preference for the cued location (Fig. 2B). To exclude the possibility that an olfactory cue in the correct sand well guided choice performance on training days, we conducted a single session of six trials in which the daily protocol was unchanged, except that no cue flavors were offered in the start box. Choice performance fell to chance (Fig. 2A, session 18), returning to above chance on the next normal session. The possibility of cryptic olfactory guidance by cues on the arena near the correct sand well was also ruled out in a later session by physically rotating the arena through 90° after the third trial of a session and back to its normal orientation after the third trial of a second session on the next day. The sand wells and intramaze cues were relocated such that their places relative to the distal room cues remained the same. Arena rotation had no effect (fig. S4, A3). With a different start box used in each session, it would appear that the animals can visually perceive their own location relative to the intra- and extramaze landmarks and use allocentric memory representations to identify the correct goal location among the six available sand wells.

If the animals develop a neocortical associative schema for this task, and if this is activated when the animals enter the apparatus, it might aid the encoding of new paired associates and their rapid assimilation into the schema. A single training session of six trials was given (Fig. 2A, session 21) in which paired associates (PAs) 1 and 6 were replaced by two new PAs, 7 and 8, hidden at two nearby locations; PAs 2 to 5 were trained normally. Note that PAs 7 and 8 received only one rewarded trial each. The inset of Fig. 2C shows how the new PAs were located near those of the now-closed sand wells. A nonrewarded probe trial was given 24 hours later to test memory for the new associates. Preferential digging was observed at the correct cued location in the arena relative to the new noncued location (i.e., less digging at location 8 for those animals on a PA7 trial, and vice versa) and to any of the original locations (PAs 2 to 5; Fig. 2D). The rapid acquisition of new PAs in a single trial, and their retention over 24 hours, are indications that the prior learning of an associative schema may aid the encoding, storage, and/or consolidation of new PAs. In contrast, animals trained on a similar one-trial task, but with novel PAs each day, showed consistent forgetting over 90 min (*13*).

Time course of memory consolidation. Hippocampal or sham lesions were then made 24 hours later—a much shorter time after training of the new flavors (48 hours) than is usually thought necessary for systems consolidation to be completed (*24*–*27*), and shorter than the usual time scale of differential changes in the patterns of glucose use or immediate early gene activation between hippocampus and neocortex after learning (*19*, *28*). After recovery from surgery, a series of nonrewarded probe tests (with

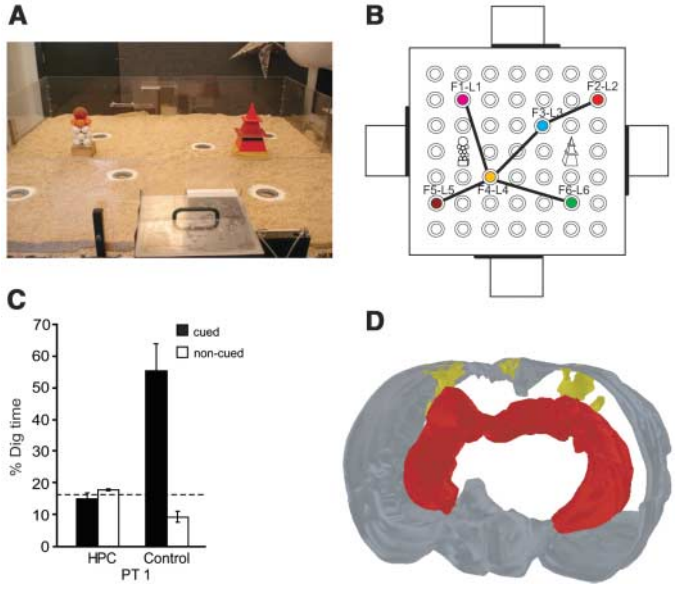

Fig. 1. Paradigm for hippocampal-dependent paired-associate (PA) learning. (**A**) The large event arena (1.6 m by 1.6 m) contains a 7 × 7 grid of locations at which sand wells can be made available and four surrounding start boxes. After being given a cue flavor in a start box, the animals recall the spatial location with which it is associated, and run into the arena to that location to secure more of that flavor of food. (**B**) The spatial arrangement of the six PAs and the "schema" this constitutes (F, flavor; L, location). (**C**) Preferential digging during a nonrewarded probe test [probe trial 1 (PT1)] by sham-lesioned but not hippocampal (HPC)-lesioned animals (*ns* = 6). Groups $t = 5.25$, df = 10, $P < 0.001$; sham versus chance, $t = 5.01$, df = 5, $P < 0.005$; HPC versus chance, not significant (n.s.). (**D**) A three-dimensional reconstruction of the volume of hippocampus lesioned in a representative rat (red), together with typical overlying cortical damage (yellow). The gray region represents the transparent volume of the rat brain.

interpolated training days using the original flavor-place pairs) was given to examine memory for the original schema and the two new PAs. These consisted of separate tests of the original PAs 2 to 5 and new PAs 7 and 8, each repeated once across a series of four sessions to enable both PA7 and PA8 to be tested in all animals. The hippocampal-lesioned group not only could successfully recall the original PAs learned over the previous month (Fig. 2D) but also, remarkably, could remember the newly acquired pairs PA7 and PA8. Because the lesions were near-complete (~90%; see Fig. 1D and fig. S2B), these two findings imply that (i) the memory traces for these PAs must be stored outside the hippocampus, probably in the neocortex; and (ii) consolidation of new associates whose acquisition is mediated by the hippocampus takes place within 48 hours.

To be more confident of these claims, it was essential to establish that the learning of further new PAs still required the integrity of the hippocampus in these same animals. Accordingly, immediately after this series of postoperative probe tests, we conducted a single six-trial training session with PAs 2 to 5 of the original schema, but with PAs 7 and 8 now replaced by sand wells containing two new flavors in nearby locations in the arena (PAs 9 and 10; Fig. 2E). The probe test conducted 24 hours later showed that sham-lesioned animals could readily learn and recall these new pairs, whereas the hippocampal-lesioned group could not. Thus, the one-trial acquisition of new PAs in this paradigm in experienced animals was still blocked by hippocampal lesions. Hence, it is unlikely that any relearning took place after the hippocampal lesions during the earlier series of four probe tests that had examined remote memory (the interpolated training was restricted to the well-trained PAs 2 to 5). The effective cued recall of the new PAs 7 and 8 introduced before the lesion must therefore reflect rapid, successful systems consolidation.

Although the animals appear to have acquired an associative schema reflecting the mapping of flavors to places in the arena, an alternative might be a response-based "win-stay, lose-shift" inference strategy in the manner of a learning set (*29*). It is not entirely clear how such a procedural strategy could be applied in this context, with six choice locations and only one trial per day to each cued location. However, as procedural strategies are generally context-independent, this account would predict that the learning of an entirely new set of six PAs in a new context would occur very quickly. In contrast, the schema hypothesis requires that a new schema be gradually learned. The same animals of experiment 2 were first trained on a new set of PAs in the same event arena (fig. S7) and then in a novel event arena in a different room with new intra- and extramaze landmarks, new flavors, and a distinct spatial geometry to the new set of sand wells (Fig. 3, A and B). Acquisition again took

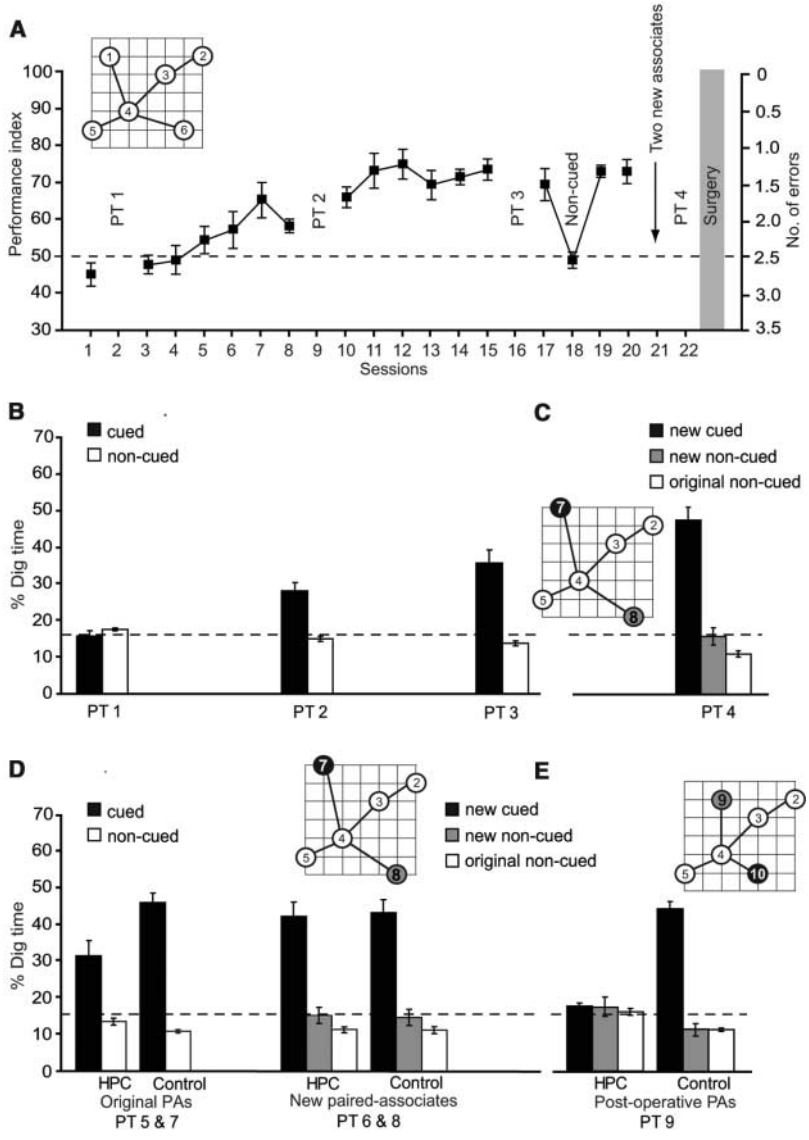

Fig. 2. Acquisition of an associative schema and its role in new learning and consolidation. (**A**) Acquisition of PAs. The animals (n = 18) made fewer choice errors over training (F = 18.24, df = 5.7/97.5, P < 0.001; Greenhouse-Geisser correction, including degrees of freedom) such that the performance index, computed as 100 − [100 × (errors/5)], was significantly above chance from session 10 onward (ts > 5.08, df = 1/17, Ps < 0.001). Removing cue flavors from the start box on session 18 resulted in performance dropping to chance and then returning to 70% correct on a succeeding normal session (session 19). (**B**) Cued-recall probe trials. Nonrewarded probe tests revealed a graded learning of the original PAs (cued flavor = solid bars) across sessions 2, 9, and 16 (F = 16.24, df = 1.54/26.22, P < 0.001; above chance in PTs 2 and 3; ts = 3.94 and 6.17, df = 17, P < 0.005 and P < 0.001, respectively). (**C**) Effective recall in PT4 of the location of the cued new PA (solid bar), coupled with avoidance of the noncued new PA (gray bar) and the remaining original associates (open bar) 24 hours after a single session of training with only one trial of each new PA (repeated-measures F = 65.28, df = 1.7/29.1, P < 0.001; cued location above chance, t = 10.29, df = 17, P < 0.001; noncued versus original, n.s.). (**D**) Postoperative retention. Both sham-lesioned (n = 8) and HPC-lesioned (n = 10) animals could effectively remember both original PAs (PTs 5 and 7) and new PAs introduced for a single trial 2 days before surgery (PTs 6 and 8). Both groups dug at the sand wells of the original associates (flavors 2 to 5) significantly more than chance (HPC t =3.60, df = 9, P < 0.01; sham t = 12.89, df = 7, P < 0.001; sham versus HPC group, t = 2.86, df = 16, P < 0.05). Both groups also dug equally at the cued locations of the new associates relative to the noncued locations (Group × Location F < 1, n.s.), and at these cued locations better than chance (ts > 8.07, df = 9 and 7, P < 0.001). (**E**) Postoperative new training. Hippocampal lesions prevented the learning of new PAs (PAs 9 and 10; Group × Location F = 60.23, df = 1.64/26.17, P < 0.001). Digging at the cued new location in PT9 was significantly above chance only in the sham group (t = 17.07, df = 7, P < 0.001) and significantly lower in the HPC group than in the sham group (t = 13.78, df = 16, P < 0.001).

place gradually, such that the learning curve of the now experienced sham-lesioned animals did not differ from the original rate of learning of the normal animals in the first event arena. The hippocampal-lesioned animals did not learn the new spatial schema despite repeated trials. Probe test performance early in training followed the same gradual pattern in the sham group, resulting in effective probe test performance only by session 67 (Fig. 3C). These findings argue against a response-based strategy, such as a learning set, because learning was no faster in the new room with new flavor-place geometry.

Completion of training in the second room offered the opportunity of returning the animals to the first arena to examine their now remote memory of the original set of PAs first learned 4 to 5 months earlier. Remarkably, the hippocampal-lesioned animals were above chance in cued spatial recall (session 68, Fig. 3D) and even showed a nonsignificant trend toward better performance than did sham-lesioned controls in a probe test as early as session 2. The sham-lesioned animals may have sustained some associative interference arising from their successful training on other sand-well arrangements in this and the other contexts, but after as few as six sessions of retraining, both groups showed effective cued recall of the original PAs (Fig. 3E). Thus, the failure to learn new PAs in a new context after a hippocampal lesion did not affect the ability to remember, after several months, information acquired before the lesion—a pattern exactly like that shown by patient E.P. in his knowledge of current and past hometown topography (*30*).

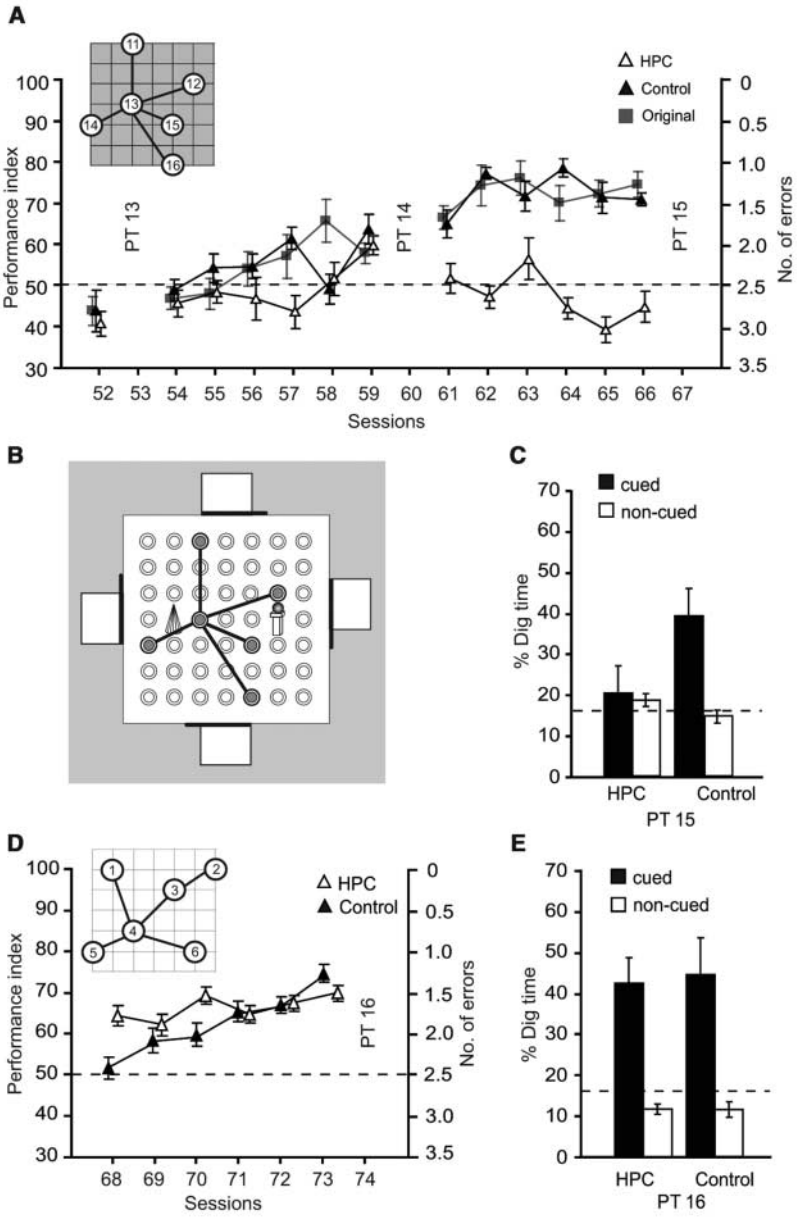

Fig. 3. Gradual acquisition of new PAs in a new context by experienced animals. (**A**) Acquisition of PAs. The now experienced sham group (n = 8) learned a new set of six PAs in the second event arena at a comparable rate to that shown by normal animals in the first event arena (Group × Session F = 1.97, df = 6.9/116.9, 0.10 > P > 0.05, treating Group as a between-subjects factor). Relative to the sham-lesioned group, the HPC-lesioned group (n = 10) failed to learn (Group F = 128.63, df = 1/15, P < 0.001; Group × Session F = 7.42, df = 5.9/89.3, P < 0.001). (**B**) Spatial arrangement of the new PAs (PAs 11 to 16) in the new event arena. (**C**) Cued-recall probe trial. Proportion of digging at the cued location relative to the noncued locations by sham- and HPC-lesioned animals (PT15, session 67). The sham group was above chance (t = 2.38, df = 7, P < 0.05); the HPC group was not (t < 1). However, the difference between groups showed only a trend toward significance (t = 1.83, df = 15, 0.10 > P > 0.05). (**D**) Return to the original event arena and flavors (flavors 1 to 6). Inset indicates transition to the original schema acquired before surgery. The HPC group is above chance at the outset (t = 3.9, P < 0.005; session 68), but neither Group nor Group × Session effects were significant for the performance index (Ps > 0.05). After six sessions of retraining, the sham group caught up, and both groups were well above chance (ts = 8.7 and 8.9, Ps < 0.001). (**E**) Performance in the probe test (PT16) indicated that both HPC and sham groups were consistently above chance in preferentially digging at the cued location (t = 4.37, df = 8, P < 0.005; t = 3.19, df = 7, P < 0.025, respectively) and did not differ from each other (t < 1, n.s.).

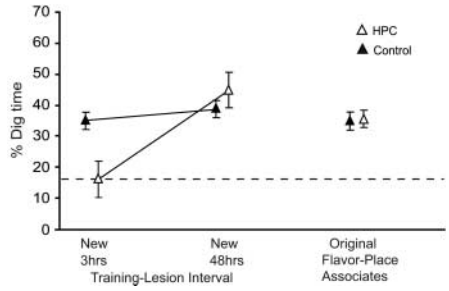

Fig. 4. Identifying the interval between training and hippocampal lesions for consolidation. A striking temporal gradient of retrograde amnesia is observed in this paradigm. HPC lesions made 3 hours after training (n = 7) on the novel flavor tested 14 days later prevented consolidation, whereas consolidation was complete when HPC lesions (n = 6) were made after 48 hours (Group × Delay F = 15.77, df = 1/13, P < 0.005). The HPC and control 48-hour groups did not differ (t < 1). The performance of the HPC 48-hour group was significantly higher than that of the HPC 3-hour group (t = 4.82, df = 11, P < 0.001), but the corresponding two control groups (ns = 9) did not differ (t < 1). The control groups were above chance at both training-lesion intervals (ts > 5.1, df = 8, P < 0.001); the HPC 3-hour group did not differ from chance (t < 1), whereas the HPC 48-hour group was above chance (t = 4.90, df = 5, P < 0.005). Separate analyses of the postsurgery memory for the original PAs learned over 14 sessions showed above-chance performance for both the HPC and sham groups (HPC t = 5.80, df = 12, P < 0.001; sham t = 9.85, df = 17, P < 0.001).

If systems consolidation within the neocortex can take place in as little as 48 hours, it becomes of interest to find out the minimal time required for it to occur. Some theoretical models suppose that a memory trace stored in the hippocampus, serving as an "index" or "pointer" to cortically encoded information, must last sufficiently long to guide the slower systems-level consolidation process that is thought to take place in sleep, requires sharp-wave activity, and has previously been shown to involve hippocampal-neocortical interactions over time (*31*–*35*). The prediction is that hippocampal lesions made 3 hours after training to animals that do not sleep during this short training-surgery interval should prevent neocortical consolidation. In experiment 3 (using a new set of 18 rats that acquired the basic schema of PAs 1 to 6 over 14 sessions as before), we compared the impact of hippocampal lesions given 3 or 48 hours after the training of two new PAs in single trials (PAs 7 and 8). This experiment used a "reverse" day-night cycle (with all testing during the animal's night) to minimize, in the case of the 3-hour interval, the likelihood of sleep episodes between the end of training and the time of the lesion. A partial within-subjects design was also used (fig. S8), with some animals having hippocampal lesions at appropriate time points soon after novel PAs 7 and 8, and others that were only anesthetized in this first phase given hippocampal or sham lesions after the later introduction of PAs 9 and 10. Cued recall was examined for the new associates shortly before surgery and was found to be effective for all animals. After surgery, cued recall for the new one-trial PAs was at chance for those animals subject to hippocampal lesions 3 hours after acquisition, but—replicating the results of experiment 2—it was effective when lesions were made 48 hours after training (Fig. 4). This is a strikingly steep upward temporal gradient of remote memory.

Causal role for schemas in learning. The final issue to consider is whether an activated schema is causally necessary for rapid memory consolidation (*5*). An alternative account of these experiments could be that the animals find it increasingly easier over the course of training to encode, store, and/or consolidate individual PAs as a result of increasing familiarity with the context of learning, with the "schema" concept being superfluous. To contrast these alternatives, we trained normal animals in two event arenas concurrently (experiment 4). In one room, they were trained on a "consistent" schema in which flavors 1 to 6 were always placed consistently at locations 1 to 6, respectively (schema 1 = PAs 1 to 6; Fig. 5B). In the other room, the animals were trained on "inconsistent" schema in which a single set of six locations (locations 11 to 16) and a set of six flavors (flavors 11 to 16) were used, but the mapping of flavors to locations was changed every two sessions (Fig. 5B). The scheduled inconsistency was therefore in the relational pairing of the items rather than the identity of the flavors or the locations of the sand wells. Moreover, a change only every two sessions did not preclude the animals attempting to learn these PAs across sessions, but would have precluded the creation of a context-specific schema. Choice performance gradually improved in the consistent schema room but not in the inconsistent room (Fig. 5A); nonrewarded probe tests also established that the animals dug preferentially in the cued location in the arena of a start-box flavor in the consistent but not the inconsistent context (Fig. 5C). This difference between the two contexts is not in itself surprising and would occur even if the animals were still trying to learn individual PAs in the inconsistent room. However, this differential rate of learning sets the stage for a last and crucial test of the schema concept.

This test involved the learning of new PAs. If animals learn PAs as isolated "facts," and if they do so ever more quickly because of context familiarity as training in this protocol proceeds, the rate of learning in the two contexts should be the same. However, if the animals bring something like "activated schema" to bear on the process of learning, a difference between the two contexts might be observed. The "consistent schema" would only be activated in its appropriate context. Procedurally, the comparison in the rate of

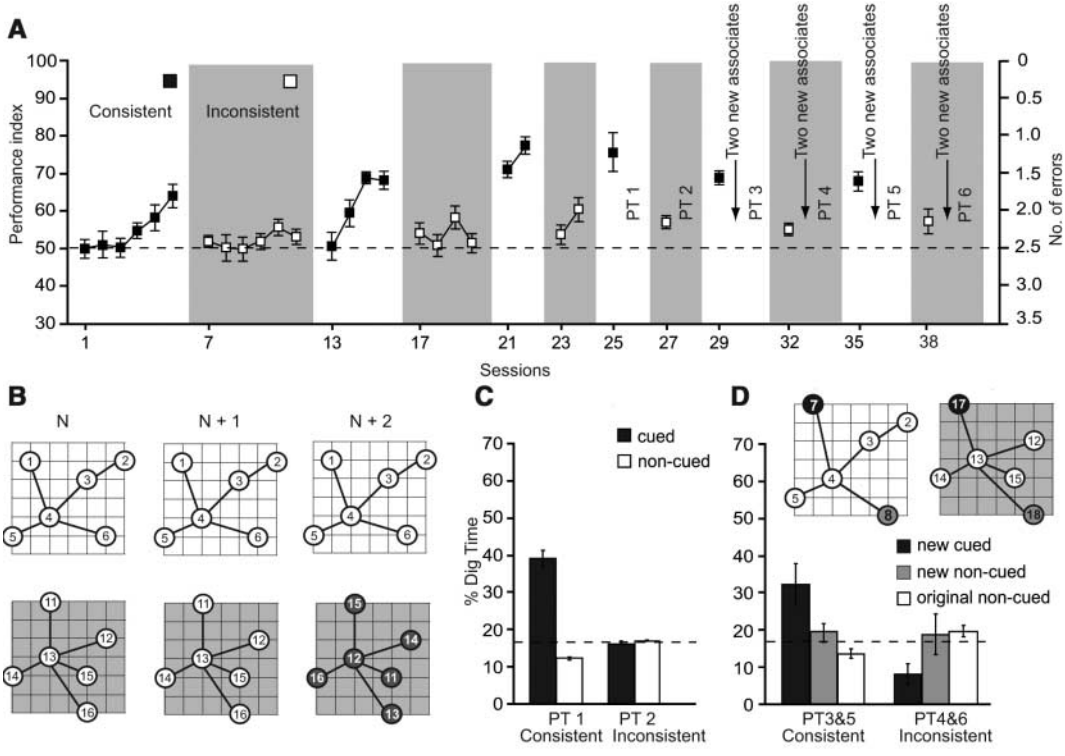

Fig. 5. A consistent activated schema promotes effective memory. (**A**) Differential acquisition of consistent and inconsistent schemata. Effective acquisition by normal rats (n = 9) occurred when mapping of flavors to places remained consistent, with six, four, two, and then single sessions (sessions 1 to 40; white background). Above-chance performance was consistent from session 15 onward ($P < 0.025$ for each comparison with chance). The same animals failed to learn a series of inconsistent schemas in the second event arena (selected days are above chance, e.g., session 27, but performance never rose above 60% correct; gray background). (**B**) With the consistent schema, the mapping of flavors to places is consistent across sessions; inconsistent schema used a common set of six flavors and locations that were associated for two sessions but then changed every third session (see N + 2, shaded gray). (**C**) Preferential digging in the probe trials at the cued locations for the consistent schema (PT1: $t = 10.9$, df = 8, $P < 0.001$) but not for the inconsistent schema (PT2: $t < 1$). (**D**) New PA probes. Performance 24 hours after exposure to the two new cue flavors and their locations when the animals would be encoding information using a consistent activated schema (PTs 3 and 5) was consistently good to the cued new location, whereas performance after use of an inconsistent schema was not (PTs 4 and 6; Group × Location $F = 13.92$, df = 1.64/26.30, $P < 0.001$). Approach latencies from the start box to the correct sand well during these probe trials were equivalent in the consistent (20.9 ± 1.9 s) and inconsistent (20.0 ± 2.5 s) contexts, indicating comparable motivation to perform each task.

learning new information had to be done in a manner that ensured an identical behavioral protocol in the consistent and inconsistent rooms. In this phase, beginning at session 29, the animals were therefore trained on four successive sequences of three training sessions beginning as follows: session 29, further consistent-context training of flavors 1 to 6; session 30, two new PAs trained in a session consisting of only two trials (PAs 7 and 8); session 31, a nonrewarded probe test for these novel associates. This three-session sequence was then repeated in the inconsistent context (sessions 32 to 34) using flavors 11 to 16, then PAs 17 and 18 followed by a nonrewarded probe test; and again in the consistent and then the inconsistent context with PAs 9 and 10 and PAs 19 and 20, respectively. The sequence ended with PT6 on session 40 (Fig. 5). The use of only two rewarded trials instead of the usual six trials per day on session 2 of this three-session sequence ensured that both the behavioral procedure and the memory-encoding demands on the animals were identical in the two training contexts on session 2. Figure 5D shows successful acquisition and 24-hour retention of these new PAs only when encoding occurred in the consistent-schema context. The apparent motivation of the animals to perform these two learning tasks was equivalent, as indexed by equivalent approach latencies to the target sand well in both the consistent and inconsistent contexts (Fig. 5D).

These findings indicate that animals—no less than people—can bring activated mental schemas to bear in a PA learning task and thereby encode, assimilate, and rapidly consolidate relevant new information after a single trial. The capacity of animals to make deductive inferences on the basis of their "mental models" of the world is, of course, far more limited than that of humans (*4*), but the principle that associative schemas can be useful in memory is not unique to humans.

In experiment 1, animals used hippocampal-dependent learning to acquire several PAs concurrently, of which one member of each pair was a spatial location in a familiar environment. This enabled the animals to treat these several associates as a connected spatial set, rather than as individual "facts," and so build up a framework in which similar new information could be stored. The construction of this "schema" took about a month—approximately the same period that several studies of retrograde amnesia have suggested is always required after learning for effective systems consolidation to occur. We observed, however, that if the several weeks of schema building was completed before new learning, the assimilation and consolidation of novel information within these neocortical schemata could be very rapid (experiments 2 and 3). We also established that the possession of an activated schema is causally important in the acquisition of new information (experiment 4). The use of rigorous control protocols (e.g., the noncued memory test, arena rotation) established that performance is mediated by PA memory rather than by cryptic uncontrolled olfactory cues. Similarly, the use of two new PAs exploring associative assimilation into a schema, rather than a single PA, ensured that the effective recall in probe trials was not an artifact of stimulus novelty.

Discussion. These findings have implications for a number of key issues in the neurobiology of learning and memory. First, they indicate that the rate at which systems consolidation occurs in the neocortex can be influenced by what is already known. In contrast, in the complementary learning systems approach (*36*, *37*), the hippocampus is said to be "specialized for rapidly memorizing specific events" (*37*) and the neocortex for "slowly learning the statistical regularities of the environment." Consolidation of memory traces in the neocortex is held to be a largely time-dependent process determined by the specific patterns of information representation, anatomical connectivity, and synaptic plasticity expression rules that it can support. Broadly speaking, this is a fair characterization of a large body of data (*27*), but it does not quite capture the potential that the neocortex has for rapid consolidation when newly acquired information is compatible with previously acquired knowledge. Given our observation that the neocortex can sometimes consolidate very rapidly, it follows that it must also be able to encode associative memory traces very rapidly—perhaps even "on-line" within sensory-perceptual systems. The widely held supposition that the neocortex is a slow learner therefore needs to be reappraised. The distinct temporal dynamics of these memory processes may contribute to the usual finding that the cortex does learn more slowly than subcortical structures—a generality that extends to conditional-associative motor learning (*38*)—but that this may not always occur.

A second finding is that the storage and recall of allocentric spatial memory can occur outside the hippocampus in the rat, even for information that has been acquired in a single trial as a consequence of hippocampal-dependent processing. This conflicts with both the cognitive-map theory and the multiple-trace theory of memory consolidation (*7*, *39*, *40*). Spatial memory has been shown previously in rats with hippocampal lesions, but the information was either acquired postoperatively and inflexibly over very extended training (*41*, *42*) or "semanticized" over many months before the lesion (*43*). The long-sought upward gradient of remote spatial memory in rats when varying intervals of time are systematically scheduled before making hippocampal lesions (*44*–*47*) is now definitively shown using a cued-recall protocol for information acquired in one trial. The temporal gradient is much steeper than might have been expected on the basis of prior work using a within-subjects design for contextual fear conditioning (*26*). Moreover, the effective remote spatial memory in hippocampal-lesioned animals upon their return to the first event arena, learned as young animals, is strikingly similar to that displayed by patient E.P. (*30*). It is unclear why effective remote spatial memory is found here but not in the water maze (*48*). One possibility is that the water maze is more "recall-like" in character (*10*), requiring an animal to generate its own reminder cues. The PA paradigm used here could allow apparent cued recall to be mediated in part by cued recognition based on proximal intramaze cues.

Third, the failure of animals with near-complete hippocampal lesions to acquire PAs over many trials of training (experiments 1 and 2) calls into question the capacity for effective "semantic-like" learning in the absence of functional hippocampal tissue. This idea emerged particularly in studies of developmental amnesia (*49*), but it has proved difficult to distinguish whether the intriguing dissociations between impaired episodic and intact semantic memory in such patients are due to intact neocortical learning of semantic information (*50*), to functional reorganization in the developing brain, or to islands of residual hippocampal function in these amnesic patients. When the medial temporal lesions are large, as in patient E.P., essentially no declarative fact learning occurs (*51*). Our findings suggest that, in animals in which it is possible to make selective 90% lesions of the hippocampus as adults, the acquisition of new flavor-place PAs is also consistently blocked and not rescued by multiple training trials. The generality of this observation beyond the spatial domain should be followed up in young animals, including primates, in order to model the situation in developmental amnesia more closely.

That the acquisition of a schema took about a month points to the possibility of it involving some kind of neuroanatomical growth process in the neocortex that creates an associative "space" in which new PAs can be rapidly stored without interference—analogous to "phase sequences" (*52*). Intercortical synaptic connections may be created or unmasked within a functional network that has only silent or baseline synaptic strengths. These could then be rapidly potentiated by relevant information when the network is in an "active" state (an activated schema). The initial growth process would necessarily take a period of days or weeks—the very time period that has hitherto been thought to mediate systems consolidation and to occur only after learning (*20*). Thus, an intriguing speculation to emerge from the present data, with conceptual similarities to the principles of synaptic tagging and capture (*53*, *54*), is that an associative space into which new information can be assimilated can be constructed before the exposure to that information. However, this construction of associative interconnections can be noncommittal or "experience-expectant" in character (*55*).

The findings bring to neuroscience a set of ideas hitherto largely discussed in the context of psychological studies of human memory. The concept of "activated schemas" has been discussed only in relation to humans (*3*), as it implies a conscious awareness that rats are unlikely

to possess. However, even if they are implicit, schemas are an economical way to characterize the gradual acquisition of an organized framework of associative "semantic-like" information from "episodic-like" events that, once acquired, allows relevant new information to be assimilated and stored rapidly. Given that animals have daily activities such as finding food and water, it is important for them to retain an organized body of knowledge about where these may be found and to be able to update such a framework rapidly, within one trial. This inferential flexibility of rodent cognition is now established in several domains (*9*).

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Supporting Online Material

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REPORTS

Nonstoichiometric Dislocation Cores in α-Alumina

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Little is known about dislocation core structures in oxides, despite their central importance in controlling electrical, optical, and mechanical properties. It has often been assumed, on the basis of charge considerations, that a nonstoichiometric core structure could not exist. We report atomic-resolution images that directly resolve the cation and anion sublattices in alumina (α-Al_2O_3). A dissociated basal edge dislocation is seen to consist of two cores; an aluminum column terminates one partial, and an oxygen column terminates the second partial. Each partial core is locally nonstoichiometric due to the excess of aluminum or oxygen at the core. The implication for mechanical properties is that the mobile high-temperature dislocation core structure consists of two closely spaced partial dislocations. For basal slip to occur, synchronized motion of the partials on adjacent planes is required.

The core structures of dislocations are critical to the electronic, optical, and mechanical properties of a wide range of materials. For most simple monometallic crystals, dislocation core termination can be determined; however, in complex crystals such as oxides, either cation or anion columns (or both) can be the terminating atomic columns even with the same dislocation character (i.e., characteristic displacement vectors called Burgers vectors, **b**). The possibility of nonstoichiometric cores also arises but has usually been rejected because it suggests the possibility of charged dislocations (*1*, *2*) and the presence of long-range Coulomb fields with a high associated electrostatic energy. This has been suggested to be the reason why the close-packed {111} crystal plane in alkali halides cannot be an easy slip system (*2*, *3*). Detailed knowledge of dislocation core structures and compositions is critical to understand dislocations in ionic crystals.

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