# BEHAVIOURAL AND NEUROANATOMICAL EVIDENCE OF EPISODIC MEMORY IN THE RAT


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## Abstract

In an attempt to test the hypothesis that rat's can perform very similar behaviours to episodic memory in humans, we here develop a novel Pavlovian conditioning procedure demonstrating integrated what-where-when representations. Rats explored two distinctive contexts, one in the morning and the other in the evening. Subsequently, either in the morning or the evening, they received a foot shock immediately upon entry into a third context that equally resembled the two explored contexts. When conditioned freezing was measured at an intermediate time of day, rats showed significantly more fear of the context congruent with the time of day of the foot shock. Thus, rats automatically form an integrated time-place memory that can be flexibly updated by future events, essential characteristics of episodic memory. Furthermore, it is shown that these memories rely upon some of the same neuroanatomical structures, including the medial prefrontal cortex and the hippocampus, as are specifically required for episodic memory in humans.

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# CHAPTER 1 - INTRODUCTION

The purpose of this thesis is to experimentally examine the idea that nonhuman animals, (specifically rats) engage in cognitive processes that closely resemble episodic memory in humans. Included in this will be an examination of whether these processes rely upon the same fundamental neural circuits in humans and in our rat model of episodic memory. This section will define some of the common terms used in the episodic memory field, then set forth the idea that episodic memory exists in nonhuman animals, particularly the rat, as a cognitive process physiologically distinct from other putative memory systems. Contrary to many skeptical claims in the literature, it is the position of this thesis that episodic memory exists in the rat and is very closely related both functionally and neuroanatomically to the process given the same name in humans.

Learning is defined as the process by which an experience results in a relatively permanent change in behaviour, while memory is the process by which information gained in that experience is retained and later recalled. Memory is not a unitary process.. It is generally separated into two distinct categories – *declarative* and *non-declarative* (Squire 1992; Squire and Cohen 1980). Non-declarative

(or implicit) memory includes procedural memories and priming, as well as some forms of learning procedures such has habituation, sensitization, and simple forms of Pavlovian and operant conditioning. By definition it involves learning that cannot be communicated to others. Declarative memory includes memories for facts and events, and involves two branches – *semantic memory*, that is comprised of general facts about the world, independent of the context in which they were learned, and *episodic memory*, that refers to a representation of experiences including rich contextual components, such that upon retrieval one has a conscious experience of relevant components of the original experience. These categories are distinct not just in terms of function, but also in the cerebral networks they rely upon to perform their operations. In humans it is for the most part accepted that episodic and semantic memories refer to separate processes relying upon distinct circuitry for both storage and retrieval (Aggleton and Brown, 1999).

The term 'episodic memory' was originally coined by Canadian cognitive psychologist Endel Tulving (1972). He originally defined it as another branch of declarative memory, but one that referred not just to knowledge, but also to a recollection of the context and time in which that knowledge was acquired. Tulving (2001) later re-defined the term to include a component of *autonoesis,* meaning that episodic

memory involved not just storage and retrieval of information, but that the retrieval was also was accompanied by an essential phenomenological experience of transporting one's conscious self mentally backwards in time, having an experience in mental space very similar to the original one which actually took place.

There were some sound reasons for this. Awareness outside the present is not a trivial thing by any means, it is central to our conceptions of reality. In fact the majority of human communication is directed via language tense either to the past or the future (Szagun, 1978 as cited in Suddendorf and Busby 2003). An important issue is that it is currently impossible to empirically demonstrate any form of consciousness in non-linguistic species, never mind a special "time traveling consciousness". The present paper then will focus first on other more recent formulations of episodic memory and their essential criteria for establishing the presence of episodic memory. Importantly these are criteria that can in principle be satisfied experimentally. Later, the issue of autonoetic consciousness, and what its existence in humans means for research attempting to show episodic memory in animal models, will be returned to and addressed from a theoretical perspective.

Tulving's claim is that only humans could possibly have episodic memories, because only humans have a sense of self or "autonoetic

awareness" with which they could perform this mental time travel. As the very term "episodic memory" was defined to include this criterion, researchers on animal models coined the term "episodic-like" to refer to memories that meet all the criteria except autonoetic awareness. Tulving proposes that the essential circuitry underlying autonoetic awareness includes specific regions of frontal cortex (Wheeler, Stuss and Tulving 1997). In contrast to his view of episodic memory and its definition based on the phenomenological aspects of subjective experiences, more recent approaches to episodic memory in nonhuman animals identify the types of information that are specifically involved. This paper will not use the term 'episodic-like' except when repeating other author's descriptions of their studies. When we read about any cognitive function of an animal it is understood that the processes are not going to be identical to those in humans.

A group of experiments reviewed in Chapter 2, and lauded as the original and still the best demonstration of episodic memory processes in animals, are the now classic scrub jay studies of Clayton, Dickinson and colleagues. In 1998 they were able to show all but the autonoetic criterion for episodic memory in a non-human species. They adopted the lesser mantle of 'episodic-like memory' to describe these behaviours in recognition of this purported deficiency. Even if scrub

jays do exhibit episodic memory in the strict sense however, they are far from the ideal experimental model from the perspective of modeling human disease that affects episodic memory systems. For one, aves and primates made their evolutionary split more than two hundred million years ago. Without convincing evidence of episodic memory in a more closely related species, one from the same class at least, it is possible that bird episodic memory is an example of convergent evolution rather than having roots in the same biology as humans. A potential consequence is that the same neuroanatomical systems may not be involved. For this and other reasons it is important to establish if another mammal, and for the practical considerations of neuroscience modeling, especially a rat or mouse, can meet the information-based criteria for episodic memory. There is no perfect model, but the rat offers many clear homologies in neural structure and functions and has been the focus of the majority of behavioural neuroscientific experimentation for many decades (Whishaw 2006).

The advantages of such a model are clear and numerous. Not only does would it challenge longstanding theory, but there are numerous types of neurological insults to which episodic memory seems particularly susceptible over and above other declarative memory processes. A model in animals then will provide a means of

detecting these subtle insults and observing the effects of manipulations which may affect changes in both humans and rats.

There are three main conditions for demonstrating the existence of episodic memory in nonhuman animals, and all will be addressed in this paper. First, animals must be able to represent aspects of an experience which include spatial and temporal dimensions bound into an *integrated representation*. In other words, what happens must be represented along with when and where. This must be explicitly demonstrated as a behavioural alteration in response to a learning episode (Clayton et al., 2003). Second, if we are to believe that this is a very similar episodic memory system to that present in humans, it should also be true that the brain regions underlying this behaviour in humans should also be implicated in nonhuman animals. Third, the historical condition which states that episodic memory recall must involve a conscious re-experiencing of the original event, must be acknowledged (Tulving, 2002). As this cannot be tested empirically, strong arguments must be presented as to why it is not a valid mandatory criterion.

In the next portion of this manuscript (Chapter 2) an experiment will be described that, on the most straightforward interpretation, indicates that rats can satisfy accepted criteria for episodic memory.

Accepted components of episodic memory will be reviewed and whether rats demonstrate these components will be evaluated.

In Chapter 3 evidence will be provided that the episodic abilities demonstrated in the first section do indeed rely upon very similar neural structures used by humans to perform the same functions. If episodic memory processes in a nonhuman animal are a close evolutionary relative to the version of the trait observed in humans, it is likely to rely upon the same conserved brain system that originally facilitated the types of computations necessary. Although there are already a number of models purported to be demonstrations of episodic memory in the rat (reviewed in chapter 2), and there is also a large body of literature pertaining to the brain areas used in humans to perform episodic memory in humans (reviewed in chapter 3), no research has yet combined these two avenues of research. Thus a purpose of the present thesis is to test for the contribution of the cortical areas used by humans, and also present in animals, to performance of episodic memory. The second function of this paper will be to conduct said experiments, testing the assertions of Tulving (2002) and Suddendorf and Corbalis (1997) that episodic memory represents an evolutionary discontinuity akin to human language. The third problem is not one that can be resolved empirically with our current technology, nor that available in the foreseeable future, and so

will primarily be addressed theoretically in the closing discussion. As per Tulving's definition of the term 'episodic memory', an awareness of self is essential. He argues episodic memory is not just the ability to recall personal experiences, it is necessary that the experience of recall involves a cognitive reenactment of the learning episode, with the person remembered as the actor, but aware that he is simply acting and not actually physically transporting himself back in time. He/she must not only be able to have a representation of himself, but a meta-representation as well. Thus, you must be able to remember yourself - your psyche. As things stand, it is impossible to prove mammals (at least lower order ones) have a concept of self; it is thus impossible to prove animals have episodic memory if this criterion cannot be rejected on other grounds. This is more a philosophical point, and will be dealt with as such.

The position of this paper is that episodic memory in animals *is* a close relative of episodic memory in humans, performing the same functions through use of the similar neural circuitry to solve similar sorts of problems. It comprises a distinct, though somewhat overlapping system from regular semantic memory, and can be defined and evaluated based on the types of information it represents and the circuits involved in doing so, without recourse to additional phenomenological or first-person experiential criteria.

## CHAPTER 2 – BEHAVIOURAL EVIDENCE FOR EPISODIC MEMORY IN THE RAT

Several commentators have suggested that the key properties of episodic memories are that they represent when an episode occurred as well as what the event relationships were and where the episode took place. Furthermore, the reactivation of an episodic memory representation can occur outside of the relevant context. Clayton et al. (2003) suggest three related criteria for establishing episodic memory competence in nonlinguistic species: what, where, when content; an integrated representation of these three elements of content; and, flexibility in updating memory representations in light of new information gathered after the original episode. It has since been argued that flexible deployment of learned information is a characteristic of all declarative memory, and hence need not be explicitly demonstrated in an episodic task that obviously cannot be solved by implicit processes (Dere et al., 2006). Replacing this criterion are several other requirements put forth by various researchers in the field, including that: the test be novel and unexpected; the test be performed at a time point that exceeds the capacity of short term memory storage (Dere et al., 2006); that the task cannot be solved by familiarity judgments or comparison of

memory trace strength (Gallistel, 1990); and that the memory be ideally formed in a single, unique, one-trial learning episode to preclude the possibility of performance being mediated by a strategy of semantic rule learning (Morris 2001).

Many believe that deficits in an episodic memory system are at the core of human medial temporal lobe amnesia, most memory disorders, and certain dementias. Certainly episodic representational processes appear to be highly susceptible to brain trauma and neurodegenerative disorders, over and above the loss seen in procedural memory (Scoville and Milner, 1957), priming (Schacter 1987) or semantic knowledge (Vargha-Khadeem et al. 1997; Janowsky Shimura and Squire 1989) from a variety of insults. A model of episodic memory in animals that can be subjected to empirical manipulations then would provide an extra level of sensitivity, so that cognitive decline in models of neurodegeneration could be detected earlier, and effects of minor physical trauma currently below our detection threshold could be discerned. Thus, understanding the behavioural processes involved in episodic memory and its possible basis in rats is of considerable general importance.

There is little doubt that humans have such memories, but do nonhuman animals? It would be justified to conclude they are absent in rats after numerous strong attempts to demonstrate them have

failed. Recently there has emerged a number of elegant demonstrations that rats can flexibly update integrated representations of what and where events occur (see for example Morris, 2006), or what, where and serial order or recency (Kart-Teke et al., 2006; Dere, Huston and De Souza Silva, 2005)), but an integrated representation that includes a "time-stamp" or explicit temporal context is not established.

Clearly the trick in such a demonstration is to show that a rat has an integrated representation of what, where and when, and that it can be adaptively updated in light of new, relevant events. We demonstrate here that rats spontaneously form such representations in the course of visiting new environments.

One way to arrange such a demonstration has been devised for the Western scrub jay (Clayton, et al., 2001). These investigators have taken advantage of the fact that these jays naturally cache both perishable and non-perishable food. It is well established that they remember where their caches are located, and because they find some foods more palatable than others, their cache site preferences reveal that they remember what was cached. By varying when the more palatable, but perishable, food (wax worms) were cached in relation to the opportunities to retrieve cached foods, one can use the cache site retrieval preferences to learn if the jays remember when they cached

the perishable, but more palatable, foods. If allowed to recover food 4 hours post-cache, jays will preferentially recover the worms. However, if the delay is extended to 5 days, long after the worms would have degraded, jays will instead retrieve non-perishable peanuts which were cached at the same time as the worms. All of the aforementioned criteria are met, and the "when" component or temporal context is shown not to be due to relative recency, serial order, forgetting, or strength of the memory. It is due to the animals being able to recall the experience of food caching. This represents a clear example of episodic memory in a bird species, though the authors give it the lesser mantle of 'episodic-like', as they do not meet Tulving's defining criterion of autonoetic awareness (De Kort, et al., 2005; Griffiths & Clayton, 2001).

To address this issue, the authors later show that jays are sensitive to espionage by other member's of their species. A jay that detects that it is being observed during food caching will later rebury its food in another location (Clayton et al., 2003). One interpretation is that the jay is acting purposely to thwart thievery and is sensitive to the future need it will experience. A more recent study shows that jays can even possess prospective planning. In a very clever experiment (which could possibly be adapted to rats as well), jays were housed in a cage with three adjacent compartments between which they could

move freely. Each morning for 6 days a bird would be confined to a single compartment for 2 hours, one that never contained food, and a second that provided access to powdered nuts, which could be consumed but not cached. On day 7 the powdered nuts were replaced with whole nuts, and it was found that jays would cache 3 times as many nuts in the food deprivation compartment as in the powder compartment, in anticipation of the next morning's predicted separation from food (Correia, Dickinson & Clayton, 2007; Raby et al., 2007). This demonstration provides a strong challenge to the longstanding Bischof-Köhler hypothesis, which states that nonhuman animals cannot take actions to serve future motivational states, only present ones (Suddendorf and Corbalis, 1997).

However, birds are evolutionarily distant from mammals including humans, and it could be argued that these episodic abilities are an example of convergent evolution specific to birds, relying on radically different computations and neural substrates. It would not be prudent to use bird episodic memory as a model for the human variant, while a more closely related model species would clearly be helpful, and thus we look to the rat, the most well investigated mammalian model for behavioural processes.

It is already established that the rat's event representations can contain information about where events occur and, based upon the

reinforcer devaluation experiments, it is clear that they remember what outcomes are expected there (see Day et al., 2003 for a very nice demonstration of this in a novel learning procedure involving where-what paired associates). What remains to be demonstrated in the rat is that the same event representation can also contain information about when the event occurred. The "when" component should not be related to simple recency, familiarity, memory strength, serial order, but rather should be analogous to a "time-stamp" as in Gallistel's (1990) notion of a temporal context being provided by endogenous circadian oscillators. There has also been a suggestion the timestamp may be represented by temporal associations between event occurrence and the age of recently born granule cells in the dentate gyrus of the hippocampus (Aimone, Wiles and Gage, 2006). It is the temporal component which defines an episodic memory as relating to a unique experience. While a given context or stimulus may be encountered any number of times in an animal's life, a moment that has passed never occurs again. The sense of subjective time is defined by the temporal order of representations of life experiences, though Eacott and Norman (2002) suggest 'occasion specifier' rather than actual timeline.

Crystal and Babb (2002) for instance used a modified version of the eight-armed radial maze to conduct an approximation of the

original scrub jay experiments using rats. Rats were trained to learn a foraging paradigm where one arm baited with chocolate flavoured pellets was replenished after a long delay (4 h), but not a short one (30 min). The rat then altered its behaviour by visiting the chocolate arm preferentially after the long, but not short delay. They could also learn to avoid the arm by pairing the chocolate with lithium chloride (Babb and Crystal 2005). This experiment confirms that rats can use combined spatial and temporal duration to solve discriminations, though it requires extensive training and thus cannot be considered to represent a one-trial learning episode. The task may possibly be solved by a semantic rule learning strategy (Hampton and Schwartz 2004).

Another recent demonstration of integrated what-where-when representations are novel object exploration experiments in mice (Dere, Houston & De Souza Silva 2005) and later in rats by Kart-Teke and colleagues (2006). The amount of time that a rat spends investigating one object over another is generally accepted to be inversely related to the strength or extent of memory for the object, all other things considered equal (Ennaceur & Delacour, 1988). Taking advantage of the fact that, given two very familiar objects, a Wistar rat will preferentially investigate the one it was least recently exposed to (showing rats can make serial order/recency judgements (Mitchell

and Laiacona, 1998, Hanneson et al., 2004), and that rats will also show a preference for an object in a new location relative to an equally familiar but consistently positioned object (Ennaceur et al., 1997). They could remember objects, object locations, and presentation order at various time points. Memory about items, context and temporal sequence are thus demonstrated. A limitation of this design however is that animals could undoubtedly solve this discrimination using recency judgments – for instance by comparing strengths of memory traces, rather then the relative position of presentations along an autobiographical timeline, particularly when the time difference was limited to 50 minutes (Yonelinas 2002). While the Kart-teke experiment avoids the pitfalls of extensive training paradigms and reinforcement, object recognition paradigms such as this are also somewhat questionable as a model for episodic memories, as the maximum delay interval between exposure and testing at which a rat can still make temporal order judgments is only 3 hours (King et al., 2004). Again, any task requiring an extensive training regimen is likely to be solved using semantic rule learning rather than event recollection. Effects were also eliminated by a pre-trial saline injection, attesting to a minimal strength of association.

A third and final recent experiment of note in rats was by Ergorul and Eichenbaum (2004). While they clearly show in their 2004

experiment that rats can infer temporal order of odour presentations by using spatial cues and remembering odours, the extensive training paradigm used to teach rats about the scents means this experiment too falls short on the stricter qualification as an episodic memory.

Other species where episodic-like memory has been demonstrated include honeybees (Menzel et al., 2006), hummingbirds (Henderson et al., 2006), pigeons (Zentall et al., 2001) gorillas (Schwartz et al., 2005) and dolphins (Mercado et al., 1998). There are actually numerous strong examples of episodic memory in primates (reviewed in Scwartz & Evans, 2001), though if this manuscript can achieve its goal of demonstrating episodic memory is also present in rodents, such processes in primates should be of no surprise.

We have developed a simple way to examine aspects of episodic memory in the rat which addresses all relevant challenges by using a variant of the contextual fear conditioning procedure. It is known that robust fear of a previously explored context is acquired as a result of a single learning episode involving foot shock and that rats can acquire fear of an explored context if foot shock is paired with retrieval of the memory for that context (Rudy & O'Reilly, 2001). Fanselow (1990) showed that if rats receive a foot shock immediately upon entry into a novel context they do not acquire a conditioned fear response to that context. However, if the day before the immediate shock episode they

explore the context for a few minutes then they *do* acquire robust conditioned fear of the context. Rudy et al. (2002) showed further that the fear becomes associated only with the mnemonic representation of the pre-exposed context, even if that context is not the same as the one the immediate shock actually occurs in. This implies that it is not actually the context which is being associated with the shock event, but rather a *mnemonic representation* of the context, likely retrieved by cues associated with transport of the animals.

The theory behind contextual fear conditioning is that a "conjunctive representation" of a context must be formed, and that it is to this representation that the aversive event (the shock) is associated. By 'conjunctive representation' we mean a single, unified representation of all the elements which compose the context (odour, luminance, texture, area, temperature, sound, etc.) (Fanselow and Rudy 1998; Young, Bonheneck and Fanselow, 1994). It is also our assertion that, in certain events, and possibly in all, time of occurrence/position along an autobiographical timeline is also one of the elements automatically encoded and bound to the other features to aid in pattern separation. Fanselow's "immediate shock effect" provides a particularly powerful means of investigating this 'context pre-exposure facilitation effect'.

The procedure is basically as follows: A naïve rat is placed in a

context in which it has never experienced, *immediately* (within seconds) upon entrance the rats receives a footshock, and then is quickly removed. If the rat is subsequently returned to the context at a later time, it will *not* demonstrate any fear memory of the context. However, if the same procedure is conducted except that it is preceded by a period of exposure to a context, followed by a delay, and then immediate shock, the animal will demonstrate robust freezing on re-exposure. Furthermore, the rat can even be immediately shocked in an entirely different context than the pre-exposed one (though one to which it is similarly transported), and it will display robust fear specifically to the exposed context and *not* to the one in which it actually experienced the aversive stimulus (Rudy and O'Reilly 2001). The idea then is that, at least in the rat, formation of the conjunctive representation requires a short, but non-negligible, period of time in which to instantiate itself into a neural trace. Without the time necessary to form this bound representation the subject has an incomplete pattern, consisting primarily of salient transport cues with a period of presence in an ambiguous context. (Rudy, Barrientos and O'Reilly). An important point to emphasize then is that it is a representation or memory of a context, likely retrieved by pattern completion of a subset of cues involved in transport (Marr, 1971; McNaughton and Morris, 1987), to which the shock is associated, **not**

the actual physical features of the shock environment. (O'Reilly and Rudy, 2001; Rudy and O'Reilly, 2001).

Rudy and O'Reilly were also able to demonstrate that the few seconds pre-shock on the conditioning day given to allow detection of enough elemental cues to support pattern completion could in fact be eliminated if specific transport cues are provided (2001). Using neurotoxic lesions of the dorsal hippocampus, Rudy Barrientos and O'Reilly demonstrated that this context pre-exposure facilitation effect is indeed dependant upon the hippocampus.

We took advantage of this "Fanselow effect" to show that contextual fear conditioning meets the criteria for episodic memory in rats in the following way. We allowed each rat to explore two distinct contexts at two different times of day (morning and night) for a total of three days. The rats subsequently received an immediate foot shock upon entry into a third, 'chimeric', context constructed out of equal elements of the morning and night contexts. Each rat received only a single shock in the chimeric box, at *either* the morning or the night. All rats were then tested for learned fear at a neutral time of day, either in the context that is congruent with the time of day that the shock occurred or in the context that is incongruent (see also detailed methods below).

## METHODS

Subjects:

Male Long-Evans rats (Charles River, Quebec; 300-350 g) were housed individually in standard laboratory cages, kept on a 12:12 light-dark cycle (lights on at 07:00) and provided with food and water *ad libitum*. Rats were acclimated to vivarium conditions for at least one week prior to beginning of behavioural testing.

Apparatus:

Conditioning boxes were Plexiglas modular test chambers with steel grid floor (ENV-008 MedAssociates, Inc., Georgia, VT). Box A had black coloured walls and was scented with the cleaning agent Quatzyl-D-Plus. Box B was white and scented with Clinicide brand disinfectant. A third context, C, was designed so as to be chimeric, containing equal elements of boxes A and B. It was half black, half white, and contained no added odour. All boxes were in the same spatial location, and the tops were left clear to allow for video recording via a ceiling mounted camera. Contextual components were counter-balanced.

Procedure:

Figure 1.1 presents the three phases of experiment 1. In Phase 1, 22 rats repeatedly explored two distinctive boxes (A or B). Box A was visited on three successive mornings and Box B on three successive evenings for 9 minutes each on Day 1, 7 minutes on Day 2 and 5 minutes on Day 3, for a total exposure duration of 21 minutes per context. In Phase 2, all rats received a single immediate shock (1.0 mA for 2 s, commencing within 2 seconds of box entry and followed by 3 seconds of exposure for a total phase duration of 7 seconds) in a 'chimeric' box composed of equal elements of Box A and B – it was half black, half white and was unscented. Exposures to all boxes occurred in the same spatial location. For half of the rats the shock occurred in the morning, for the other half the shock occurred in the evening. Exposure boxes and shock times were counter-balanced such that neither environment was better represented at the time of the shock across groups. In Phase 3, freezing was measured at a time midway between the morning and evening sessions in a box that was congruent with the time of the immediate shock for half of the rats, or incongruent for the other half.

Experiment 2, as shown in figure 1.2, involved the same procedure describe for experiment 1, except with all phases occurring at mid-day. In phase 1, 12 rats were consecutively exposed to boxes A and B. To establish a baseline assessment level for freezing, an

additional 4 animals was run without receiving a footshock during the chimeric exposure in phase 2. The procedure in experiment 3 was also identical to that for experiment 1, with the exception that one group of 6 rats was tested for freezing in Box C (figure 1.3) and compared to 12 treated as in experiment 1.

**EXPERIMENT 1: Episodic Memory Model**

If rats acquired an integrated representation of time and place at the time of exploration that is retrieved by cues related to the time of occurrence of foot shock, then fear of the congruent context should selectively emerge during fear testing. If the rat "automatically" changes its behaviour in the presence of the context with the same time-stamp as the shock, without any further direct experience with the context, we will have demonstrated that rats have an integrated representation of when, where and what that can be flexibly accessed and adaptively altered..

Results and discussion:

A repeated measure ANOVA was conducted on the percent of time spent freezing on the test day (Figure 2.1). Our observed power to detect a difference between congruent and incongruent groups was 0.7. Rats showed robust freezing on the test day that declined reliably

over the first three minutes of re-exposure to the contexts ($F(2,40) = 8.0$, $p < .001$). The rats placed in the context that they had explored at the same time of day as the subsequent foot shock in the ambiguous context showed more freezing at each minute of testing compared to rats placed in the incongruent context ($F(1,20) = 45.0$, $p < .001$). The freezing shown by the rats in the incongruent context, especially during the first minute, likely reflects generalization. It is thus confirmed that rats can acquire the types of representations defined by previous investigators as constituting episodic memory processes (Clayton et al., 2003; Dere et al., 2006). Furthermore, that the learning episode consists of a single trial eliminates the possibility that performance of the task is mediated by rule learning or other semantic information, as would be suspect in tasks requiring an extensive training paradigm.

**EXPERIMENT 2: Test for Requirement of Temporal Cues**

It was predicted that without any temporal cues available to distinguish between the pre-exposed contexts animals would not show differential freezing between contexts during testing. The temporal cue should be necessary for retrieval of representations for the association of fear.

Results and Discussion:

Repeated measures ANOVA on freezing revealed that there is a significant difference in freezing between groups ($F(2,13) = 4.7$, $p = .03$). There also a significant difference across the three minutes of testing ($F(2,13) = 18.1$, $p < .001$). *Post hoc* analysis by Tukey HSD detected no difference between conditioning to context A or B ($p = .940$). A borderline significant increase in freezing in the white box versus the unshocked rats was seen ($p < .06$), with a more robust difference observed between the black context tested animals and the unshocked rats ($p < .05$). This supports our interpretation that the cue used to discriminate between the two contexts was indeed temporal in nature, and that contextual components were not being used to differentially associate fear to the two boxes.

**EXPERIMENT 3: Test for Conditioning to Shock Context**

This control experiment was conducted to confirm Rudy and O'Reilly's (2001) postulation that it is the conjunctive representation of the pre-exposed context that is retrieved during the immediate shock phase, and that it is this representation to which the fear becomes associated.

Results and discussion:

Repeated measure ANOVA again confirmed a significant effect of freezing between groups ($F(2, 13) = 7.11$, $p < .008$). *Post hoc* analyses using Tukey HSD showed significantly less freezing in the chimeric condition as compared to the congruent ($p < 0.05$), with no differences between the chimeric and the incongruent groups ($p = 0.5$). That more fear is shown to the congruent context than the one where shock actually occurred also addresses the possible concern that rats in Experiment 1 simply conditioned to a box that somehow shared the most elements to the conditioning context.

## GENERAL DISCUSSION

Like all classical conditioning paradigms, this task shows that a rat can learn information that can then be used to make predictions about future life events (Gallistel 1990), addressing to some degree the flexible deployment criterion set forth by Clayton, Bussey, Dickinson and De Kort (2003).

Suddendorf and Busby (2003) also require for true episodic memory that "(t)he memory should be shown to use a generative, reconstructive process at retrieval," even claiming that "accuracy is not imperative". While they may be correct in their further claim that the scrub-jay experiments do not meet this criterion, our experiments

very clearly depend on event reconstruction by way of the fact that conditioning is in some respects to a false memory. The only way this could possibly be occurring is if the memory truly was a reconstructive process as opposed to retrieval of an unalterable snapshot. As to their requirement of meta-representation and that episodic memory can be used to plan for the future, to some degree this is inherent in the fact that freezing, a response to an anticipated aversive event, will be expressed only if an aversive event would be expected in the given context. The "what, where and when" content criteria are clearly demonstrated, and structure is seen in the fact that the memory is a reconstructed integrated representation.

The actual Pavlovian conditioning trial was under 10 seconds in duration, with a single aversive stimulus to which emotional associations would be formed. Though pre-exposure sessions were necessary for the animal to learn about the contexts in which the event occurred (or in which they thought it occurred), it is clear that these exposures do not qualify as extensive training. The integration of what, where, and when was clearly achieved. The *only* way the rat could possibly have differentially attributed fear to the pre-exposure contexts is if it had a memory for the time of day at which it had been shocked (when) and that that cue served to pattern complete a unified memory which included the conjunctive context representation

(where) that was activated at the moment of shock (what). Experiment 2 precludes the possibility that somehow the test context was more similar to one pre-exposure context than the other – time is the critical factor. We focused on the temporal component as that is the most unique factor making up any episodic experience (the same time can only occur once) (in the case of our experiments however it is unclear whether simply time of day is being conditioned).

One conditioning session involving a single foot shock immediately upon entry into a context can lead to significant conditioned fear responses in similar, explored contexts. In preliminary work (data not shown) we found that rats receiving the same immediate shock episode but without prior context exploration did not show a learned fear response, confirming the reliability of the Fanselow effect observed by others (Fanselow, 1990; Rudy et al., 2002). Significantly for our purposes, the rats in the present study discriminated between the two test contexts, in line with the episodic memory hypothesis. That is, they displayed significantly more conditioned freezing in the context that they had explored at the same time of day as the immediate foot shock session. It is important to emphasize that the non-temporal cues present at the time of the immediate shock were composed of equal elements of the two test contexts, thus the temporal cue (morning or evening) must have

provided a basis for discrimination at the time of learning. Furthermore the next day, at the time of testing, the temporal cues were completely ambiguous, thus only the nontemporal elements of the context could serve as a basis for discrimination.

The fact that the rats showed clear learned fear of the context congruent with the time of day of the shock supports the following notions. During exploration rats acquire a memory that contains an integrated representation of the elements of the context, including the time of day. The time of day cue during the immediate foot shock session selectively retrieves the memory of the congruent context. This memory is updated by the association with foot shock, such that when the rats are exposed the next day to the elements of the context at a neutral time of day, they respond defensively to the congruent context. Because of the design of this experiment we know that rats are not using differential familiarity, recency, memory strength, or serial order of the contexts or interval timing to solve this problem. Instead, a parsimonious account is that rats have an integrated representation of place and temporal context that can be accessed via temporal or non-temporal cues, leading to adaptive future behavior.

There is some uncertainty about the nature of the temporal cues due to a lack of research, but one possibility is that they are based upon information from endogenous circadian oscillators as suggested

by Gallistel (1990). Howvever, it can not be the case that circadian rhythms are the sole temporal cue as this would allow for only a 24 hour cycle of uniqueness. Another suggestion is that the time-stamp may be represented by temporal associations between event occurrence and the age of recently born granule cells in the dentate gyrus of the hippocampus (Aimone et al., 2006). Whatever the nature of the 'time-stamp" contributed by the mPFC, it is likely an integrated association of various cues which together form a distinct representation of a unique moment in subjective time, just as the hippocampus integrates various spatial elements into a single context representation. It is important to note that while the midday time-point may not be exactly equally different from morning and night tests in terms of contextual elements, there was no difference between animals within congruency groups, regardless of the time of day at which they were shocked. Thus, the temporal cue outweighs slight contextual discrepancies in determining which context becomes associated with the shock episode. This is supported by the control experiment in which the temporal cue was removed by conducting all pre-exposures and the immediate shock at mid-day. This resulted in rats being unable to differentially attribute fear to the test contexts.

The task described here meets the criteria for episodic memory in rats. It combines the advantages of previous investigations while

addressing some of their shortcomings. We conclude from this that rats have a sophisticated representational process in which temporal cues are integrated with "where" and "what" information to form distinct autobiographical memories of past experiences. With the exception of Tulving's (1983) criterion of 'autonoetic awareness', a phenomenological quality that is currently un-testable in non-linguistic species, this task meets the qualifications required for a demonstration of episodic memory in the rat.

# CHAPTER 3 – NEUROANATOMICAL EVIDENCE FOR EPISODIC MEMORY IN THE RAT

Chapter 2 presents evidence that rats have a memory system that resembles aspects of the human episodic memory system. In this chapter we seek to extend the resemblance from purely behavioural criteria to the level of neural structures that underlie this system in rats and humans. Establishing strong similarities at the behavioural as well as neuroanatomical levels would increase our confidence that manipulations of the rat model will have validity in respect to human conditions. The neural circuits underlying episodic memory in humans have been investigated in behavioural studies of brain damaged individuals, as well as functional neuroimaging studies involving PET and fMRI. The findings will be reviewed here with specific attention to fundamental features that can be tested in the rat model of episodic memory presented in the previous chapter. If it holds that the same brain regions that are important in human episodic memory make comparable contributions to performance of our episodic task, this will provide an additional source of support that the model may be useful in modeling human memory disorder and treatments. There are also a number of unresolved issues about the nature of differences between semantic and episodic memory which could benefit from study of an

animal model. To our knowledge, this is the first empirical attempt to bridge the gap between the robust findings on neurophysiology of episodic memory in humans and a potential model in a non-primate species.

The first brain region frequently associated with declarative memory or contextual representation is the hippocampus. This large piece of archicortex has been investigated so extensively with respect to these functions that it has become nearly synonymous with them. It is also frequently stated that the hippocampus and adjacent cortices are critically involved in memory for events in people's lives, or episodic memory (Eichenbaum and Cohen, 2001). The hippocampus, in concert with adjacent parahippocampal cortices, is generally understood to be an information binder, responsible for taking multimodal perceptual representations of any one experience, and then integrating and storing them as conjunctive representations or propositions (Sutherland and Rudy, 1989; Anagnosteras et al., 1999, Cohen and Squire, 1980). Additional important regions associated with explicit memory operations are found scattered throughout the neocortex, with the prefrontal cortical areas, or PFC, being of particular relevance to episodic memory. Numerous theories have been proposed to explain how the various physiological and functional

components of the explicit memory system operate, though only three will be addressed in this paper.

**Theories of Explicit Memory:**

The first and probably most well known theory is referred to simply as "Declarative Memory Theory", and was initially proposed by Cohen and Squire in 1980. In its most simple form, this theory states that declarative memory formation and storage initially requires the hippocampus, in concert with other medial temporal lobe structures, in order to bind together all the many elements of a learning experience into a retrievable representation. Over time however, associations formed within neocortical areas are sufficient to mediate recall, via a process of consolidation directed by the hippocampus (Squire & Alvarez, 1995; Squire et al, 2004). The nature and necessity of this consolidative process is still somewhat unclear, though McNaughton's group probably provides the most plausible explanation, supported by computational models, in that the hippocampus is required to play a short term role in binding and maintaining various neocortical representations that are initially highly plastic and thus subject to decay and interference (McNaughton et al., 2003). The neocortex may acquire some simple, elemental memory representations through perceptual processing, but actual facts and events are initially

recorded solely within the medial temporal lobe (Squire and Zola-Morgan 1991). Squire's theory does not recognize an anatomical distinction between semantic and episodic memory, claiming any differences are purely categorical, that is simply different components of a unitary function. In terms of processing then, both are simply propositional representations dealt with by the medial temporal lobe in the same manner. Squire does however concede that some propositional information necessary for source memory may be stored in the frontal cortex, as indicated by studies of amnesic patients (Shimamura and Squire, 1987).

"Configural" or "conjunctive" theory, proposed in its original form by Sutherland and Rudy in 1989, stated that the hippocampus plays a unique and essential role in combining incoming multimodal perceptual elements into unitary conjunctive representations. In light of new experimental evidence the theory was later modified (Sutherland and Rudy 1995) to allow for a role for the neocortex in forming conjunctive representations with storage and binding during retrieval being directed by the hippocampus. In contrast to Squire's theory, configural theory holds that *two* types of memory representations are set for any given experience – one which is rapidly established and stored in the hippocampus automatically and indiscriminately, and a second which is more slowly and purposefully set in various regions of the neocortex.

The idea is that the hippocampal representation influences and enhances the neocortical representations by rapidly separating out similar memory patterns so as to prevent interference between and increase the efficiency of associations between traces (Sutherland and Rudy, 1995). The function of the hippocampus then is to separate out which of the elemental representations set in the neocortex need to be bound together during recall into the appropriate unitary representation (O'Reilly and Rudy 2001).

This theory does an excellent job of explaining the observation that while standard contextual fear conditioning (association of fear to a context in which an aversive stimulus has been experienced) can be observed in the absence of a hippocampus (Wiltgen et al., 2006), associations to context by way of Fanselow's (1990) immediate shock paradigm cannot. During the extremely short pre-shock interval an animal without the rapidly conjunctive hippocampal system available will be unable to efficiently retrieve the context representation to enable association with the shock. Neocortical systems can independently, but inefficiently, acquire different bits of elemental information and form associations between them, allowing for context-fear associations if given enough time, but they do so much more slowly. Similarly, it is well documented that simple associations such as the ones formed in non-conjunctive classical conditioning tasks are

hippocampus independent (Beggs et al., 1999), whereas associations between events separated in time (i.e. trace conditioning) are not (Moyer et al., 1993). An episodic memory system must always be on line recording and updating events, due to the impossibility of predicting when and where an experience worth remembering might occur (Morris and Frey, 1997). Configural theory would then predict that damage to the hippocampus will result in severe impairment of episodic faculties, whereas it would have only a small to moderate impact on semantic functioning that can still be mediated, albeit less efficiently, solely by neo-cortical sites.

In Squire's model episodic and semantic memory are like two sides of the same coin – inseparable components of the same process that works to carry out the broader function of declarative memory. In contrast Tulving proposes a "Serial Parallel Independent" model (serial recording, parallel storage, and independent retrieval, SPI) (Tulving, 1993, 1995; as cited in Tulving and Markowitsch 1998), in which incoming perceptual information is initially represented semantically, but that a second, episodic, representation is formed from the semantic one. Once both representations are set (during retrieval for example) they are basically equal and independent, though able to interact via reactivation into working memory (Baddeley 2001). In terms of damage induced retrograde amnesia the SPI model allows for

a complete dissociation of episodic and semantic knowledge (either can be impaired to any degree regardless of the other), though in the anterograde direction it predicts semantic impairment must necessarily cause episodic impairment, though the same does not hold in reverse. This is in contrast to Squire's theory, which allows no dissociation whatsoever. Tulving's theory is a purely cognitive one, developed primarily from observations of amnesic humans, and does not specify what circuits are involved at each level of processing.

In light of these theories, there are a number of different ways in which the large body of research pertaining to the nature of episodic and semantic memory, their differences, and how they interact.

**The Hippocampus and Episodic Memory:**

The first clear indications that the hippocampus and related medial temporal lobe structures played a role in memory are found in Scoville and Milner's (1957) classic studies on the patient H.M. He exhibited profound and selective amnesia for declarative memory following a medial temporal lobe resection undertaken to control intractable epilepsy. H.M.'s damage was quite widespread however, and thus specific contributions of the structures within the medial temporal lobe to memory could not be dissociated. In 1997, Vargha-Khadeem et al. described 3 case studies in which children with early

life anoxic trauma localized bilaterally to the hippocampus (i.e. not including parahippocampal cortices) were still capable of acquiring a large amount of semantic knowledge (with school performance even approaching normal). Despite this sparing of learning general knowledge they had a complete inability to describe specific episodes in which they acquired their wealth of knowledge. One patient, K.C., for example could learn to play new games or memorize sentences without any recollection of the learning experience. They had profound anterograde amnesia for episodes, concurrent with normal (or at least non-pathological) semantic retention. A separate group of patients ranging in age from 6-14 at time of onset of hypoxic damage showed a similar pattern of severe episodic memory impairment coincident with comparatively mild semantic deficits (Kitchener et al. 1998; Holdstock et al. 2000). The young age of the children is an important part of the challenge this work presents for Squire's declarative model, as it precludes the possibility that a large repertoire of semantic knowledge could have already been consolidated in the neocortex before onset of the pathology. In line with Conjunctive and SPI theories, and patently pertinent to episodic memory, sparing for individual item memories was seen, but not for more complex associations involving relations between many items or the context in which they were located. This indicates that the dissociation between episodic and semantic memory

is not simply a categorical one, but may have a true neuroanatomical underpinning. Their findings support a model localizing semantic functions to parahippocampal cortices but not to the hippocampus itself. It is in hippocampal circuitry that critical episodic functions are proposed to be instantiated (Tulving and Markowitsch 1998). Vargha-Khadem, Gadian and Mishkin (2001) conclude, based on this evidence and an additional review of 11 other amnesic patients that, "... regardless of age at onset of hippocampal pathology, there is a pronounced dissociation between episodic memory, which is severely impaired, and semantic memory, which is relatively preserved." (p. 139).

These observations seem to be a significant blow to Declarative Theory, though in a commentary Squire and Zola (1998) argue that the results can also be interpreted in light of the possibility that enough hippocampal tissue remained to support traces of online event memory capacity which, though barely detectable, was still sufficient to facilitate formation of semantic traces. Configural association theory on the other hand is clearly supported by Vargha-Khadem's observations of a critical and dissociable role of the hippocampus proper in episodic versus semantic memory.

Tulving and others infer from these studies that the hippocampus plays a critical role in episodic, but not semantic

knowledge. Semantic memories depend upon the adjacent medial temporal lobe cortices (Tulving and Markowitsch, 1998). More extensive medial temporal lobe damage then, such as that seen in H.M., would not show a dissociation between semantic and episodic processes, and for the most part this is in fact the case (Penfield and Milner 1958; Scoville and Milner, 1957). It has also been stated by a number of researchers that hippocampal damage is necessary and sufficient to cause anterograde amnesia for of episodic memory in humans (O'Keefe & Nadel 1978; Morris et al. 1982; Maguire et al. 1996; Rosenbaum et al. 2000). Rats with hippocampal damage however can still acquire some configural associations if given enough exposure (Rudy and Sutherland 1995; Bussey et al 1998; Davidson et al. 1993), emphasizing the importance of the requirement for an episodic model to avoid extensive pre-training and ideally limit the learning episode to a single, short experience (Dere et al., 2006), as is the case in the model given here and in Chapter 2.

**The Prefrontal Cortex and Episodic Memory:**

Episodic memory requires binding of the many elements comprising an experience into a conjunctive contextual representation, a requirement all evidence indicates would depend on the hippocampus. Some form of temporal sequencing or time-stamping of

these representations would also seem to be necessary. A contextual representation might be akin to a snapshot, fixed in time, whereas an episodic memory is more like a film clip, a sequence of events with a specific order of flow from beginning to end, and having a specific temporal position in relation to other such events which have been stored. Evidence stemming from functional neuroimaging (Knutson et al., 2004) and lesion studies (Canavan et al., 1989; Hanneson et al., 2004 McAndrews & Milner 1990; Shimamura et al., 1990) suggests that this temporal sequencing capacity is critically dependent upon the medial prefrontal cortex (mPFC) via its numerous connections with medial temporal lobe memory systems (Aggleton & Pearce 2001). Fuster et al. (2000) for instance has demonstrated an essential role of the mPFC in association of stimuli separated in terms of both time and modality. It has also been demonstrated to be necessary for trace fear conditioning (Runyan et al., 2004). With temporal aspects being one of the things uniquely added in episodic representations, the prediction follows that damage to mPFC could allow another kind of dissociation of episodic and semantic memory functions.

In a commentary paper reviewing the findings of a number of clinical and neuroanatomical studies of amnesic patients, including the Vargha-Khadeem studies, Zola and Squire (1998) suggest that prefrontal effects are dissociable, with episodic-type memories being

far more susceptible, if not uniquely susceptible to damage, although these two types of memory are not dissociable on their view in medial temporal lobe amnesia. There *is* an emerging consensus then that the PFC plays a critical role of episodic memory in humans (Wheeler et al., 1995; Nyberg et al., 2000; Burgess et al., 2002; Wheeler and Stuss, 2003; Schacter, 1987; Squire 1987).

While it is fairly certain that the PFC plays an important role in episodic memory, the exact nature of its involvement in formation and recall is somewhat unclear (it is possible that it plays a role solely in the latter). Lesions restricted to the frontal cortex do not generally result in identifiable amnesia, defined as an inability to acquire and later explicitly reproduce knowledge (Milner 1964). Deficits that are encountered typically have been attributed to the loss of mnemonic strategies or organizational capacity (Smith and Milner 1984), rather than loss of a site of information storage. The dorsolateral prefrontal cortex is also thought to provide essential circuitry underlying working memory (Goldman-Rakic 1987), and it may be that this type of short-term memory is required for dealing with retrieved conjunctive representations of contexts and the additional information that must be bound to them as part of the memory reconstruction during episodic recall. It has also been proposed that the medial temporal lobe structures support the representation of the context and possibly

associations of events to that context, whereas the prefrontal areas are responsible for association of temporal cues to the contextual representation (Nadel and Moscovitch 1997, 1998).

Tulving and colleagues claim that in humans the role is facilitation of an autonoetic consciousness that is essential for mental time travel during retrieval (Wheeler, Stuss & Tulving, 1997). He described for instance a frontal lobe patient lacking not only episodic memory, but also any capacity to imagine either past or future (Tulving 1985). This was taken as evidence that the PFC housed a faculty of special autonoetic conscious awareness necessary for representing one's self in mental space. Specifically, it is said to provide the ability to perceive one's own position along an autobiographical timeline, and mentally project in either direction away form the present "stream of consciousness". These issues have not yet been resolved in humans, and so should not be expected to be solved here, though the emergence of animal models of episodic memory may in future shed some light on these questions.

Further clarification of the frontal lobe's role in memory is provided by cases of *source amnesia,* in which factual information can be acquired without conscious recollection of where and when the actual learning experience occurred (Schacter, Harbluk and McLachlan, 1984). Interestingly, it seems to be one of the only forms of memory

impairment consistently demonstrated in lesions restricted to the frontal lobes. While subjects have no problem identifying items which had previously been presented to them as familiar, they have far more difficulty than controls in recollecting where the item was encountered. They make numerous false positive responses in which the learning location is misattributed. Consistent with the idea that episodic information is particularly susceptible to impairment, problems in source attribution (Glisky, Polster, & Routhieaux, 1995) or recency judgements (Milner Corsi and Leonard, 1991 as cited in Moscovitch 1992) typically precede problems with recognition in normally aging individuals.

In a 1995 paper, Wheeler, Stuss, and Tulving conduct a fairly extensive empirical review of memory deficits in frontal lobe patients studied up until that point. The main findings included a small but detectable recognition impairment, possibly explained by minute septal damage, or a loss of organizational strategies. More drastic and important was a consistent trend of major impairment in recall above and beyond recognition. Similarly, in the animal literature, Hanneson Howland and Phillips (2004) have demonstrated that medial prefrontal lesions in the rat impair the ability to make temporal order judgments for objects they have explored, in the presence of intact object recognition ability.

**Neuroimaging Studies:**

Functional neuroimaging studies provide an additional powerful means of examining the relationship between semantic and episodic processes. By monitoring brain activity during very similar tasks, one semantic in nature and the other episodic, one can identify all the shared areas of activity and subtract them, identifying the specific regions uniquely involved in one and not the other.

The most common means of testing for episodic/semantic distinctions in humans involves distinguishing between *remembering* and *knowing* using word lists. Participants are given a list of words to remember, and then at a subsequent time are presented with a new list containing some of the words from the original list mixed in with new ones. The participant is then asked to decide whether each word had appeared on the list or not, and for words they claim were on the original list, they are asked whether they remember the actual event of seeing the word on the list, or if they simply 'know' it was there (Tulving 1985; Gardiner and Java 1991). Thus confidence is used as the prime indicator of whether the memory was episodic or not. It is seen that the *remembered* items are subject to the same sorts of variables that are widely accepted as affecting episodic memory (Wheeler 2000). When these types of tests are conducted, it is

consistently found by a variety of investigators that the frontal cortex is active during episodic, but not semantic representation.

In general, evidence obtained from such functional imaging investigations shows that episodic memory processes consistently involve activity in the PFC, whereas semantic processing does not (Nyberg et al., 1996; Schacter 1987; Tulving 1986; Kapur et al., 2004). This confirms the trend observed in the lesion studies reviewed above. Specifically, episodic retrieval is selectively associated with increased blood flow in the right hemisphere PFC. A hemispheric asymmetry in acquisition has also been observed, with an increase in left medial frontal gyrus blood flow during episodic encoding, but not semantic (Fletcher et al., 1995; Nyberg, Cabeza & Tulving 1996). Right frontal blood flow was equivalent (Kapur et al., 1995; Nyberg et al., 1995). Tulving states that the frontal lobes are involved in creating an 'episodic retrieval mode', an autonoetic state where the brain is primed to use retrieval cues specifically for experiences involving autonoetic consciousness (Tulving 1983).

The pattern of lateralization noted is also seen in other item recognition tasks, and is referred to as HERA (hemispheric encoding/retrieval asymmetry (Tulving et al., 1994; Habib et al., 2003). Lateralization is not as prominent in non-primate mammals such as the rat however, and so was not tested for in this study.

Just as the circuitry and function of the rat hippocampus is known to be very similar to that of humans, the basis for innumerable models in the memory domain (Whishaw & Kolb 2004), PFC circuitry and function between rats and humans also appear to be conserved (Dalley et al., 2004 for review). As reviewed above, the PFC seems to play a specific role in episodic memory in humans, while it makes minimal or no contributions to non-temporal or recollective, semantic aspects of declarative memory processes (McDonald et al., 2006; Wheeler et al., 1995; Squire 1987).

Curiously, given the large number of recent animal models purported to be true demonstrations of episodic memory, there is virtually no literature on the effects of frontal cortex lesions on episodic memory performance outside of humans or primates. From what has been reviewed concerning their specificity of effect on episodic type processes over semantic ones in humans, performing anterograde tests of prefrontal dependency would seem a valuable and informative test of whether or not one's episodic model taps into the same brain regions involved in human episodic recall. An impairment in performance would provide some evidence that a task is not solved via some sort of semantic rule learning or simple associative strategy. In an effort to test our model for not just behavioural, but also

neuroanatomical, parallels with human episodic memory, we here administer such a test in Experiment 6.

We also tested a conditioning procedure that closely resembled our basic episodic memory task, but we modified it so that it did not require an integrated what-where-when (episodic) representation. If the modified task does not require prefrontal involvement, but our basic episodic task does, this would provide good evidence that our model taps into very similar processes as in humans. We are then viewing the episodic memory system as an extension of the semantic system, both neuroanatomically and likely evolutionarily. An episodic representation will require the same types of configural/conjunctive associations required for forming semantic representation (mediated by the hippocampus and neocortex), plus additional information, or at least direction, from the PFC. Thus, it should be possible to observe a deficit in episodic memory in the presence of PFC damage, while semantic abilities remain intact. According to conjunctive theory, new associations amongst non-linear configural representations such as are required to associate conditioning to *memory* of a context during immediate shock will also necessarily require the hippocampus (Rudy et al., 2002), though Declarative Theory also predicts an essential hippocampal contribution.

**METHODS**

Subjects:

Subjects were male Long Evans hooded rats (Charles River Laboratories) approximately 75 days of age (250-350 grams). Animals were housed in pairs in standard plastic housing tubs (45 cm x 25 cm x 25 cm) on a 12 h light/dark cycle (lights on at 7 a.m.). Food and water was provided *ad libitum,* and a single piece of PVC tubing was provided in the cage for enrichment.

Apparatus:

Plexiglas modular test chambers (ENV-008 MedAssociates, Inc., Georgia, VT) with steel bar floors were used as the context boxes for all experiments. In experiments 5 and 6, boxes were modified so as to provide three distinct contexts. Context A had black walls and was scented with Quatzyl-D-Plus brand disinfectant. Box B was white walled and sprayed for odour with Clinicide disinfectant. Box C was designed to be equally similar to boxes A and B – the walls were half white, half black, and the chamber was left unscented (a small amount 100% ethanol was used for cleaning in between trials, and allowed to completely evaporate). Chamber ceilings were left clear to allow for top-down video recording of behaviour. Scents and wall shading were counterbalanced. Experiment one used only a single context, either A

or B depending on the subject. All boxes were in the same spatial location.

Surgery:

Nearly complete lesions of the hippocampus were created by stereotaxic infusion of n-methyl-D-aspartate (NMDA), conducted under isoflurane induced general anaesthesia (2% isoflurane delivered in 2 L/min oxygen). Rat's heads were shaved and the skin was cleaned by three alternating applications of hibitane (4% chlorohexidine gluconate) and 70 % alcohol. 0.1 mg/kg temgesic was given s.c. immediately prior to stereotaxic mounting (David Kopf Instruments) as an analgesic, and .2 mg/kg diazepam was given immediately prior to NMDA infusion for the purpose of controlling seizure activity. Drugs were re-administered following recovery from anesthesia for the same purposes. An incision was made through the skin and periosteum, which were retracted from the skull and held using hemostats. Small holes were drilled above the desired injection sites into which 30 gauge cannulae connected via PE50 plastic tubing to a 10 ul Hamilton syringe (Hamilton, Reno, NV) mounted on an infusion pump (Harvard Apparatus PHD2000, Holliston, MA) were lowered to the desired depth. Infusion rate was set to 0.15 ul/min, and 0.4 ul total of 7.5mg/ml NMDA in phosphate buffered saline was infused over 2 minutes and 40

seconds to each of 7 sites per hemisphere. Injection cannulae were left in place for 2 minutes 20 seconds to allow for diffusion from the tip before retraction. Injection co-ordinates from bregma with skull flat were as follows: (AP -3.1, ML +-1.5, DV -3.6), (AP -4.1, LV +-3, DV -4), (AP -5, ML +-3, DV -4), (AP -5, ML +-5.2, DV -7.3), (AP -5.8, ML +-4.4, DV -4.4), (AP -5.8, ML +-5.1, DV -7.5,), (AP -5.8, ML +-5.1, DV -6.2).

Medial prefrontal cortical lesions were performed using a very similar surgery procedure and drug regimens as used for the hippocampal lesion. 10mg/ml NMDA was infused at 0.1ul/min for 4 minutes followed by 2 minutes diffusion time for each of 5 sites per hemisphere. Injection co-ordinates from bregma with skull flat were: (AP +4, ML +-0.7, DV -4.8), (AP +4, LV +-0.7, DV -2.8), (AP +2.7, ML +-0.7, DV -5.6), (AP +2.7, ML +-0.7, DV -3.5), (AP +1.7, ML +-0.7, DV -3.2).

Sham surgery was performed on all control rats by performing the hippocampal lesion procedure with the omission of skull puncture or NMDA infusion. For Experiment 4, 6 hippocampal lesions, 8 mPFC lesions and 8 sham surgeries were conducted. Experiment 5 used 10 hippocampal lesioned rats, and 10 shams; Experiment 6 subjects were 12 mPFC lesioned rats and 12 shams.

Procedure:

Rats were allowed 10 days of post surgery recovery time before training began. Experiment 4, as illustrated in Figure 4, was effectively just a replication of Fanselow's immediate shock protocol (figure 1.4), except with multiple pre-exposure sessions. During phase 1, animals were placed in a single context (A or B) for successive three days , with exposure times of 9, 7 and 5 minutes for days 1, 2 and 3 respectively. In phase 3 (day 4) animals received a single immediate foot shock in the same context. They received a 1.0 mA footshock of 2 seconds duration. There was a 2 second shock free interval before and a 3 second shock free interval after the shock. The shock was delivered through the floor bars. Phase 3 was a 3 minute exposure period in the shock context during which freezing behaviour was recorded and the percent of time spent freezing for each minute was calculated by an automated computerized video system. All phases were conducted approximately mid-day (between 3 and 5 p.m.).

Experiments 5 and 6 involved the same procedure as described in Chapter 2 and illustrated in Figure 1.1. Phase 1 involved two pre-exposure phases per day, for each rat, over a three day period. One pre-exposure session was in box A and conducted between 9 and 11 a.m., the other was in box B and given between 9 and 11 p.m. Exposure times were the same per context per day as in experiment 4

(for a total duration of 21 minutes per context in phase 1). Phase 2 was an immediate shock as described exactly for Experiment 4, but it was given in the chimeric context (Box C). For half of the rats the shock was provided in the morning exposure time, for the other half it was provided in the night. Phase 3 was a 3 minute test period scored for freezing using the same automated procedures as in all other experiments. Half of the rats were tested in the context that had been previously paired in Phase 1 with the time the animal ended up being shocked at in Phase 2 (i.e. the *congruent* context), the other half were tested in the *incongruent* context. Exposure contexts and times were counterbalanced across and within groups.

Histology:

After testing animals were euthanized by an overdose of sodium pentobarbital (Euthansol, Schering, Kenilworth, NJ) and then perfused intra-cardially first with 200 ml phosphate buffered saline (PBS) and then an equal volume of 4% paraformaldehyde in PBS. Brains were extracted and left in a 30% sucrose, 4% paraformaldehyde in PBS solution for at least 48 hours prior to being sliced coronally at 40 um on a cryostat (MICROM HM560, Waldorf, Germany) and stained for cresyl violet. Section samples were taken to the full extent of the lesions.

## EXPERIMENT 4: The hippocampus, but not frontal lobes, is required for context conditioning using immediate shock

Hippocampal damage should disrupt acquisition of fear of a pre-exposed context using the immediate shock procedure. However, unlike its predicted effect on time plus context fear memory, mPFC should not disrupt acquisition in this experimental task. This is based upon the idea that mPFC is hypothesized not to be important for context representation or fear association, but are necessary for permitting the use of temporal cues to distinguish between contexts. Our work here is essentially a successful attempt to systematically replicate previously published studies showing that the hippocampus is responsible for contextual fear conditioning, and that the medial prefrontal cortex is not (Gewirtz, Falls & Davis, 1997; Rudy and O'Reilly 2001). Here we use a set of conditioning parameters that are different from published work, but that are directly relevant to conditions in our previous experiments.

### Results and Discussion

Results are presented graphically in Figure 3.4. A main effect of lesion on freezing behaviour was evident based on repeated measure ANOVA ($F(2, 15) = 4.71$, $p < .05$). The amount of freezing did not

significantly change across the three minutes of testing. *Post hoc* analysis indicated significantly less freezing was exhibited by the hippocampal lesion group compared to the Sham control group ($p <$ .05) or medial prefrontal cortex (mPFC) lesioned animals ($p = .037$) .No difference, however, was observed between the Sham control and Prefrontal lesion groups.

The hippocampus is thus confirmed to be necessary for the type of fast conjunctive/configural association involved in the immediate shock effect (Rudy and O'Reilly 2001), but the mPFC is not necessary for mediating learned fear of a context in a task with very similar parameters to our episodic memory task, with the exception that there is not a time-place conjunction.

**EXPERIMENT 5: Hippocampal requirement of episodic model**

In this experiment we test Sham control and hippocampal damaged rats in the episodic memory task described in Experiment 1 (Figure 1.1). Without an intact hippocampal formation it is predicted that rats will be unable to show discriminative learned fear involving the two contexts. Based upon the results of Experiment 4, it is hypothesized that rats with hippocampal damage should not even have a contextual representation that could be associated with fear in the immediate shock procedure, and so these rats should show equivalent conditioned fear responses to the congruent and incongruent test

contexts, albeit at a reduced magnitude. With episodic memory requiring a multimodal conjunction of allocentric spatial context and integration of multiple elemental cues, the hippocampus should obviously play an essential role. As the hippocampus has been conclusively shown to have an important role in regular contextual fear conditioning (Maren et al., 1998; Phillips & LeDoux, 1992), as well as the immediate shock or "Fanselow effect" variant (Rudy and O'Reilly 2001), it is predicted to be required for expression of fear in our model of episodic memory. The procedure used is the same as described in Figure 1.1 and discussed in detail in Chapter 2. Rats are given an exposure session to one context in the evening and a second in the morning for a period of three days. On the fourth day they are given immediate shock in *either* the morning or evening

Results and Discussion:

All rats displayed robust freezing behaviour which was seen to decline over the three minute test period (Figure 3.5). Repeated measures ANOVAs were conducted on the two different lesion groups tested either in the congruent or incongruent contexts. There was a significant difference in freezing between groups ($F(3, 16) = 10.3$, $p = .001$). The *Post hoc tests* showed that the Sham control group

tested in the congruent box showed significantly more freezing than any of the other three groups (all *p's* < .007).

As has already been demonstrated in Experiment 1 intact rats show strong learned fear of a context previously paired with the time of day of a foot shock. As predicted, rats with hippocampal damage do not show this effect. Thus, the hippocampus is essential to this form of episodic memory. This should not be surprising given the results of Experiment 4 showing that the hippocampus is essential to simple contextual fear conditioning using the "Fanselow effect".

**EXPERIMENT 6: Frontal lobe requirement for episodic model**

We hypothesize that damage to the mPFC will impair performance in the episodic memory task to the same extent as that observed in rats with hippocampal damage. Episodic memory is understood as a unified representation of both a conjunctive contextual component along with some form of as yet unspecified temporal information provided by the mPFC. In the absence of this temporal information, the time appropriate context will not be retrieved to be associated with foot shock.

Results and Discussion

As seen in Figure 3.6, mPFC lesions produced a similar pattern of effect on freezing behaviour as that observed with the hippocampal lesions conducted in Experiment 5. All rats displayed robust freezing behaviour which was seen to decline over the three minute test period (Figure 3.6). Repeated measures ANOVAs were conducted on the two different lesion groups tested either in the congruent or incongruent contexts. There was a significant difference in freezing between groups ($F(3, 16) = 7.8$, $p = .002$). The *post hoc tests* showed that the Sham control group tested in the congruent box showed significantly more freezing than any of the other three groups (all $p's < .05$).

From what we observed in Experiment 4, we know that this pattern of impairment is not due to the same reasons as seen with hippocampal lesions in Experiment 5. Frontal cortex damaged animals are still capable of quickly acquiring a conjunctive context representation and associating it with shock, but here it is shown they cannot use a temporal cue to differentially attribute fear between pre-exposed contexts at time of testing. The medial prefrontal cortex then must mediate specific processes involved in our episodic model, but not very similar processes not requiring a what-where-when conjunction.

**General Discussion:**

In the previous experiments we have demonstrated a form of context + time memory in rats that we interpret as a close relative of human episodic memory. We demonstated that both hippocampal damage and mPFC damage disrupt performance in our rat episodic memory task. Furthermore we demonstrated that hippocampal damage but not mPFC damage disrupts the rat's ability to form a contextual fear memory in the immediate shock procedure even when the contextual memory conditioning is independent of time. This supports the idea that performance of episodic memory in rats, as described in Chapter 2, is dissociable from semantic or episode-independent memory by way of exclusive involvement of the mPFC regions in the former, as compared to dependence of both memory types on an intact hippocampus. Impairment of episodic memory in the presence of intact semantic capabilities replicates the same pattern of results observed in brain damaged humans. This is the first demonstration of such a parallel in a rodent model of episodic memory.

We propose that episodic memory is an extension of the medial temporal lobe semantic system, dependent upon the same stimulus conjunctions used to represent declarative memory of the non-episodic variety (Sutherland and Rudy 1995; Rudy and O'Reilly 2001), but with

the additional requirement of PFC circuitry necessary for associating the temporal components which distinguish the uniqueness of individual experiences. The addition of a distinct episodic memory system built upon a semantic framework is amenable to the Serial, Parallel, Independent theory of declarative memory progression proposed by Tulving (1993), although the present work is in obvious opposition to Tulving's notion that episodic memory is a uniquely human trait involving dependence upon phenomenological characteristics accompanying recall ((Tulving, 1989, 2002). Our findings are also in accord with the notions of Nadel and Moscovitch (1997; Nadel et al., 2000) that episodic memory involves co-ordination of a contextual representation provided by the medial temporal lobe, and a temporal trace localized to the prefrontal cortex. We do not find support for the longstanding conjecture that declarative memory represents a single medial temporal lobe system dealing indiscriminately with any form of semantic or episodic knowledge heralded by Squire and colleagues (Cohen and Squire, 1980; Squire, 1992; Squire & Zola Morgan, 1998). (Tulving, 1989, 2002). It seems somewhat contradictory that Squire maintains his position of a single declarative memory system, while publishing manuscripts attesting to the role of the frontal cortex in episodic but not semantic memory (Squire 1987). Though the claim can be made that the frontal lobes

are simply assisting in performance of recall type processes but not recognition, with formation and processing solely mediated by a single medial temporal lobe network, the very fact that the frontal lobes can interact with one but not the other type of memory would seem to imply these types of memory are in fact distinct and dissociable.

Future work with this model should allow for a more in-depth investigation of specific brain regions involved in mammalian episodic memory as well as how it changes with age and pathologies. Precision lesions unethical to administer to humans may also allow investigators to test the assertion of Declarative Theory (Squire and Cohen 1980) that semantic and episodic memory are not differentially represented in the medial temporal lobe, by taking advantage of histological techniques that could remove any doubt as to lesion locale or extent.

An additional experiment of interest would be to conduct place cell studies in which hippocampal cell assemblies representing each pre-exposure context are noted and conditions of their individual retrieval examined. It would be very convincing, for example, if a cell assembly associated with a context was reactivated even without re-exposure to the context, upon provision of retrieval cues such as removal from the home cage at the time of day associated with pre-exposure. Such a demonstration may convince even the most steadfast of skeptics that episodic memory is a shared characteristic of

rats and humans, perhaps even accompanied by a richly imagined re-experiencing of the event context accompanying recall. Finally, sensitivity of this task to frontal lobe impairment provides a novel means of assessing integrity of this structure in rats, which may be useful in combination with other models of prefrontal dysfunction.

In summary, we have shown here that the model for episodic memory as described in the previous chapter exhibits very similar general neuroanatomical underpinnings to those serving episodic memory in humans. Not only does our task meet relevant behavioural criteria for the various definitions previously set forth by a number of different researchers (reviewed in the previous chapter), but it also relies on some of the same neural circuitry as human episodic memory. To our knowledge this is the first time specific brain regions underlying episodic memory in a rodent model have been investigated. Based on this, and in accord with observations in the human domain, we conclude that both the hippocampus and medial prefrontal cortex are necessary for episodic memory function in the rat. Excepting the issue of autonoesis in animals (Tulving 2002), all characteristics of human episodic memory have been shown to be present in our rat model, and we feel that appropriate protocols now exist for doing animal research in the area that will be applicable to human disorders of episodic malfunction.

In a recent and extensive text summarizing nearly all of the research to date concerning the hippocampus (aptly entitled "The Hippocampus Book"), a section reviewing theories of function concludes with the statement, "... we still do not understand the precise role of the hippocampus in episodic and semantic memory or, within the domain of episodic memory, in familiarity and recollection... [This] points to the need to develop new animal models of these forms or memory and of retrieval to help resolve the issues." (Andersen et al., 2007 p. 617).

It is our hope and belief that the model described here will contribute substantially to that project.

# CHAPTER 4 – CONCLUSION

In the previous two chapters it has been shown that: 1) the rat can demonstrate the behavioural characteristics associated with episodic memory in humans, and 2) the execution of these behaviours depends critically on at least some of the same cortical regions already known to sub-serve them in humans. Though that would seem sufficient to now state unequivocally that rats have episodic memory, one problem, which may prove to be insurmountable, still remains. That is the issue of autonoetic consciousness.

When Tulving first presented the concept of episodic memory in 1972, he defined it based on the type of information it represented. He asserted that it was a form of declarative memory that was distinct from purely semantic knowledge in that it represented not just rules, or verbally expressible information, but also a rich spatio-temporal structure. He further asserted, quite correctly, that these differences were no small matter, and in fact were so distinct that they would comprise two separate, though overlapping systems, with episodic memory requiring all that underlies semantic memory plus additional circuits to deal with these spatiotemporal associations. However, in 1983 Tulving revised the definition of episodic memory so that it must also necessarily include certain phenomenological qualia. If one

retrieves an episodic memory, according to Tulving, he has the experience of reliving the event in the mental world, as the person it actually happened to, at the time it actually happened (though obviously as a mental representation of one's self rather than a physical being). He called this "Mental Time Travel". If we introspect on this, it has some intuitive appeal. If one thinks about the non-episodic question, "What is your favorite breakfast cereal," it is likely that a word, and just a word, is recalled, say 'Cheerios'. If, on the other hand one is asked "What did *you* have for breakfast this morning?" the recollective experience may change, such that instead of a list it is as if you have a mental image of actually eating the breakfast, within the spatial context of one's home, and with a sense of knowing that it is *that* morning and no other, while still of course remaining aware that one is really now at work, answering a question.

From this example we start to see why Tulving argues for three additional features of episodic memory to be explicitly shown, all implied by the concept 'autonoetic awareness': A sense of temporal awareness that centers on the individual, referred to as subjective time; the awareness of self as an acting agent; and autonoetic awareness, the ability to represent one's self so as to be able to create a second, mental self which can operate in mental space (Tulving, 1983; Tulving and Markowitsch, 1998). Unfortunately (though

arguably not so importantly for our purposes), it is technically impossible to demonstrate these qualities in non-linguistic species. It is in fact not known whether these limitations are only technical. One simply cannot arrange to have a nonhuman animal tell you about its breakfast experience, nor prove that it has autonoetic awareness. Tulving claims there are not even analogues of these abilities outside humans, based on a lack of empirical evidence. One could however make the equally incontrovertible claim that humans without communication do not have autonoetic awareness either, though common sense seems to preclude this.

Tulving, however, does concede that, "In many ways, the relation between autonoetic consciousness and episodic memory can be thought of as much a matter of definition as a matter of empirical facts; we have defined episodic memory in terms of its dependence on autonoetic awareness." (Wheeler, Stuss and Tulving, 1997 p. 343). His solution to the seeming lack of room for empirical testing or support of this assertion is to outline the correlation in development of a sense of awareness and the advent of episodic memory functions. Children do not usually start expressing episodic memory until after certain benchmarks of development that can be interpreted as conscious self awareness or a theory of mind (Piaget, 1997). Most of this however is simply correlational, and correlating one trait that is found in animals

and humans alike (episodic memory, as defined by function) to another that arguably is not (conscious awareness) does not prove one is required for the other.

Tulving does hypothesize that the circuits underlying autonoetic consciousness essentially involve the frontal lobes of humans (Wheeler, Stuss and Tulving, 1997), and performance of our task has been shown to be critically dependent upon a similar area in the rat (Chapter 3).

There is no consensus about non-linguistic behavioural indicators of conscious experience (Griffiths et al., 1999). However, one that would reasonably seem to indicate consciousness is the apparent presence of dreams in animals. Many people have anecdotes of their animals looking like they are acting out dreams in their sleep, but Louie and Wilson's (2001) group at MIT have given a much more convincing demonstration. They recorded cell activity in the hippocampus as animals ran a circular maze for food reward. Later, as animals were in REM sleep it was seen that the exact same patterns of cell activity recorded during the task was also seen in the dreaming animals, with firing rate and changes in firing patterns occurring at the same speed as was observed in the awake rats while running. Although the realm of dreaming is still considered by most to be outside the realm of empirical science, if

this study's conclusions can be accepted that may actually solve the (unnecessary) problem of proving animals have a self concept, and even a meta-representation of themselves. If not, then who is dreaming? Frith et al. (1999) furthermore show that many of the other accepted neural correlates of conscious experiences are also present in animals. Thus it may even be the case that animals do exhibit some form of autonoetic consciousness, though lack of sufficient proof of this to date should be no more valid a reason for limiting animal models of episodic memory than it should models of pain or age related dementia.

Virtually any cognitive process can be said to be accompanied in humans by some sort of special awareness, but this is not essential, and may often not even be important to its function. It could be said when a person feels pain there is an essential qualia of agony that is additional to the outward manifestations such as grimacing, yelping, and subsequent avoidance of associated contexts, and that while animals may exhibit all the same observable features of pain, what they are experiencing is only 'pain-like' due to a lack of accompanying intangible phenomenological qualities. Even if this is true, it makes no difference in terms of the benefits that can be derived from using animal models of pain for research. If we did not, all of humanity would be many years behind in medical science and quality and

duration of life.

Most certainly vision is not experienced the same way in humans and animals, nor pain or olfaction, yet we still refer to them by the same name in both species as they serve the same function using very similar, but not identical structures. Likewise we do not include 'being aware that one is viewing' as mandatory in animal models of vision. It may be that the animal just operates as a stimulus-response automaton to different patterns of photons on the retina, or it may be that it experiences a rich visual motion picture such as we do, but this difference has not been in any way an impediment to vision research. If the same functions can be shown to be served by very similar brain networks, that should be sufficient to advance claims about the utility of the nonhuman model for human processes. Autonoesis may not be an essential component of the function of episodic memories in humans. To say that we cannot use nonhuman animals to model episodic memory, given the considerations raised by the experiments conducted and the literature reviewed in this manuscript, is counterproductive to say the least.

It is important then to recognize that we are not claiming to have demonstrated autonoetic awareness in the rat, as would be required to classify the processes we are observing as "episodic memory" according to Tulving's definition. What we do show is that

rats are capable of using the 'when' component of an experience to determine the 'what' and 'where', suggesting these components are linked together in an integrated representation. It is not difficult to see that non-human animals could benefit from a system that allowed them to discriminate life events, and remember specific experiences marked by time and place rather than just simple cause and effect generalizations about the world. It is also rare that a complex trait would suddenly occur in only a single species with little relevant evolutionary precursors or foundation.

While it may currently be impossible to show animals can meet the autonoetic criterion of Tulving's episodic memory, it does appear that at least two species other than humans can form memories that consist of an integrated representation of time, place and event nature (Chapter 2). Furthermore, these representations rely upon at least some of the same neural circuitry in nonhuman animals as they do in humans (Chapter 3). Like all animal models this one is only an approximation of the human processes, but this makes it no less useful in permitting experimental manipulations relevant to humans and possibly in elucidating the evolutionary history of the trait.

As to the importance of this work, episodic memory models are of particular clinical relevance because it is these memories which are most susceptible to nearly all forms of insult. When we think of

memory loss resulting from neurodegenerative diseases such as Alzheimer's (Small et al., 2005), or even acute trauma such as a closed head injury, patients do not typically lose their lexicon or forget how to walk – they are most likely to lose recent portions of their autobiographical record in both the retrograde and anterograde direction. They may forget where they were, having met another individual, or what they had to eat. They will very rarely, however, forget how to eat, the name of their country, or the capital of France, etc.. (Evans et al., 1993; Duffy and O'Carroll, 1994; Greene et al., 1996). A similar pattern of susceptibility is also seen in normal aging (Herlitz and Forsell 1996; Nilsson et al., 1997; Tulving & Markowitsch 1998). Tulving and Markowitsch (1998) for example describe episodic memory as a system which develops late and decays early.

Memory deficits in autism have also been described to include a lack of ability to form episodic memories, while still maintaining an intact semantic repertoire. Autism is a developmental disorder which results in dysfunctional limbic-prefrontal connections, while largely preserving the integrity of the rest of the limbic system (Ben Shalom, D., 2003). Korsakoff's syndrome, attributed to damage of a system including the mammilary bodies and anterior thalamic nuclei that are important in connecting hippocampus with prefrontal cortex (Aggleton

& Pierce, 2002), is an additional condition associated with specific episodic deficits.

It is possible that the episodic memory model presented in this manuscript could be combined with models of neurodegenerative disease or acute trauma in rats. It would be particularly convincing if it was shown that this behaviour, and other demonstrations of episodic memory in the rat or jay, are more susceptible to removal (failure? breakdown?) in models of dementia and cognitive decline both pathological and due simply to normal aging, as is clearly the case in humans. Combined with all the previous studies reviewed above, it can now more confidently be stated that episodic memory does exist outside of humans, and we should take advantage of this fact by using animal models for the benefit of human health and to further our understanding of human memory. The debate about autonoetic awareness may have benefit in a different realm of scholarly activity.

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## FIGURES

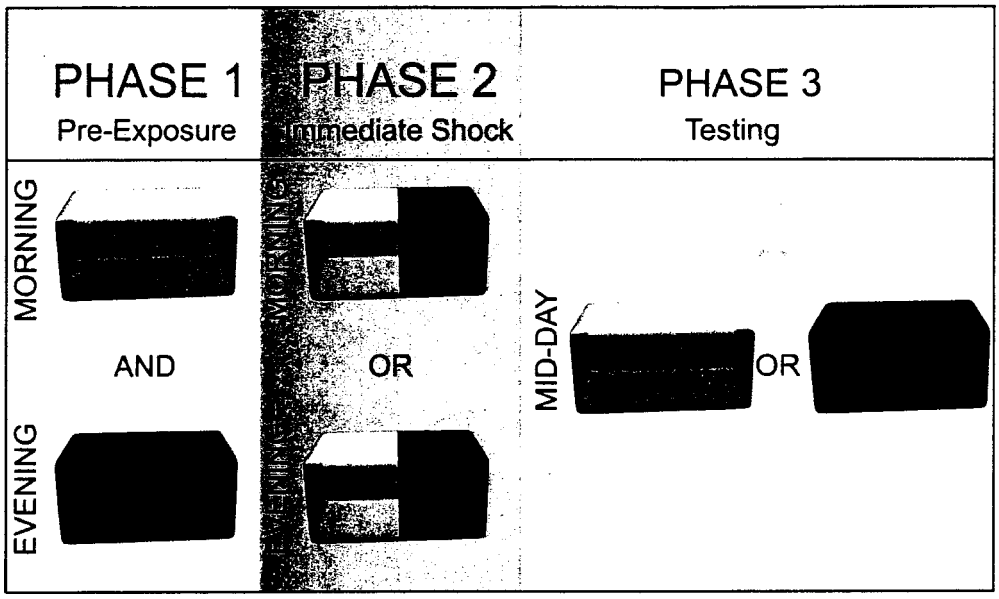


**Figure 1.1.** In Phase 1 rats are exposed to one context in the morning, **and** a second in the evening for 3 days. During Phase 2 rats are shocked immediately either in the morning **or** evening in a context which equally resembles the two contexts from Phase 1. Phase 3 is an exposure at mid-day video recorded to monitor freezing behaviour of a subject in either the context experienced during the mornings of Phase 1 **or** the evenings.

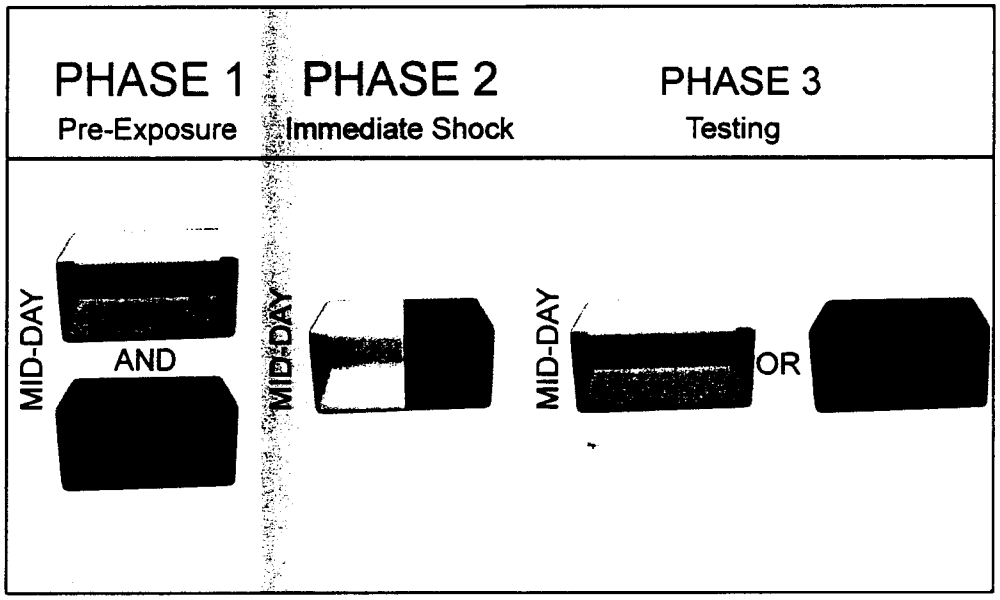


**Figure 1.2.** In phase 1 rats are exposed consecutively to two different contexts once a day for three days. During Phase 2 they are immediately shocked in a context comprised of equal elements of both Phase 1 contexts. Phase 3 is a motion recorded exposure session in either of the phase 1 contexts. All phases are conducted at approximately mid-day.

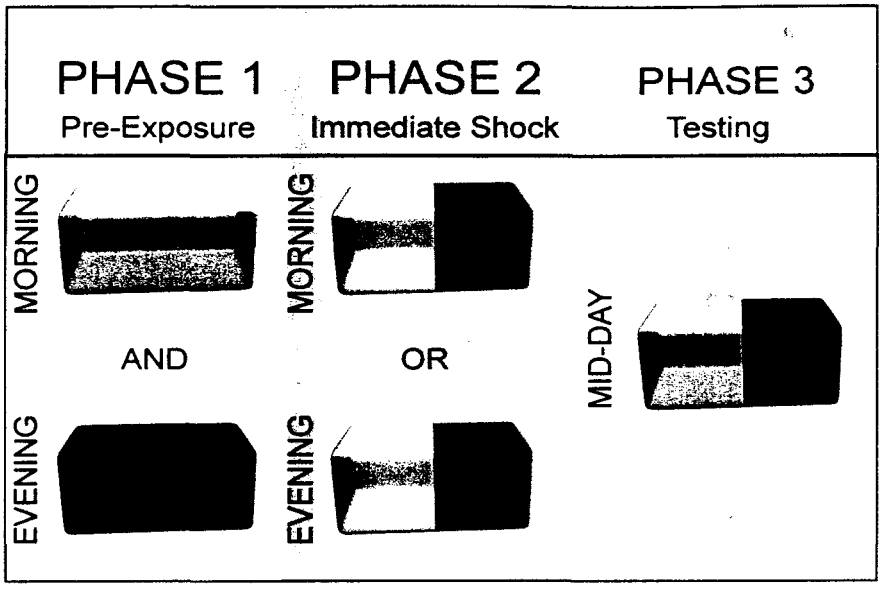


**Figure 1.3.** In Phase 1 rats are exposed over three days to one context in the morning and a second in the evening. For Phase 2 they are immediately shocked either in the morning **or** evening in a context equally similar to the two phase 1 contexts. At Phase 3 rats are tested for freezing in the same context they were shocked in during Phase 2.

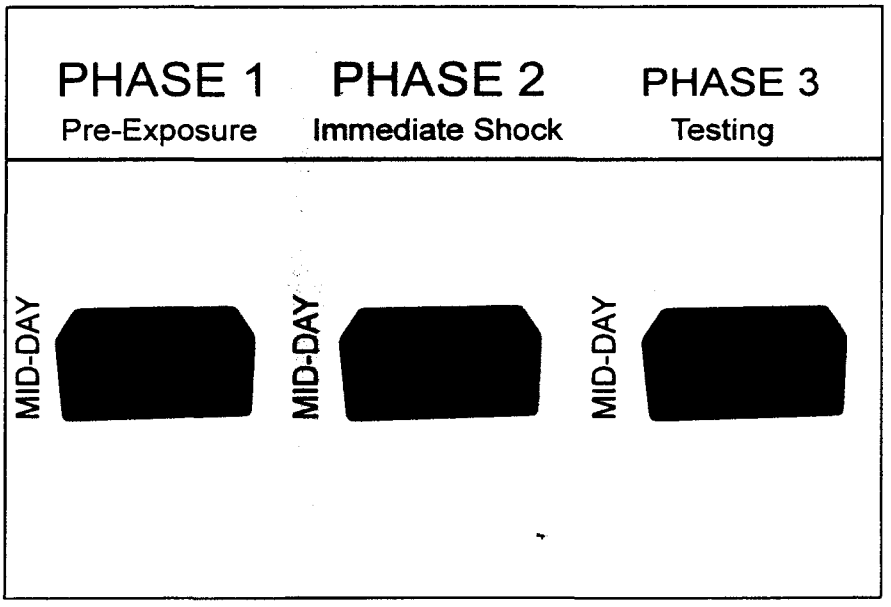


**Figure 1.4.** Rats are exposed to a single context once a day for 3 days (Phase 1), then given an immediate shock in that context during Phase 2 and subsequently tested for freezing behaviour in Phase 3. All exposures occur at the same time of day.

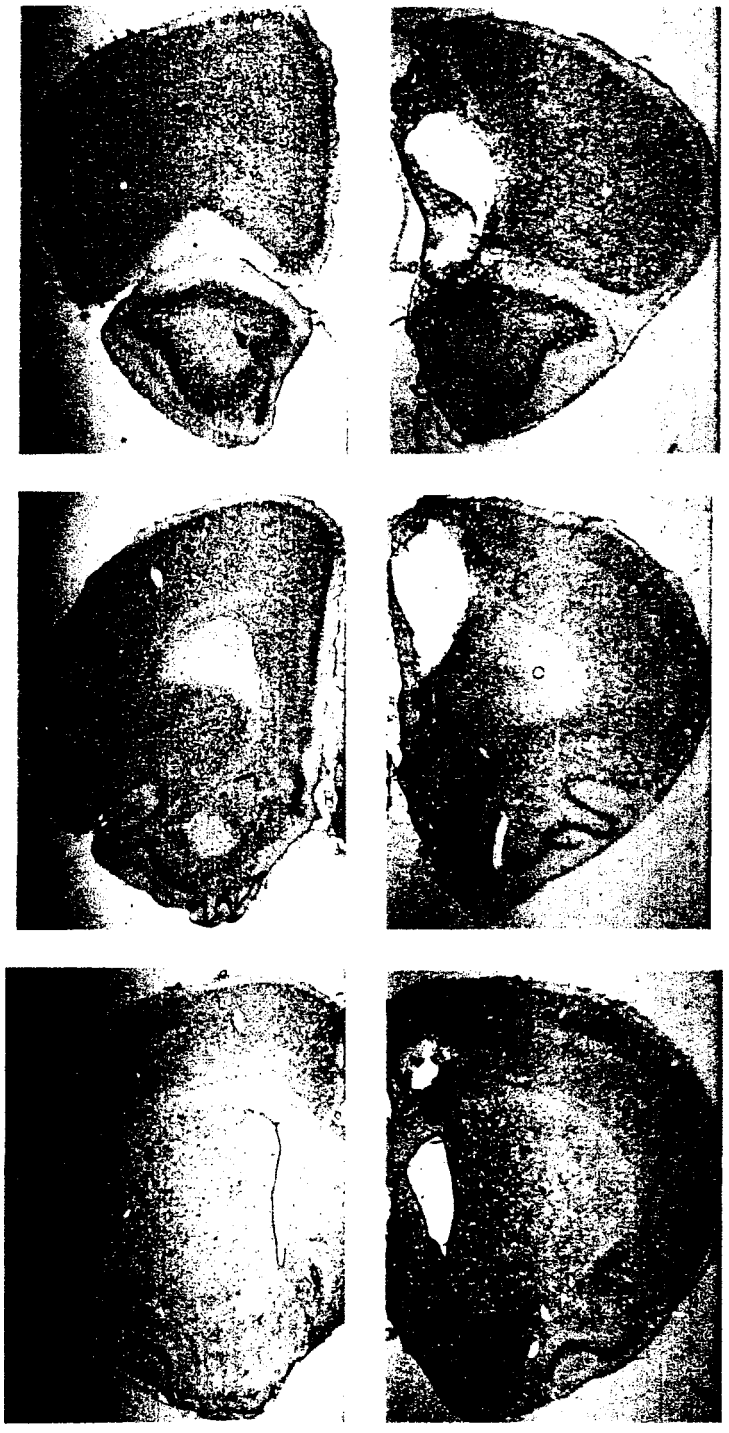

**Figure 2. 1.** Tissue sections stained using Cresyl violet demonstrate typical lesion extent observed in animals given NMDA infusions to the medial prefrontal cortex (right) as compared to shams (left), arranged anterior to posterior in descending order.

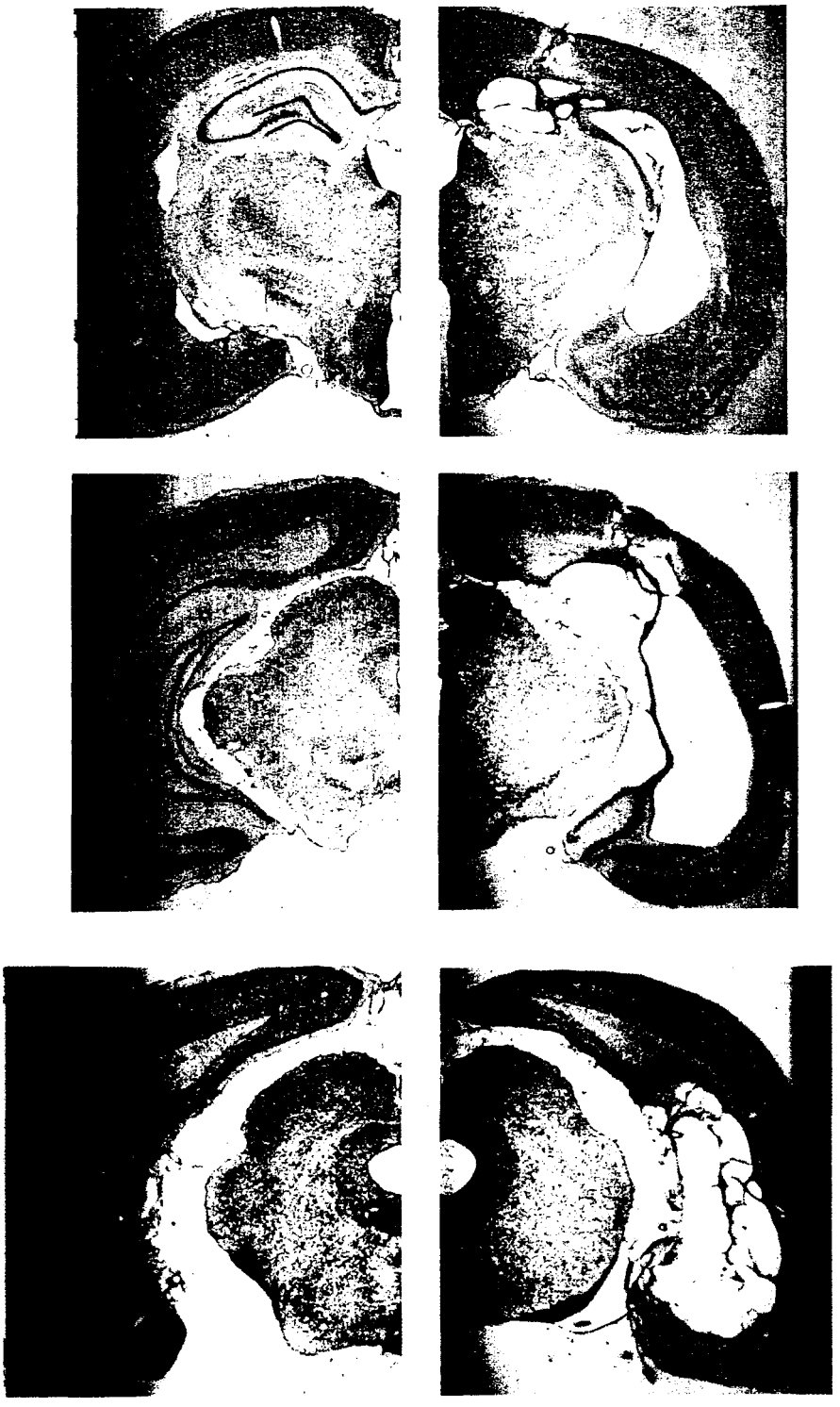

**Figure 2. 2.** Cresyl violet stained section demonstrating typical lesion extent observed in animals subjected to NMDA induced hippocampalectomy (right) as compared to shams (left), and arranged from anterior to posterior down the page.

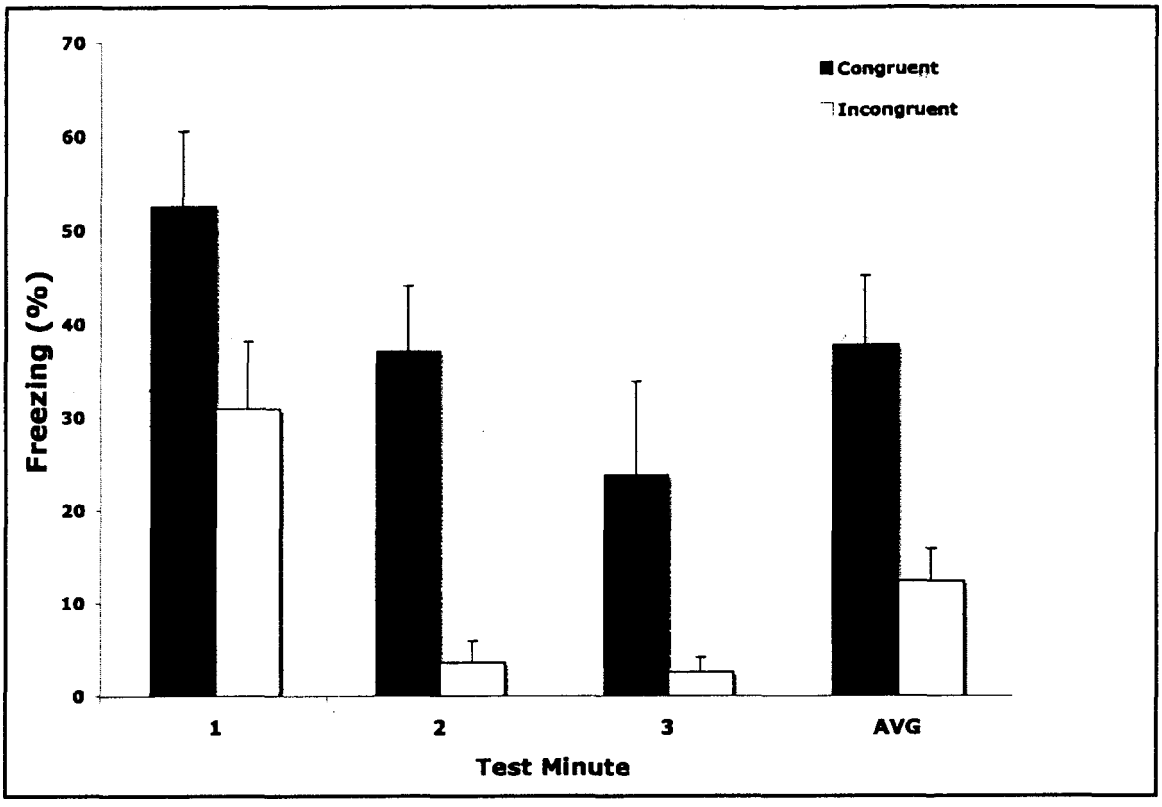


**Figure 3. 1.** Rats subjected to the episodic memory task illustrated in figure 1.1. show far more fear memory of the pre-exposure context congruent to the time of day at which they were shocked than the incongruent one, as indicated by percentage of time in which they exhibit freezing behaviour. Bars represent standard error of the mean.

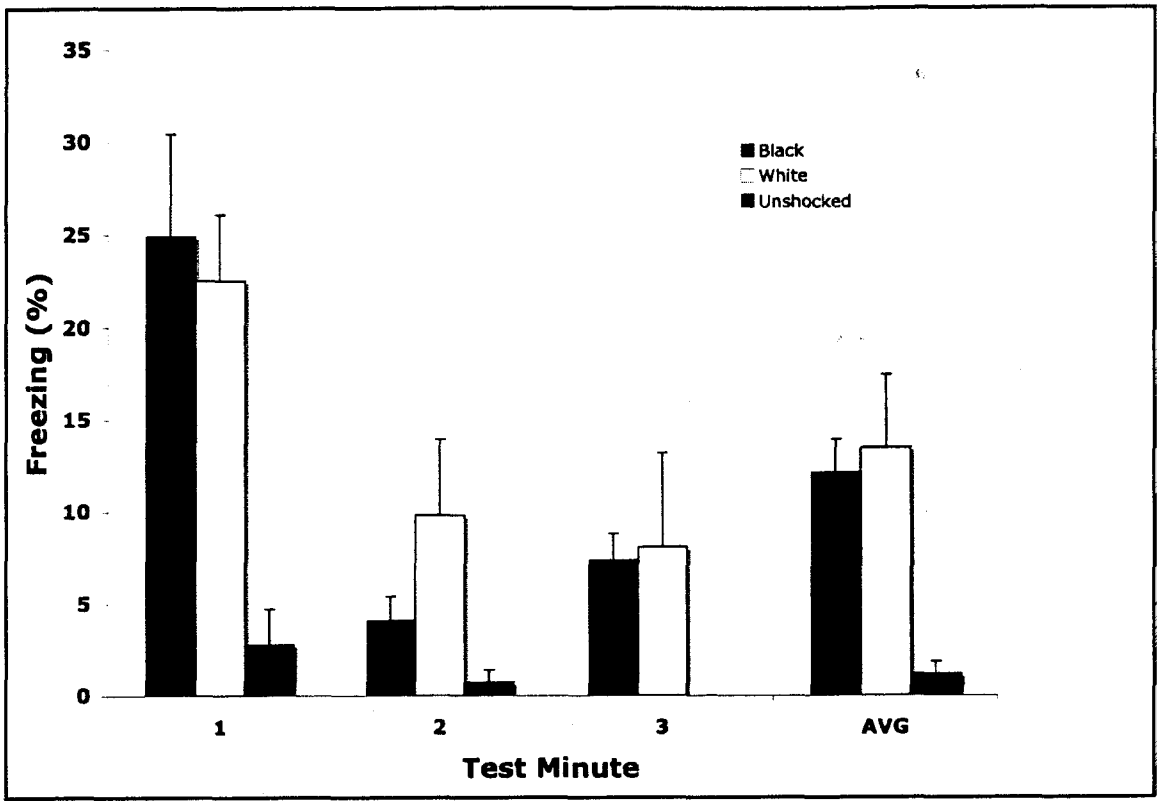


**Figure 3. 2.** Rats given the same duration and order of exposures as in the episodic memory task, but without a temporal distinction between contexts (as seen in figure 1.2.) do not differentially attribute fear to one pre-exposure context over the other, though they do learn a generalized fear association.

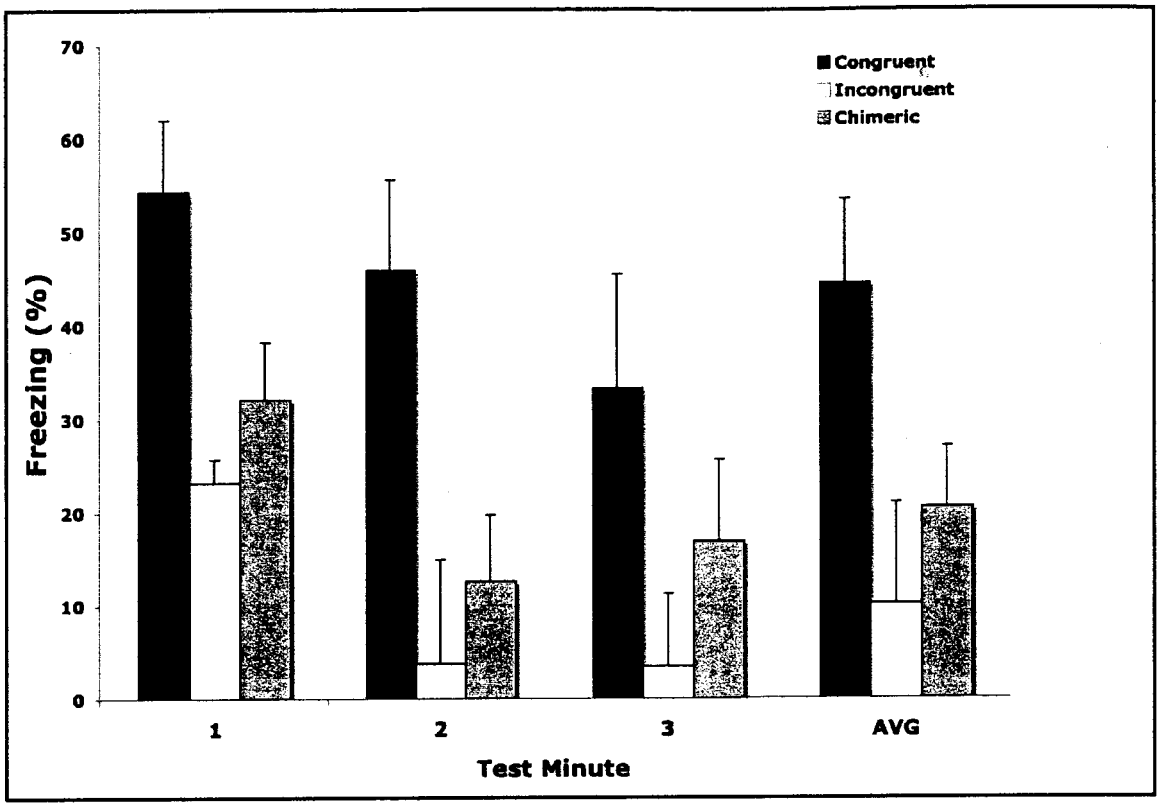


**Figure 3.3.** Rats exhibit more fear memory to the context in which pre-exposure time was congruent to shock time than to the chimeric context in which the immediate shock was actually delivered (procedure illustrated in figure 1.3).

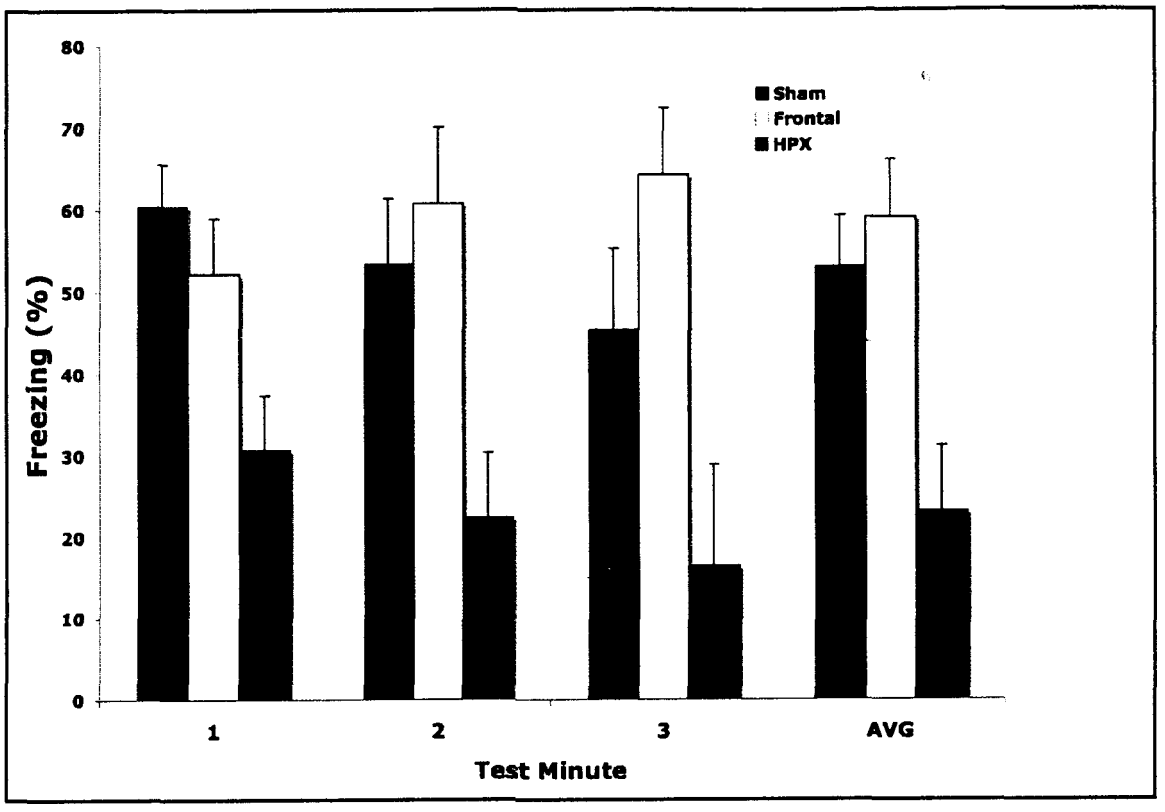


**Figure 3. 4.** When performing a version of immediate shock which does not involve a temporal association to context (as per figure 1.4), rats with hippocampal lesions show impaired fear memory while those with damage to the medial prefrontal cortex do not.

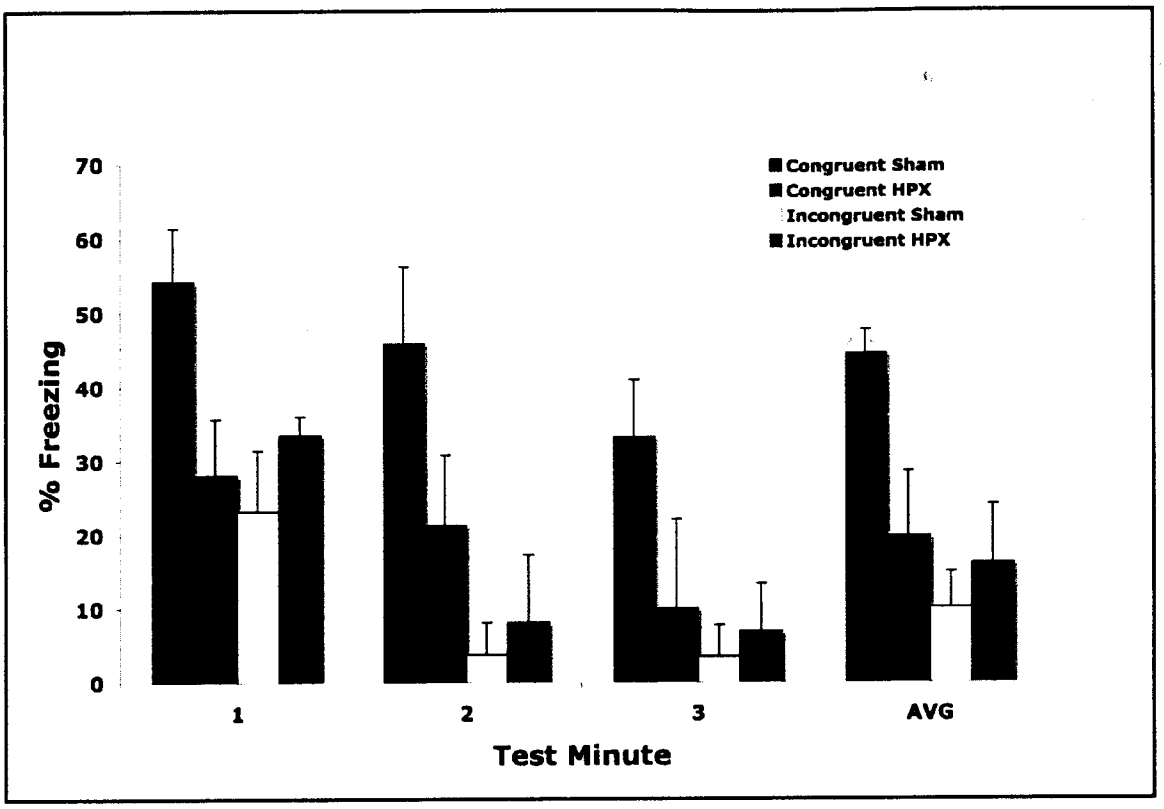


**Figure 3. 5.** Rats with hippocampal lesions do not discriminate between congruent and incongruent shock contexts in the model of episodic memory illustrated in figure 1.1.

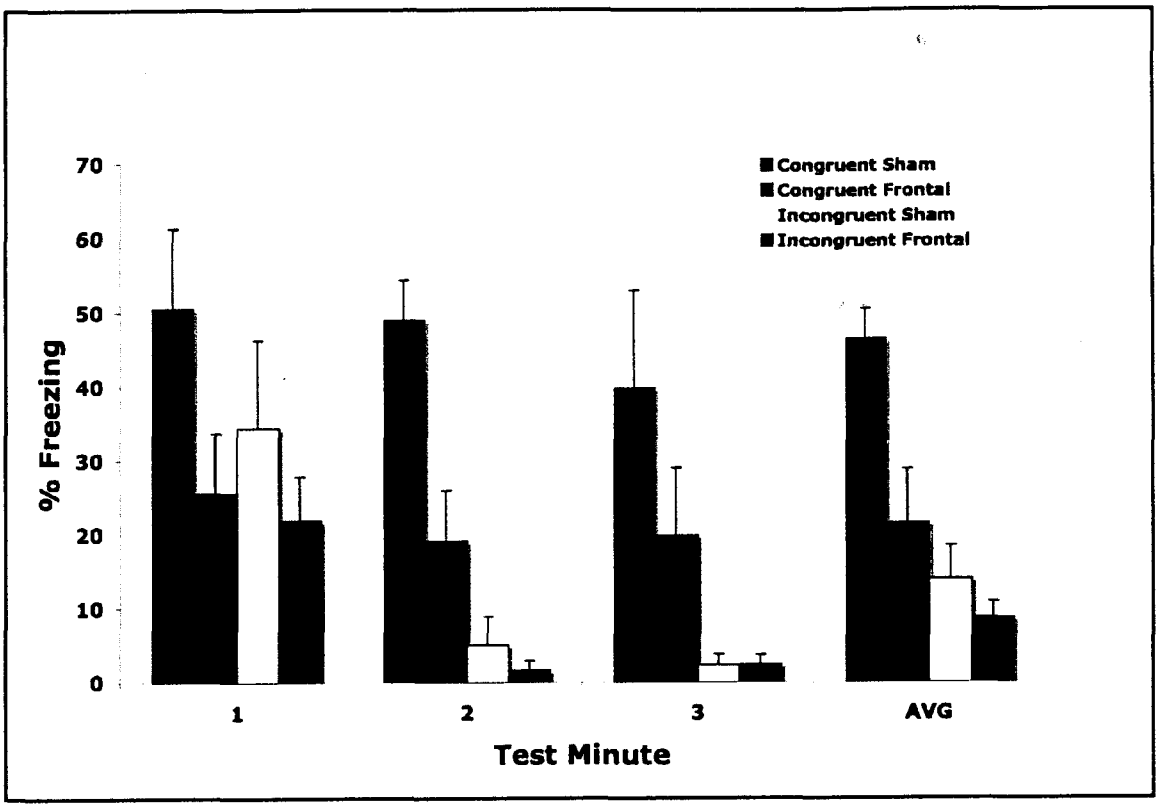


**Figure 3. 6.** Rats without an intact medial prefrontal cortex are impaired in the test of episodic memory described in figure 1.1.