Episodic-like event recollection in the rat: E-maze task, memory retention accuracy and the role of the fornix.

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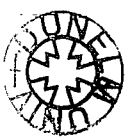


Abstract

Episodic memory was proposed to be a memory system unique to human (Tulving, 1972, 2002), until Clayton and Dickinson (1998) presented evidence form their study of the scrub jays that animals were capable of recalling episodic-like past events.

To date the evidence of episodic-like memory was demonstrated in avian behaviour but not in the non-human mammals. Therefore, this thesis set out to establish a similar "what" "where" "which" episodic-like memory model in rats. Research findings showed that rats can learn a behavioural task encompassing information about specific objects, placed in a given location at a particular time in the recent past, which is evidence of episodic-like recall.

Furthermore, rats were capable of retaining the newly attained episodes for at least 15 minutes and performed the episodic-like test with a decreased degree of accuracy subsequent to sustaining bilateral fornix section. Hence these tests of episodic-like memory in the rat provided evidence that the capability of the non-human memory has potential for further research, proving useful in the general understanding of the episodic memory system.

Contents

Chapter 1: Introduction

1.1 General Introduction

Despite a dramatic increase in understanding of memory in the recent years not all mechanisms underlying memory encoding storage and retrieval are yet fully understood. Here the main theme of investigation followed the evolving concept of episodic memory. This memory system was proposed to facilitate an individual's ability to recall events which had occurred earlier, making mental re-experience of one's own past, through autonoetic awareness, possible (Tulving, 1972, 1987, 2001, 2002). The subsequent text will concentrate on establishing an animal model of episodic-like memory.

Despite numerous objections to the existence of such a memory system (e.g. Squire, 2004), and doubts as to its evolution in an animal cognitively inferior to a human being (Tulving, 2002) this thesis argues that rats can demonstrate memory abilities which have many episodic memory characteristics. In this thesis, I aim to show a behaviourally elicited recollection of specific objects, placed in a given location depending on specific past occasion in non-human animals. The ability of an animal to recall such trial unique episodes were taken to indicate memory ability similar to that of episodic memory in humans. Earlier findings indicated that some animals, scrub jays (Aphelocoma californica), were able to incorporate memories collectively encompassing the concepts of "what" "where" and "when". Consequently, scrub jays were argued to have an ability to produce memories which, in their content, were episodic-like (Clayton and Dickinson, 1999).

The evidence of episodic-like memory however, was thus far limited to behavioural experiments in birds. Research findings discussed here will focus on learning a behavioural task able to investigate the memory of "what" "where" and "which" in a common laboratory animal the rat (Rattus norvegicus). This research also analysed the animal ability to retain such memories by testing the effects of delay intervals on recollection. Also, an involvement of the medial temporal lobe (Aggleton and Brown, 1999), and in particular it's information relay via the fornix neural

bundle (Eacott and Norman, 2004), in episodic-like memory was considered. Building on the earlier episodic-like memory model proposed by Eacott and Norman (2004) this research will focus on producing a task based on event recollection in the rat. Therefore, this investigation should provide further grounds for understanding and research in the general field of episodic memory.

1.2 Taxonomy of memory

Memory is considered to be an area of great complexity, involving many different processes (Murdock, 1970). An ability to acquire, retain and retrieve different types of information allows animals to take advantage of previous experiences in solving multiple problems in their environment. Therefore, different memory systems evolved to link behavioural demands and selection pressures with the solution to new environmental problems, and qualitatively distinct memory systems are thought to have arisen in the course of evolution, to accommodate this memory demand (Sherry and Schacter, 1987).

Increasing complexity of memory systems, from lower to higher animals, became one of the widely assented views (Jarvis et al., 2005). The intricacy of memory capabilities was thought to follow similar progressive linear pattern in the hierarchy of animal kingdom, crowned by 'the most evolved' human (Tulving, 2001), although some recent publications dispute the latter view of the linearity of memory evolution, (Northcutt, 2001; Jarvis et al., 2005). The question of whether different types of information are processed by different memory systems persists to the present day. The same fundamental memory processing was thought to apply to all forms of information, (Roediger, 1990). On the other hand, cognitive neuroscientists have postulated that there are multiple memory systems sub-served by different neural structures (Tulving, 1972, 2002; Gaffan, 1974; Schacter and Tulving, 1994; Squire and Zola-Morgan, 1991; Eichenbaum et al., 1994; Schacter, 1996; Vargha-Khadem et al., 1997; Aggleton and Brown 1999).

In accordance with this view a cognitive neuroscience taxonomy of human memory (Tulving, 1972) was developed (Figure.1). This taxonomy depicted memory as a complicated

hierarchical structure subdivided into ever more specialised memory systems. Here *memory system* referred to an interaction among acquisition, retention and retrieval mechanisms (Sherry and Schacter, 1987).

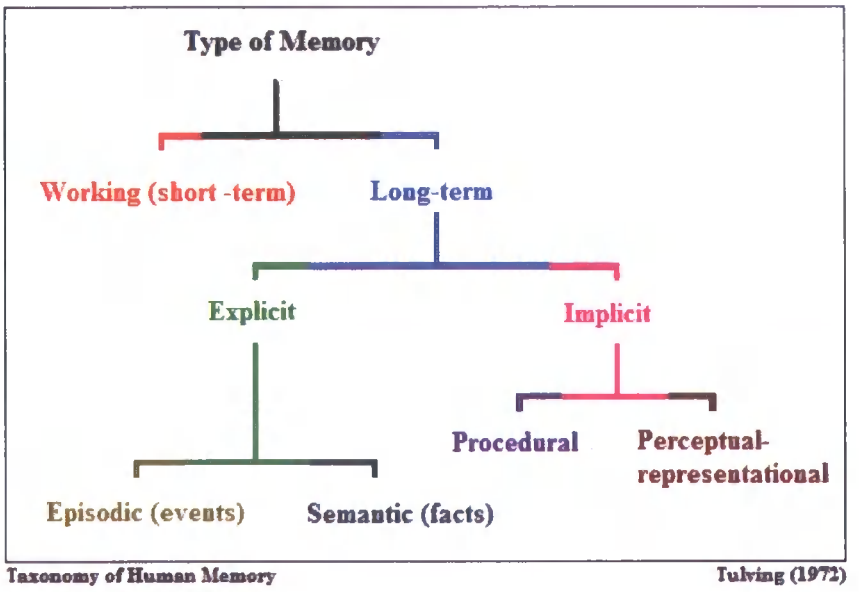


Figure.1 Taxonomy of human memory, from Tulving (1972).

It was the explicit form of memory which was directly relevant in our line of investigation. My research focused on aspect of memory geared to storing facts and events, which in the taxonomy was branded explicit memory (Tulving, 1972). Typically of any memory system, explicit or declarative memory was subject to forgetting and thought to be capable of lasting retention of information (Squire et al., 2004). Although this taxonomy was first put forward to describe human memory, it was also useful in classifying animal memory. For instance, an animal can improve on its task performance and then remember the improvement-reward association (Pearce, 1991). While this division of memory was disputed at first, it served in aiding neuroscience, since each type of memory was thought to be sub-served by different types of neural substrates, medial temporal lobe in the case of the declarative memory system (Tulving, 2002).

Within the declarative memory a distinction was made between a memory system supporting gradual or incremental learning, involved in acquisition of general knowledge and skills, and a system supporting rapid one-trial learning necessary in formation of memories representing specific situations or episodes (Sherry and Schacter, 1987). Therefore, declarative memory was further subdivided to isolate semantic and episodic memory types (Tulving, 1972). Semantic

described a memory system encapsulating the knowledge of the world (Baddeley, 1997). Episodic memory was "a system that receives and stores information about temporally dated episodes or events, and temporal-spatial relations among them" (Tulving, 1983, p.21). Importantly, according to Tulving (2002) this memory system also involved conscious recall of past events (Corkin, 2002).

It is important to point out that this view was not universally acknowledged (Mayes and Montaldi, 2001). Some argued that although intuitively possible, episodic-semantic distinction was not supported by their experimental evidence. A more parsimonious account placed the two forms of memory as different aspects of the same system, (Parking, 1999). This view was put forward most forcefully by Squire and his colleagues, (Cohen and Squire, 1980; Squire, 1987) they suggested that the term 'episodic' and 'semantic' memory should have remained classed as the declarative memory system. Nevertheless, evidence stemming from case studies of localised brain damage (Delay and Brion, 1954; Delay, Brion and Elissalde, 1958a, b), resulting in specific aspects of memory loss (Vargha-Khadem et al., 1997; Temple and Richardson 2004) were interpreted by some as backing Tulving's (1972) episodic-semantic memory system partition.

In accepting episodic-semantic dichotomy, episodic memory was defined as a "neuro-cognitive system, uniquely different from other memory systems, making past experience recollection possible... recently evolved, late-developing, early-deteriorating and past-oriented memory system, more vulnerable to neuronal dysfunction than other memory systems, and unique to humans" (Tulving, 2002, p.5). Episodic memory was proposed to "mark the possibility of mental time travel through subjective time, thus allowing one to re-experience, through autonoetic awareness, one's own past" (Tulving, 2002, p.5).

Tulving (2002) proposed that episodic memory required, and went beyond, the functioning of the semantic memory system. He suggested that at some point in human evolution "episodic memory emerged as an "embellishment" of the semantic memory system. Hence, it represented an instance of the so-called Baldwin effect (Baldwin, 1902)" (Tulving, 2002, p.7). Therefore, the

essence of episodic memory lay in the conjunction of three concepts; self, autonoetic awareness, and subjectively sensed time (Tulving, 2002).

Tulving's (2002) view stipulated that episodic memory was a distinguishing feature between humans and lower animals "an evolutionary "frill" necessary for mental time travel... animals do not have the same ability to travel back in time in their own minds because they did not need to" (Tulving, 2002, p.2). Furthermore, Tulving (2001, 2002) considered episodic memory to be autonoetic (existence of knowledge of one's-self) and such memory was argued to require consciousness. However, if consciousness was necessary to show episodic memory, such capacity would have been very difficult to demonstrate in animals. In the absence of a well developed language system it was deemed unclear what would have constituted the evidence for consciousness (Zentall et al., 2001) as animals, unlike humans, could not easily reflect on their conscious retrieval of past events.

However, since the initial objection to the possibility of an episodic memory system in the non-human animal, (Tulving, 1987, 2002) the presence of an analogous "episodic-like" (Clayton and Dickinson, 1998, p.272) memory processing became evident in some mammalian and avian experiments. Spearheaded by Clayton and Dickinson (1998), an extensive study of caching behavior in scrub jays showed episodic-like recollection of one trial events. Such recollection was exhibited in terms of avian ability to identify the location of specific objects at a given past time (Clayton and Dickinson, 1998; Clayton, et al., 2001). I will allude to this point in greater detail in the subsequent text.

1.3 Amnesia and disruption of episodic memory

Before returning to the concept of episodic-like recall in non-human animals, neurological bases of episodic memory must also be consider. Isolating specific functions of neural structures was one means of identifying the domains of memory sub-systems (Sherry and Schacter, 1987). Episodic memory was proposed to have been based in a widely distributed network of cortical and

sub-cortical brain regions which were overlapping with and extending beyond other memory system networks (Tulving, 2002).

Difficulties in episodic recollection were initially discovered in assessing memory impediments associated with the syndrome of amnesia (Pribram and Broadbent, 1970). The syndrome resulted from damage to the structures of the medial temporal lobe (Whitty and Zangwill, 1977). It was anterograde amnesia, the inability to recall or encode newly experienced episodes (episodic memory), which was demonstrative of episodic memory loss (Vargha-Khadem et al., 1997).

One of the most prominent cases was that of patient H.M. (Scoville and Milner, 1957), where a bilateral removal of the medial temporal cortex and underlying limbic structures (amygdala, hippocampi and their associated fiber connections) resulted in, amongst other deficits, severe anterograde amnesia. Here, the detailed description of the neuroanatomical damage in H.M., made at the time of surgery, marked this case as relatively unique for its time. Subsequently, an extensive MRI investigation revealed an inconsistency between the surgery notes and the actual neurological damage (Corkin, 2002). However, other case studies, F.C, P.B and N.T., (Penfield and Milner, 1958; Milner, 1966; Teuber, Milner and Vaughan 1968), have further implicated damage to the medial temporal structures in memory impairments remarkably similar to those of H.M.

Furthermore, anterograde memory loss has resulted from damage to diencephalic structures (mamillary bodies, anterior thalamic nuclei, medial-dorsal and parataenial thalamic nucleus) though this type of amnesia appeared to be more complex than the aforementioned medio-temporal cortex one (Korsakoff, 1887; Gudden, 1896; Yakovlev, 1955; Parking and Leng, 1993; Clarke et all., 1994). Unfortunately, the proximity of these neural structures impeded the likelihood of attaining a human case study with localized damage to just one of these neural areas (Aggleton and Brown, 1999).

1.4 Early evidence and investigations of neural basis of memory in animal.

Evidence from the human case studies pointed to medial temporal lobe and structures of the diencephalon as neural regions implicated in episodic memory (Whitty and Zangwill, 1977). The bases for these assumptions were initially limited to accidental and untargeted instances of brain damage, as in the case of H.M. (Scoville and Milner, 1957). It was not until the 1980s that possible animal models of amnesia had become available (Squire and Zola-Morgan, 1991).

In testing primate visual memory, memory of, and the ability to distinguish between, previously viewed objects was demonstrated (Gaffan, 1974). The same ability was extended to identifying unfamiliar objects (Mishkin and Delacour, 1975). In these memory tests, rhesus monkeys were shown objects and later presented that same object which was paired with a novel one. The animal was rewarded for identifying familiarity (Gaffan, 1974) or novelty (Mishkin and Delacour, 1975). However, it was claimed that these were tests of semantic memory (Tulving, 1972), much like that of semantic memory in humans (Pearce, 1991). After developing such behavioural tasks, which were subsequently branded delayed match to sample (DMS) (Gaffan, 1974) and delayed non-match to sample (DNMS) (Mishkin and Delacour, 1975), much of the ensuing animal testing involved targeting, and subsequently compromising, various neural formations within the medial temporal lobe; attempting to induce anterograde amnesia in the DMS and DNMS task performance.

Although initially Gaffan (1974) thought to have disrupted DMS performance by severing the fornix which "functions as a tract which conducted cholinergic connectivity to the hippocampus from the medial septum, as well as hippocampal efferents to the diencephalon, striatum, and prefrontal cortex" (Aggleton and Brown, 1999, p.427) these findings were unsubstantiated by later research in monkeys, (Bachevalier et al., 1985a; 1985b; Gaffan et al., 1984; Zola-Morgan et al., 1989). Also, fornix section did not affect DNMS performance in rats (Aggleton et al., 1990; Rothblat & Kromer 1991; Shaw & Aggleton 1993), or have any effect on spontaneous tests of object recognition (Ennaceur & Aggleton, 1994; Ennaceur et al., 1997). In fact it was the extensive lesions of the hippocampal formation and adjacent cortical structures (parahippocampal, perirhinal

and entorhinal cortices, as well as the amygdala) that produced disruption of object familiarity. However, the amygdala was judged not to contribute to the type of memory dependent on the medial temporal system (Squire and Zola-Morgan, 1991). In fact, disruption of the DNMS task was later attributed to compromising the tissues of the perirhinal cortex (Gaffan and Murry, 1992; Eacott et al., 1994; Norman and Eacott, 2004).

Effectiveness of DMS and DNMS tests in assessing the function of structures such as fornix, were subsequently called into question (Aggleton and Brown, 1999). This became apparent when DNMS tests were administered after lesioning the mamillary bodies (innervate by the fornix) had occurred. It had long been accepted that mamillary body degeneration resulted in anterograde amnesia (Wernicke, 1881). However, mamillary body lesions in animals did not disrupt DNMS performance (Aggleton & Mishkin 1985; Aggleton et al. 1990; Zola-Morgan et al. 1989). As these findings failed to support the role of the mamillary bodies in anterograde amnesia, similarly they might have failed to provide evidence for specific function of the fornix.

In accepting that DMS and DNMS tasks were not sufficient to study episodic recollection, as these memory tests could be solved through sense of object familiarity alone (Aggleton and Brown, 1999) alternative tests were necessary to demonstrate event recollection. One attempt to address this problem was to introduce recall of "scenes" composed of an array of different features (Gaffan, 1994). Here, hippocampal lesions impair scene recognition (Gaffan, 1994; Moser et al., 1995). It was therefore proposed that memories of scenes served as evidence for animal recall analogous to that of human episodic memory (Gaffan, 1992). Evidence that stimuli rearrangement in testing after lesions to the rat hippocampus (which was proposed to be analogous to a fornix section) spared novelty recognition, but caused a failure in detection of or response to changes in the learned association between pairs of cross-modal stimuli (Honey et al., 1998) offered further backing for Gaffan's (1992) view. This research demonstrated that the properties of episodic memory in humans evolved from the capability to organize memory representation in the non-human animals (Eichenbaum, 1997). Importantly, although the new approach distinguished

memories in terms of scenes it was not providing a task that tested animal ability to recall one-time unique episodes, as defined by Tulving (1983, 2002) in his description of the criterion for the human episodic memory.

1.5 Neurological structures underlying recall and familiarity

Nonetheless, a recently published model of episodic memory (Aggleton and Brown 1999), provided great aid in understanding the structural bases of the neurology described above. This model was composed using information derived from clinical and experimental (lesion, electrophysiological, and gene-activation) studies. This model differed from others by placing critical importance on the efferents from the hippocampus via the fornix to the diencephalon (mamillary bodies and the anterior thalamic nuclei): the "extended hippocampal system", (Figure.2).

Although fundamentally accepted by many (c.f. Squire et al., 2004), this model was only reflecting the understanding of the research findings acquired up to the date of its formulation, and further research advances in the area had thrown up additional elaborations. For instance, (Eichenbaum, 2000) proposed supplementing the model by considering the role of the neo-cortex. Nor was this model devoid of its critics. Squire, Stalk and Clark (2004) fundamentally disregard involvement of diencephalon and concentrated entirely on the functions of the temporal lobe, Figure.3. Moreover, in the majority of human amnesia both hippocampal - anterior thalamic and perirhinal - medial dorsal thalamic systems were compromised, making deficits in recall and recognition apparent, (Aggleton and Brown, 1999). This provided a further case for targeting specific neural structures, backing the inclusion of animal studies in episodic memory research.

In accepting this model as a depiction of neurological bases of episodic memory it was considered that the "extended hippocampal system" formed the basis for encoding and subsequent recall of one trial unique episodes, or episodic memory. This same "hippocampal system" was irrelevant in item recognition, otherwise thought of as ability to judge items as familiar. Familiarity

judgements were attributed to an independent process, a distinct system, involving perirhinal cortex of the temporal lobe and the medial dorsal nucleus of the thalamus (Aggleton and Brown, 1999).

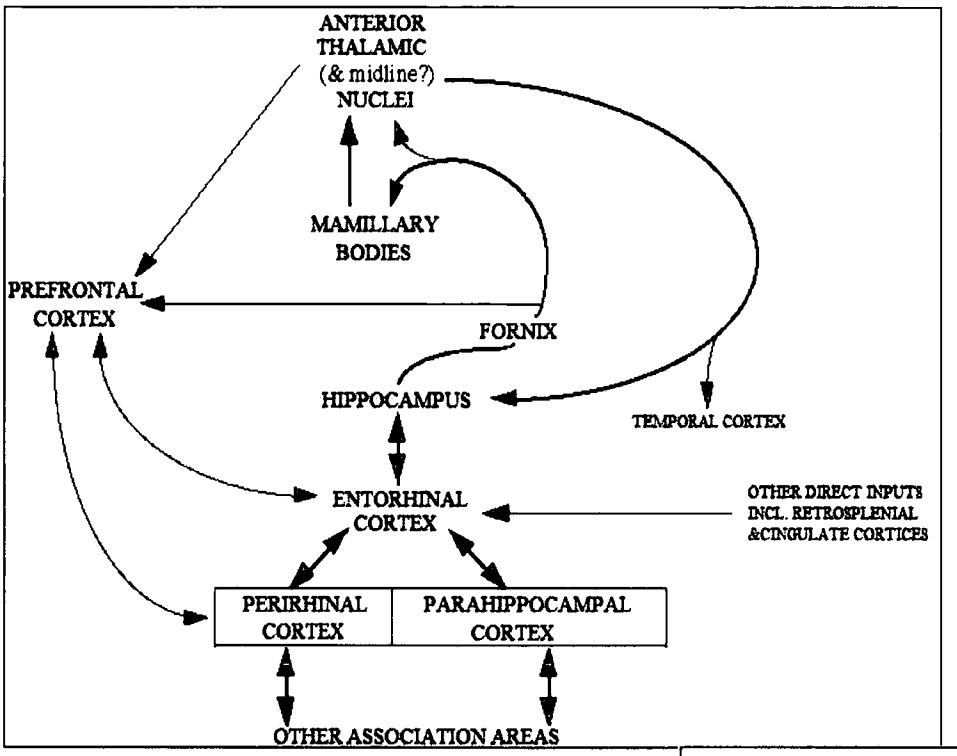
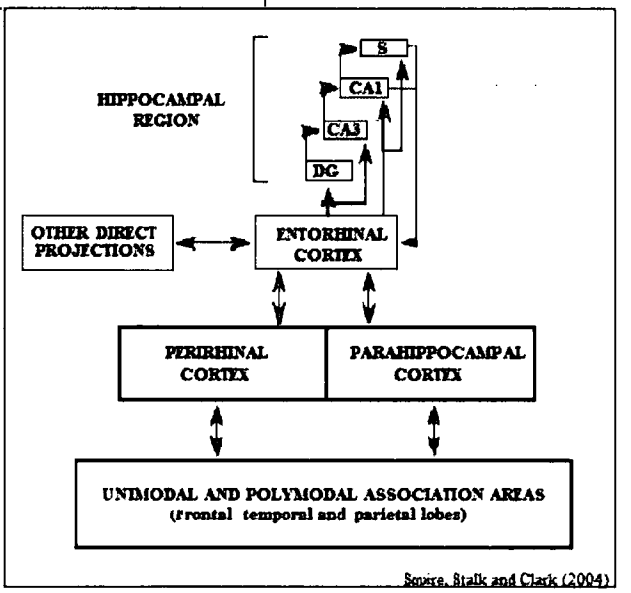


Figure.2

Episodic Memory Model (schematic diagram of the principal pathways that underlie encoding and recollective information pathways) Aggleton and Brown, (1999).



Schematic view of the medial temporal lobe structures important for declarative memory S, subicular complex; DG, dentate gyrus; CA1, CA3, the CA fields of the hippocampus (Squire, Stalk and Clark, 2004; as adapted from Burwell et al. 1996).

Figure.3

From the theoretical standpoint familiarity was analogous to the precise match between a current stimuli and stored memory (equivalent to a semantic memory). Recollection was said to involve retrieval of time dependent, associative and contextual information about an event (episodic memory), (Yonelinas, 2002). Human recollection tasks usually stipulated recall of specific events or episodes (Gron and Riepe, 2003). Animal models however, often failed to demonstrate recollection rather than familiarity (Eacott and Norman, 2004; Ergorul and Eichenbaum, 2004). To present a

quantifiable measure of recollection in non-human animals the episodic memory was classed as; happenings in particular places at particular times, or "what," "where," and "when" (Clayton & Dickinson 1998, Nyberg et al. 1996). However, as the integration of the three concepts, as it is manifested in the human recollection, could not be proven and the presence of autonoetic awareness remains an unanswered paradigm in animal memory (Tulving, 2002) animal event recollection was branded as "episodic-like" by Clayton and Dickinson (1998). It was this later type of episodic-like recall that our experimental methodology attempted to address.

1.6 Animal episodic-like recollection

The question of whether any non-human species displayed episodic memory was controversial (Pearce, 1991). It was suspected that certain animals possess an episodic-like memory system, because a variety of learning and memory tasks were developed which, though not meeting the strict criteria required for episodic memory, did have an 'episodic-like' character (Morris, 2001). These included certain one-trial learning tasks, scene-specific discrimination learning, multiple reversal learning, delayed matching and non-matching tasks and, most recently, tasks requiring recollection of "what", "where" and "when" an event happened (Gaffan, 1974; Mishkin 1975; Aggleton, 1985; Clayton and Dickinson, 1998; Morris, 2001; Eacott and Norman, 2004; Ergorul and Eichenbaum, 2004; Fortin and Eichenbaum, 2004). Another reason was that the neuronal architecture of the brain areas thought to be involved in episodic memory (including the hippocampal formation) were relatively similar in mammals and, arguably, all vertebrates (Jarvis, 2005).

Episodic-like memory abilities in a lower animal were first demonstrated by Clayton and Dickinson, in their scrub jay memory research, published in 1998. Clayton and Dickinson (1998) showed that food hoarding birds could remember spatial-location and the content of caches. Furthermore, food hoarding birds could learn to adapt their caching and recovery strategies to the perishability of food, which suggested that this behaviour was subject to memory flexibility. Hence,

the "what" of food and "when" perishability was associated with the memory of "where" particular food items were cached, fulfilling behavioural criteria for episodic-like memory in non-human animals. This distinction differed from that of the episodic memory in humans, as it did not attempt to claim the presence of the autonoetic experience in the animal's event recollection (Griffith et al., 1999).

However, it has been argued that this study demonstrated memory but not "mental time travel" as the study did not claim that the birds had the ability to mentally revisit the time of the original event, and did not project their memory of the event into the future to guide their behaviour (Eacott et al., 2005). A number of subsequent studies attempted to recreate episodic-like memory conditions. Recently, signal detection techniques in rats showed that odour recognition had characteristics of both familiarity and recollection, (Fortin and Eichenbaum, 2004). Ergorul and Eichenbaum, (2004) provided rats with spatial location and odour cues to distinguish the time of the event occurrence following a sequence of event presentations. However, here "where" and "what" cues could have been used independently. Elaborate experiments were devised to test rodent's ability to discriminate between objects "what" in different special location "where", given interchanging context of the enclosure "when", (Mumby et al., 2002). Yet, these tests did not demonstrate rat's ability to use recollection in the absence of familiarity, nor was it shown that these animals could use recollection to plan future behaviour.

Nonetheless, mounting evidence acquired in search of "what" "where" and "when" recollection of unique personal experiences in animals, did fulfil the general criteria for Tulving's (1972) proposed episodic memory, and were thus termed episodic-like (Clayton et al., 2001). However, these studies had not as yet provided unambiguous evidence that this capacity was based on the recollection of learned episodes (Eichenbaum and Fortin, 2003).

1.7 Leading to Eacott and Norman (2004)

Although episodic memory exists in humans, this form of memory is particularly prone to alteration and inaccuracy (Schacter, 1995). In particular with relation to the "spatio-temporal"

continuity of self, used to determine "when" in the relative past an event had occurred (Campbell, 1994; 1997). To recollect an event is reasonably common, but to be sure of the exact time when an event had occurred, in the "time travel" analogy (Suddendorf and Carbollis, 1997), remains a common difficulty (Eacott et al., 2005). Clayton and Dickinson (1998) managed to demonstrate that scrub jays were able to remember "when" the caching episodes had occurred, within the realms of episodic-like recall, the same episodic-like ability hadn't been experimentally demonstrated in other species; mammals or birds alike. This was particularly true when attempting to show the evidence of the time passage ("when") as one of the integrated element of the "what" "where" "when" triad, (Eacott et al., 2005).

To address the issue of experimentally distinguishing the concept of "when", in an episodic memory task, Eacott and Norman, (2004) devised a study which presented animals (rats) with a choice of distinct sequential time periods during which an episode had occurred. This was done by means of introducing rats into two pictorially and visually different enclosures (which Eacott and Norman, (2004) labelled as "contexts"). Animals were placed into either black and white (context 1), or wire mesh and wood (context 2) fitted enclosures. The ability of the animals to discriminate between events which took place in each of the two enclosures was argued to be demonstrative of the concept of "when" (now substituted with the simplified concept of "which" occasion – context 1 or context 2?).

In testing episodic-like recall animals were presented with two distinctly different objects "what" (A and B). These objects were used only once, thus making the experience trial-unique. Importantly, the two objects were placed on the opposite sides of the enclosure (for instance A on the left and B on the right side of the arena) thereby establishing the location concept of "where". Rats were subsequently placed into context 1 and then 2 (object location; context 1:A-left&B-right and context2:B-left&A-right), the context order was randomised throughout test trials. In the test phase the rats were entered into one of the two contexts selected at random, which contained a pair of either objects A or B. Thus, one object in the pair would always be uniquely experienced, in a

given position, within a given context. This created a one trial unique episode synonymous to an episodic-like event, Figure.4.

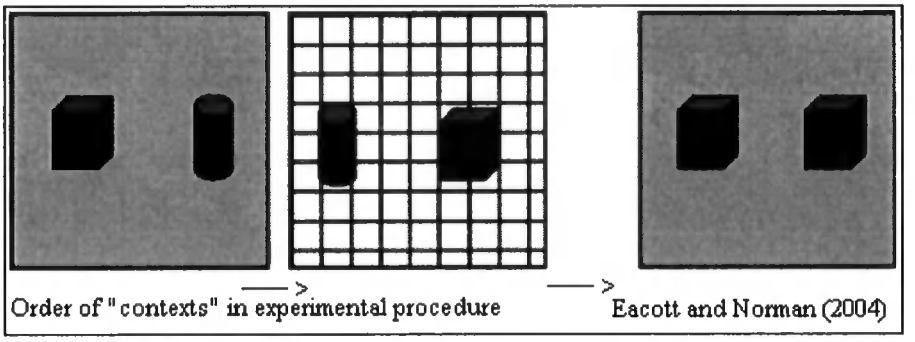


Figure 4.

Experimental procedure, from Eacott and Norman, (2004).

Unlike humans, animals couldn't be asked an experimental question, as no language exists enabling such discourse to take place. However, in the experiment by Eacott and Norman, (2004) language was taken to mean a set of commonly held rules which allowed questions to be asked (and answered), to ensure that the task 'instructions' were understood, (Zentall et al., 2001). In the above experiment rats were trained to acquire a greatly simplified set of behavioural rules.

Earlier behavioural experiments demonstrated that rat memory could be assessed based purely on object exploration time (Ennaceur and Delacour, 1988). When rats were presented with various combinations of neutral objects the distinguishing feature of animal exploration lay in relative novelty of objects. Notably exploration of novelty was by far longer lasting than that of familiar objects. Therefore, evidence for remembering an object was displayed by the duration of the object exploration time (Ennaceur and Delacour, 1988).

Ennaceur and Delacour (1988) considered memory to be a unique event based the on repetition of the same conditions. Hence, stemming from the earlier DMS and DNMS procedures in the monkey deemed insufficient in episodic-like memory research, Ennaceur and Delacour (1988) produced object exploration criteria for one-trial based learning of experimental rules. Eacott and Norman (2004) incorporated this approach into their experimental setup, producing an alternative approach to investigating behavioural manifestations of episodic-like memory in a rat.

Experimental findings backed this approach. Rats demonstrated a marked preference for objects in a novel configuration, suggesting that rats were indeed able to distinguish novel combinations of "what" "where" "which". Eacott and Norman (2004, p.1948) proposed that this test

was providing "a possible model of episodic-like memory" in a rat. In fact rats were shown to demonstrate "memory for two highly confusable episodes after single exposure over delays of up to 1 hr" (Eacott and Norman, 2004. p1953). This model was based on natural exploratory behaviour, following a non-reward criterion and was therefore argued to provide a true manifestation of memory free from any additional physiological manipulation (Ennaceur and Delacour, 1988).

However, the setup of the experimental arena was such that an increased exploration of 'novel' situation could have come about through the implicit association and not recollection. Some critics suggested that this experiment was not testing free recall of stimuli, as just a sense of familiarity was enough to identify test objects in the open field, (Squire et al., 2004). Familiarity alone, as mentioned above, was said to be insufficient to demonstrate all aspects of the episodic-like memory, (Tulving, 2002; Squire et al., 2004).

Consequently, and in view of the familiarity problem in the Eacott and Norman, (2004) task, a reliable behavioural manifestation of episodic-like memory in the rat, had not yet been ascertained. Based on the open field exploration tests of Eacott and Norman (2004) further elaborations of the experimental procedure and setup were undertaken to produce an alternative model. As a result an E-maze (Eacott et al., 2005), comprised of interchanging visual and tactile contexts, was used to extrapolate the recall element of episodic-like memory in the rat.

1.8 *Fornix*

Importantly Eacott and Norman's (2004) study had also addressed the question of the neurology underlying the episodic-like memory model they proposed. As part of their exploration, specific areas of the rats' medial temporal lobes were compromised and the animals were assessed for the effects on event recollection (or recognition, as argued by Squire et al., (2004)). Of particular interest was the effect of fornix section on the ability to recall the novel situations of the test. Fornix transection resulted in a severe impairment of the task. This effect was not observed in the animals with sham, perirhinal or postrhinal lesions (Eacott and Norman, 2004).

In fact within the "extended hippocampal system" the fornix had been a site of particular interest in memory research. Particularly as some viewed fornix severation as an equivalent to a hippocampal disconnection (Olton et al., 1982, Aggleton et al., 1986b, 1992). This was because structurally the fornix conducts cholinergic connectivity to the hippocampus from the medial septum, as well as hippocampal efferents to the diencephalon, striatum, and prefrontal cortex (Aggleton and Brown, 1999). Consequently, it forms a "vital bridge between medial temporal and medial diencephalic regions" (Aggleton and Brown, 1999, p.427), areas implicated in anterograde amnesia.

The implication of the fornix in amnesia was noted, in human case studies, as far back as 1939 by Campbell and Biggart. As mentioned in **1.4** fornix transection was reported to cause impairment in the delayed match to sample memory tests (Gaffan, 1974). However, a number of later DNMS and DMS studies found that fornix transection produced little or no recognition deficit in monkeys (Bachevalier et al., 1985a; 1985b; Gaffan et al., 1984; Zola-Morgan et al., 1989), and spared DNMS performance in rats (Aggleton et al., 1990; Rothblat & Kromer 1991; Shaw & Aggleton 1993). Hence, fornix was dismissed as insufficient for the induction of anterograde amnesia (Squire & Zola-Morgan 1991; Zola-Morgan et al. 1989).

However, human case studies of fornix damage (Aggleton et al., 2000). And more recently animal studies geared at episodic-like recall (Eacott and Norman, 2004) produced evidence that fornix is indeed implicated in episodic memory function (Aggleton and Brown, 1999). In view of the criticisms of the Eacott and Norman (2004) task, in that the preferential exploration of objects could have arisen due to the sense of familiarity and not necessarily recall, in devising a methodological elaboration on Eacott and Norman (2004) task in this thesis, the animal memory ability would also be tested after the fornices of the rats were compromised.

1.9 Aims of my Thesis

Based on the work of Eacott and Norman (2004), a further elaboration of the open field tests will be devised. The all encompassing aim is the development of an experimental procedure and

apparatus aiding in establishing a memory model of episodic-like recall in the rat. As in the Eacott and Norman (2004) object preference will be induced by means of habituating individual animals to the experimental objects, where every trial will be presented as a novel trial-unique event, as in Ennaceur and Delacour's (1988) research. Here however, animals will be introduced to a maze search, not an open field setup, as was the case in Eacott and Norman (2004). To achieve this an E-shaped maze will be developed, and in addition a single object habituation stage introduced to induce relative-novelty preference in later testing.

The aim is to achieve four experiments. Initially, testing "what" and "where" exploration to determine whether the animals could remember and identify the location of objects in the E-maze. Here the crucial "when/which" will not be addressed. Such testing will present animals with two directions in which they could turn. An animal is expected to seek out hidden non-habituated objects within the arms of the E-maze. The memory of the object location should therefore be based on remembering object-place association from the previous maze visit. Natural preference of novelty exploration is expected to be of sufficient enough reinforcement power to induce such behaviour.

Were the initial testing to prove successful the subsequent test will aim at establishing an equivalent of Clayton and Dickinson (1998, 1999) "what" "where" and "when" episodic-like memory model for use with the rat. Based on Eacott and Norman (2004) the concept of two pictorially and visually different contexts will be used to facilitate the concept of "which" (argued in Eacott et al., (2005) to represent a broader notion of "when") one of the occasions, marked by the contexts X or Y, represented a given past episode. Object ("what") location ("where") would be associated with a given context, forming a trial-unique episode that the rat will recall, to find the E-maze arm containing non-habituated objects. Here preferential turning toward relative novelty is predicted. We also considered the effects of object exploration time.

In the third phase of testing the "what" "where" and "which" task further investigated by introducing a delay interval between initial exploration and test condition. Providing delays ranging

from a minimal delay of less than a minute up to one hour, this is proposed to test the endurance of the newly acquired episodic-like memories. As Eacott and Norman (2004) demonstrated event memories to be relatively long-lasting, we predict that novelty preference will also endure, with diminishing strength, over time.

Finally the role of the fornix in an episodic-like recollection will be tested. Fornix-sectioned rats will be introduced to the "what" "where" "which" test. It is predicted that the fornix sectioned animals will have an impaired episodic-like recollection compared with the sham-operated rats. The fornix sectioned animals should be unable to distinguish "which" of the contexts represented the relevant episode and their ability to predict the object location would be compromised. Therefore, fornix severed animals would not display preferential turning to novelty, but the control animals would. This research will thus attempt to develop new parameters for a laboratory test of episodic-like memory in a rat.

Chapter 2; Object in Place recognition (*Test 1*)

2.1 *Introduction*

Human episodic memory was defined as "a system that receives and stores information about temporally dated episodes or events, and temporal-spatial relations among them" (Tulving, 1983, p.21) in humans. However, despite extensive study episodic recall was not comprehensively demonstrated in the non-human animals, (Tulving, 2002). Such criteria as consciousness or autonoetic awareness were difficult to pinpoint behaviourally in assessing animal recollection, as quantification of such memories were obtained linguistically in humans (Zentall, 1997; Zentall et al., 2001). Given that animal memory, prior to the findings reported in this thesis, could only be tested behaviourally Clayton and Dickinson (1998) proposed that animal recollection was expressed through knowledge of "what" "where" and "when" a past episode had occurred. A definition taken from Tulving's (1972) original description of (what, where, when) information was stored as a representation of the human episodic memory. In animals such memory was branded episodic like, introducing the term episodic-like memory. Episodic-like memory did not incorporate evidence of conscious (or autonoetic) awareness integrated into episodic memory definition by Tulving in 2002, but described the integration of "what" "where" and "when" and became a recognised classification in the recent animal memory research, (Clayton and Dickinson, 1998; Clayton, at al., 2001).

Thus far, scrub jays were demonstrated to possess episodic-like memory, (Clayton and Dickinson, 1998). However, no other animal species has been shown to exhibit the same ability to recall trial-unique events (Tulving, 2002). Eacott and Norman (2004) designed a behavioural task, aimed at testing episodic-like memory in the rat (see Chapter 1.7). This experiment however, was unable to demonstrate indisputable event recollection. The experimental setup was based in an open field arena and on a recognition paradigm, therefore some argued that the animals could have been influenced by a sense of familiarity (feeling of knowing) and not remembering a specific episode experienced in their earlier exploration, (Squire et al., 2004). Since demonstrating the ability to

recall is central to episodic (Tulving, 2002) and episodic-like memory (Clayton et al., 2001, 2003) Eacott and Norman's (2004) task required procedural changes to create experimental conditions capable of yielding episodic-like recall.

Therefore, as in Eacott and Norman (2004) the first set of behavioural trials, performed as part of this research, was designed to test spontaneous object exploration. In this experiment the habituation methodology of Ennaceur and Delacour (1988) was used. Ennaceur and Delacour (1988) demonstrated that rats explore novel objects much longer than those they had explored in the recent past, therefore presenting behavioural evidence for remembering the previously seen, and therefore "habituated" objects. Ennaceur and Delacour (1988) used object exploration time during the final object exploration stage as the measure of object familiarity. However, in the current experiment the objects were hidden in the arms of the E-shaped maze, so the animal would have to choose the correct object location based on the recollection of the past event. One additional modification was an introduction of the "object habituation" stage, which was necessary to differentiate between the two objects hidden in the arms of the E-maze. Hence, the animals spent some time exploring one object independent of their maze exploration experience.

Procedurally this test was simplified, as compared to Eacott and Norman (2004) to provide a preliminary memory test before episodic-like memory was tested. This experiment tested object-place ("what" "where") memory, which was experienced in recent past. The concept of "context" discrimination was not used here, as we did not test animal ability to distinguish between "which" of the two past episodes had occurred, unlike Eacott and Norman (2004). Thus, not all aspects of episodic-like memory were encapsulated in this test. Rats were expected to be able to identify the arms of the E-maze containing non-habituated objects. It was predicted that experimental animals would prefer turning toward the arm of the maze containing relative novelty. It was also established that rats spent more time exploring novelty (Ennaceur and Delacour, 1988; Eacott and Norman, 2004), therefore exploration time (during 'test' phase) itself would be a factor. Animals partaking in longer object exploration were predicted to show more reliable event recollection.

Finally, in this experiment rat predisposition to novelty exploration was the only motivation behind driving this behaviour to occur. So, unlike most tests of "where" and "what" that used physiological rewards such as food, water or invoking a fear responses (Morris et al., 1986, 1998; Bontempi et al., 1999; Vann and Aggleton, 2003; Kennedy and Shapiro, 2004; Canal et al., 2005) and penalties (Fanselow, 1980; Anagnostaras et al., 1999) this experiment observed naturalistic exploratory behaviour. Thus, our test would present evidence of spontaneous object-location association memory. Also, this trial would serve as a prerequisite control task for "which" "where" "when" experimentation, to be discussed in the later chapters.

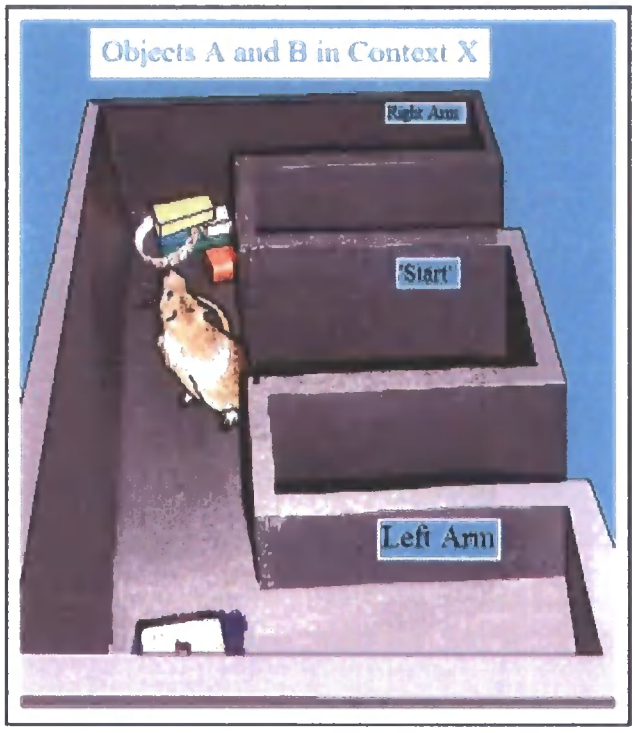
2.2 *Method:* What, Where Task *(Behavioural Methodology)*

2.2.1 *Subjects*

Five experimentally naïve male rats *(*Rattus norvegicus), Dark Agouti strain were used. At the start of testing they were aged 12 weeks, length 180-200mm (nose to tail) and weighed 180-250g. They were housed in sets of two or three in diurnal conditions (12h light/ 12h dark cycles) and tested during the light cycle. Throughout the study all animals had ad libitum access to food and water. All experiments were conducted according to Animal (Scientific Procedure) Act (1986) and as permitted by the Home Office Project License.

2.2.2 *Apparatus*

The E-maze used in the behavioural test (60x37x21centimetres) had objects placed in the peripheral (left and right) arms of the maze (Figure.5). The central stem, or the 'start' chamber (15 centimetres in length), was used to let the rats into the maze. A trap-door separated 'start' enclosure from the main body of the maze. The walls and floor of the cage were smooth and coloured black. The E-maze was covered by a transparent plexiglass lid (61x43cm). On the outer surface of the lid two sets of white lines were drawn.



(E-maze) Objects A and B in the "what" "where" habituation test phase

Figure.5

These lines encompassed the area of the maze around the 'start' enclosure. When the rat remained inside the area marked by the white lines it was unable to see the objects hidden within the right and left arms of the maze. Therefore, as long as the animal's head was inside the marked territory the objects in the peripheral arms were obscured from the animal's view. During the 'test' phase (see later), ones an animal had crossed one of these lines, it was judged to have made a choice (left or right turn).

During every trial the rats were given two different objects to explore (objects A and B). Every experimental object was used only once throughout habituation and testing. In total 40 novel objects were presented to every animal. In testing, three copies of every object were used, so during three stages of each experimental trial an animal was exposed to a different copy of the same object. Such object duplication prevented any possible scent marking, which could have aided rats in their object search.

To avoid interference with naturalistic exploration the experimenter was hidden from the animal's view. Rats were filmed on video (Panasonic Hi-Fi stereo VHS) and the footage was stored on JVC compact video cassettes. The camera was positioned on a tripod (Velbow CX 440) suspended 150 cm above the 'start' chamber of the E-maze, and the rats were habituated to the presence of the video-camera.

To habituate the animals to one out of the two experimental objects, which were presented on daily basis trials the 'habituation chamber' was used. It was a rectangular box (40x 28x 28

centimetres). The walls and the floor of the enclosure were opaque white plastic. The lid was transparent plexiglass. Four corners of the chamber were marked a, b, c and d to indicate the location of the habituation objects, which were presented randomly at every trial. Daily procedural protocols were created to randomise the order and location of object presentation. Thus, creating one-trial unique testing procedure for every experimental animal (appendix b).

2.2.3 *Design*

In this study all animals were exposed to the same E-maze and object habituation, and the same 18 days of experimental procedure trials, although the factors of the position of the (left or right) and the direction of the 'correct' turn was balanced across days and rats. This prevented any possible object preference when the search for non-habituated object was performed during the 'test' stage (Figure.6).

Therefore, in this experiment the location of a non-habituated object (A or B) became the experimentally manipulated independent variable. Consequently the dependent variable in this study became the direction of turning (toward the left or right arms of the maze) performed by rats, in search of the preferred non-habituated object. Also, object (A and B) object exploration time in the first experimental phase (Figure.6) presented an independent variable, which was hypothesised to influence the later search for the non-habituated object, an experimental dependent variable.

2.2.4 *Procedure*

a) *Trial phase*

The experimental procedure was subdivided into a succession of habituation to the E-maze and experimental objects, and 18 experimental trials. The following were the order of stages given to our rats: During *Stage 1* animals were placed in the E-maze in pairs in order to accustom them to the maze environment. During this stage the E-maze did not contain any objects. Animals received four such habituation trials, each trial lasting 30 minutes.

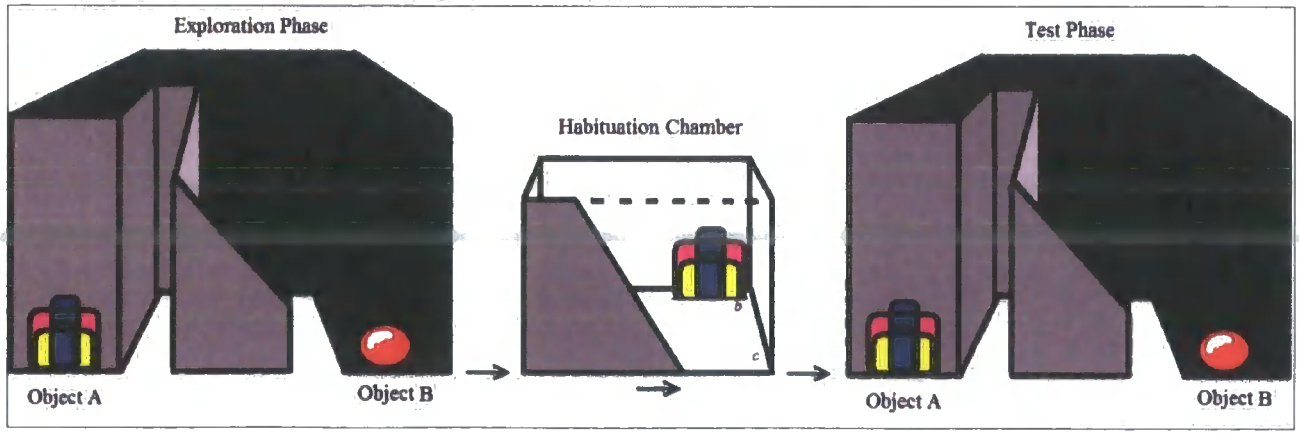
In *Stage 2* animals were placed into the E-maze individually. Rats received two trials in which they spent five minutes in the maze. Animals were exposed to two objects and allowed to explore them in order to accustom them to the maze and object exploration. Objects at this stage of habituation were placed in the visible corners of the E-maze. The animals were then placed in a separate holding cage for five minutes (no objects present) before finally being returned to the maze for five minutes with objects remaining in their previous location. This stage allowed the rats to experience that the location of the objects in the E-maze remained stable within a day.

The proceeding *Stage 3* was the main experimental stage (Figure.6). Here the animal was placed in the start box and released to explore the E-maze. The objects were placed at the end of the right and left arms, visible only upon entering the arms themselves. The rats remained in the maze for 5 minutes. Subsequently, rats were placed into the 'habituation chamber' containing one of the two experimental objects (counterbalanced). The object habituation stage lasted for five minutes, after which the animal was returned to the E-maze, initiating the 'test' phase of the trial. Upon exiting the start-box the animal could turn either towards the position of the now-highly familiar habituated object, or towards the relatively novel unhabituated object. The direction of the first turn (toward habituated or non-habituated objects) was the experimental measure.

Stage 4 was an additional memory test. The procedure was the same as in *(Stage 3.)* with the exception that during the 'test' phase both objects were removed from the E-maze, controlling for any possibly of unintended cues, such as odour cueing.

Figure.6

"What" "where" test procedure (exploration followed by habituation and test)



b) Post experimental data collection phase

The rat behaviour was filmed and the video footage subsequently scored. When the rat turned left or right, after leaving the 'start' box and crossing the vector past which the hidden object had become visible, the rat was judged to have chosen an object. Turning toward non-habituated object first was scored as remembering location of relative novelty on that trial (appendix a).

When object exploration was recorded, the rats were judged as exploring, when they held their nose at a distance of ≤ 1 cm from the object and/or touched it with their nose. Turning around or sitting on the object was not considered exploration. Hence, the basic measures were the directions of preferential turning and the total time spent by rats in the exploration of an object during the individual exposure periods.

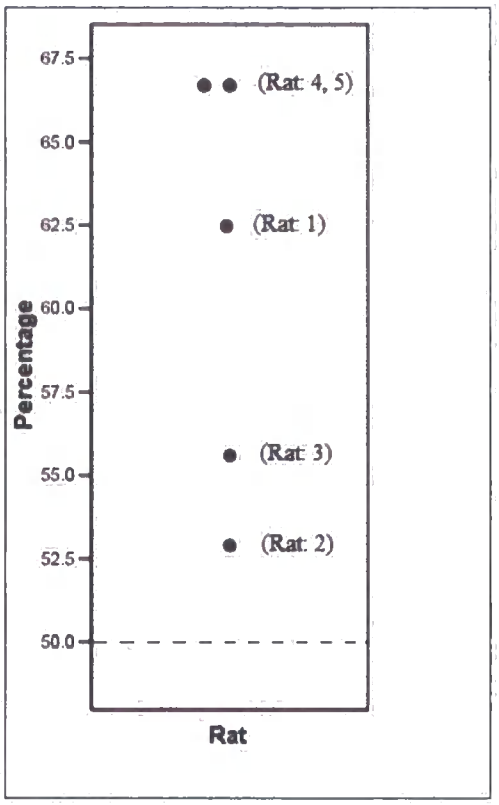
2.3 *Results*

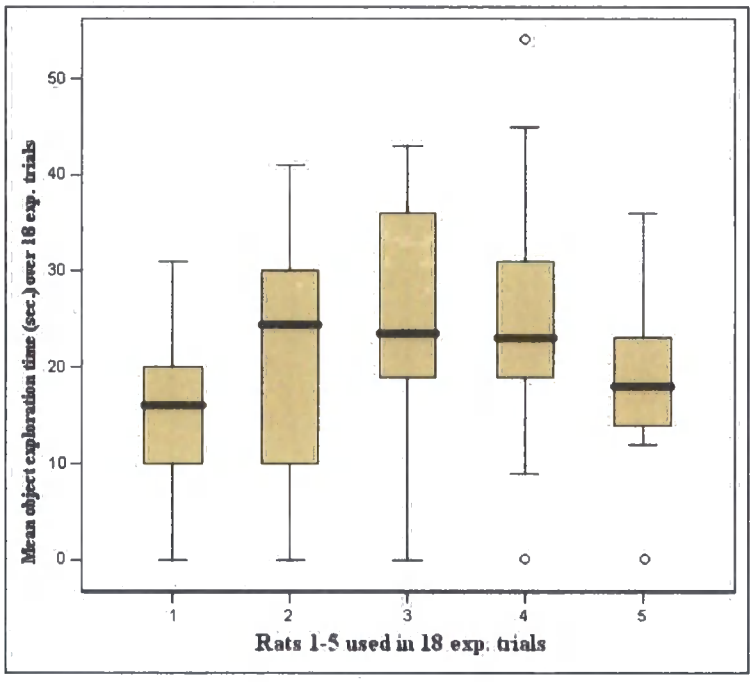
Over 18 experimental trials all five rats tended to preferentially turn toward the novel objects (or in the case of the probe trials, its previous positions). The probability of first turns made toward the E-maze arm containing non-habituated objects exceeded 50 percent, averaging 60.9 percent across all animals (Figure.7) ranging between 52.9 and 66.7 percent. Overall, this represents a significant preference for choosing the arm which contained the non-habituated object significantly more often ($t_4 = 3.824$, $p < 0.01$).

Overall object exploration time during the exposure phase varied significantly between experimental animals, ANOVA ($F_{1,4} = 3.052$, $p<0.02$), Figure.8. and there was no correlation between mean exploration time and the number of correct turns ($r = -0.186$, $p=0.8$). It is worth noting however, that N=5 is a relatively small population size, relative to the numbers recommended to be used in analysing parametric statistics. Therefore, the test might not have been powerful enough to detect the relationship between exploration time and turning toward the novel objects.

Percentage of turns toward the E-maze arm containing the non-habituated object.

Figure.7





Mean object exploration times for the five experimental animals

Figure.8.

2.4 *Discussion*

Experimental findings demonstrated that rats were able to retain memory of object location within the E-maze, which was consistent with the findings of T and radial maze studies (Bontempi et al., 1999; Kennedy and Shapiro, 2004). Thus, showing that rats could retain "what" "where" types of memory, as the sample of animals used in this test was turning toward the hidden, non-habituated object significantly more often than not. Most importantly, these findings confirmed that the object habituation paradigm was a reliable method of identifying object preference. Habituation therefore, presented a reliable test of memory for place object association in a rat.

Furthermore, this research showed that using first turn as an indication of memory for the location of an object within a single context in an E-shaped maze could be developed as a useful indicator of rat memory of unique events. Also, this experiment demonstrated that spontaneous

exploration may provide an alternative way to study event memory, providing an alternative to experiments which use physiological reward (e.g. Kennedy and Shapiro, 2004).

Therefore, it could be possible to use this behavioural methodology to test more complex types of memory. This test confirmed that naturalistic exploration of relatively novel objects was a strong enough reinforcement for rats to prompt a search for hidden objects. Rats could retain memory for previously experienced episodes and predict the location of the hidden object, based on this past recollection. Interestingly, in this sample of animals object exploration time during the 'test' phase did not correlate with memory accuracy. This suggests that in "what" "where" association, additional object exploration did not aid subsequent recall however, given the very small sample size this may be usefully be explored further.

This initial set of test trials did not encompass one concept vital to episodic-like memory, the ability to distinguish between past experiences; the notion of "when" (Clayton and Dickinson,1998) or "which" (Eacott and Norman, 2004). Therefore, the following experiment will incorporate the criteria of "which" by following the methodology of Eacott and Norman's (2004) experiment. This will be achieved by introducing a visuo-spatial context for an event, providing the "which" element of episodic-like memory described in Chapter 1.7.

Chapter 3; "What" "where" "when" context specific object-in-place recognition (*Test 2*)

3.1 *Introduction*

As discussed in Chapter 2, thus far, the behavioural methodology developed there was unable to model all attributes of episodic memory in animals. Clayton and Dickinson (1998) suggested that the capacity to remember "what" "where" and "when" a specific event had occurred provided evidence for episodic-like memory. Those authors argued that food-caching behaviour in scrub jays meet the essential criteria for episodic-like memory (Clayton et al., 2003). However, such episodic-like memory was demonstrated in avian species, separated from mammals by hundreds of millions of years of evolution (Jarvis et al., 2005). Earlier primate studies addressed combinations of "what" "when" (Hampton, 2001; Fortin et al., 2002), "where" "when" (Chiba et al., 1994) and "what" "where" (Day et al., 2003) memories. These studies however, were not incorporating all necessary components required in the formation of an episodic-like memory (Ergorul and Eichenbaum, 2004). It was the "when" element of the episodic-like triad that proved particularly problematic (Eacott and Norman, 2004).

Subsequently, Ergorul and Eichenbaum (2004) attempted to incorporate "what" "where" and "when" in their sequence learning of odour and location. However, the design of the test was such that, although the combination of odour and location provided more accurate memories, the two cues could have been coded independently and not in an integrated manner. Eacott and Norman (2004) demonstrated that in an open field rats were able to discriminate between two different objects ("what"). Importantly, rats were able to distinguish between object-place episodes over time. Eacott and Norman (2004) proposed that introducing a contextual change in the open field provided a marker of a particular unique occasion. Two different contexts ("what") became markers of two different occasions which may be functionally equivalent to Clayton and Dickinson's (1998) concept of "when". However, due to its reliance on recognition Eacott and Norma (2004) study could not provide unambiguous evidence for recall. Squire et al., (2004) stipulated that here rats could have used familiarity and not object recollection to perform this memory task.

Therefore, an elaboration on the Eacott and Norman, (2004) task was devised, to address the problem of demonstrating event recollection. In our tests the open field was replaced with an E-maze, described in Chapter 2. In Chapter 2, using only one context of the E-maze, we demonstrated that rats could recall the location of a non-habituated object when objects were hidden in the peripheral arms of the E-maze. Furthermore, research described in Chapter 2 showed that naturalistic object exploration and subsequent object habituation was sufficient to induce reliable search for the relatively novel object.

In the present study, a task was developed to assess memories for events from a single episode. Amalgamation of memory for objects ("what"), their location ("where") and the contexts in which they were found ("which") were similar to combinations used by Eacott and Norman (2004). Here rats had to use recall in order to trace the location of objects hidden in the arms of the maze. It was predicted that as rats possess an episodic-like memory ability, they would be able to combine the information from the "what" "where" and "which" triad, and preferentially approach the arm containing the non-habituated (relatively novel) object.

3.2 *Method*

3.2.1 *Subjects*

Sixteen experimentally naïve male rats (Dark Agouti strain, age 2-9 months, length 180-250mm weights 200-400g) were used. They were housed in sets of four in diurnal conditions (12h light/ 12h dark cycles) and tested during the light cycle. Throughout the study all animals had ad libitum access to food and water.

3.2.2 *Apparatus*

The apparatus was the same as described in Chapter 2. However, in this study the E-maze could be altered to form two different contexts. Two possible contexts were distinguishable both visually and tactilely (henceforth to be referred to as contexts X and Y). Context X incorporated the original walls of the E-maze enclosure described in Chapter 2 (walls and floor were smooth and

black), whereas context Y consisted of three inserts; Grey mesh which served to cover the walls of the original context X, also an insert to cover the 'start' chamber and the mesh to line the floor of the E-maze. Y context inserts were painted grey and overlaid with black wire mesh, while the floor insert was an E-shaped black wire mesh.

As before the E-maze was covered by a transparent plexiglass lid marked by two sets of white lines on the outer surface. When the rat remained inside the area marked by the white lines it was unable to see the objects hidden within the right and left arms of the maze. Therefore, as in Chapter 2, as long as the animal's head was inside the marked territory the objects in the peripheral arms were judged as obscured from view.

During every trial the rats were given two different object to explore (objects A and B). Every experimental objects was used only once throughout habituation and testing. In total 144 novel objects were presented to every animal. In testing, four copies of every object were used, so during four stages of each experimental trial an animal was exposed to a different copy of the same object. Object duplication prevented any possible scent marking, which could have aided the rats in their object search.

As before, the experimenter was hidden from view of the animal and the rats were filmed on a video camera, which was suspended 150 cm above the E-maze 'start'. The footage was stored on JVC compact video cassettes. As in Chapter 2, to habituate the animals to one object the 'habituation chamber' was used. As in the previous study the location of the objects (left/right) and the habituated object was counterbalanced across animals and days. In addition for this study the order of presentation of the context (X and Y) was similarly counterbalanced (appendix c&d).

3.2.3 *Design*

In this study, all animals were exposed to the same E-maze and object habituation. The study had two stages. The first stage, consisted of 16 days of experimental trials when the objects were visible immediately after leaving the 'start' box. The second stage consisted of 16 trials when

the objects were hidden, in the outer arms (as in the previous study) and 2 probe trials in which the objects were removed at test.

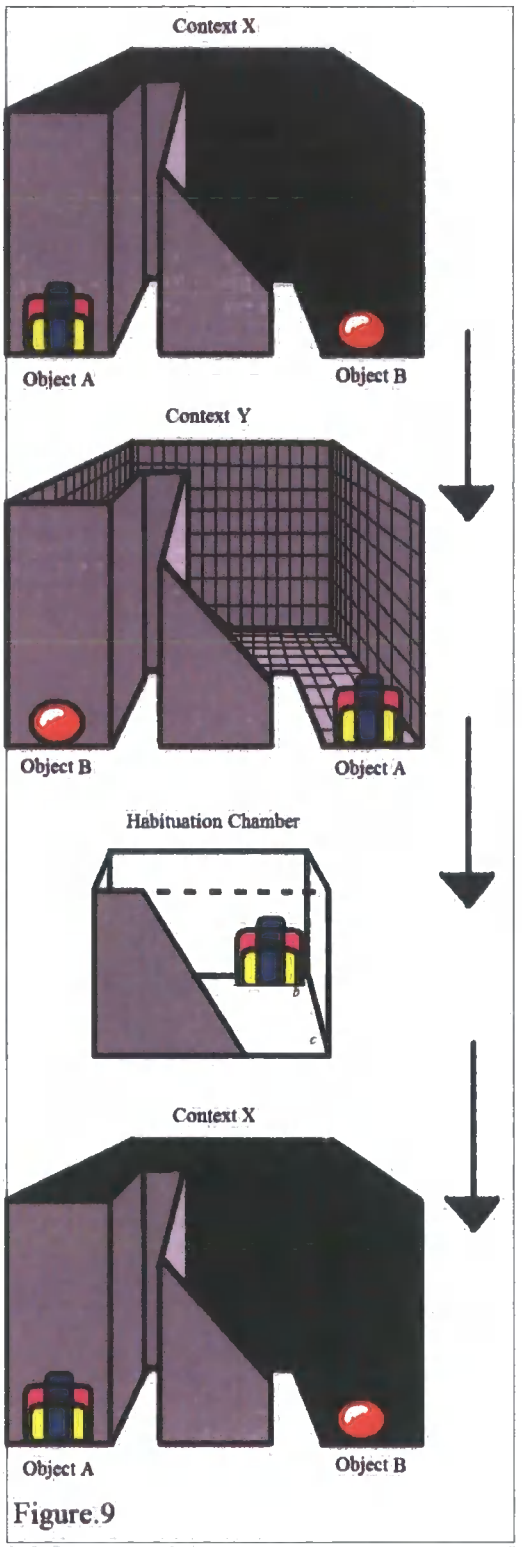


Figure.9

As the context used at 'test' phase could be either first or the second explored context, one additional factor, alluded to in the introduction, was the delay between original and 'test' object exploration. This was either 5 or 8 minutes, depending on the order of the test context presentation. This delay variation presented an additional factor which could influence the recollection accuracy; the shorter the delay the higher the rate of turning toward novelty.

3.2.4. *Procedure*

a) *Testing phase*

The experimental procedure was subdivided into a succession of habituation to the E-maze, and experimental objects, 16 trials of objects visible, 16 trials of objects hidden and 2 object hidden probe trials (where at test stage exploration objects were removed from the E-maze). The following were the order of stages given to our rats: During *Stage 1* animals were placed in the E-maze in pairs. During this stage the E-maze did not contain any objects. Animals received four trials in context X followed by four trials in context Y, each trial lasted 30 minutes.

Stage 2. Over the following two sessions E-maze exploration time was reduced to 10 minutes. As before, both contexts were explored; however during these two sessions rats were habituated to the maze individually. Furthermore, at this stage experimental objects were present

inside the E-maze, placed in the position immediately visible after the rats left the 'start' chamber. Eight subsequent habituation sessions were the same as the test procedure, except habituation objects were still presented as visible. Hence, during *Stage 3* animals received two trials (one in each context) in which they were placed in the E-maze for a five minute period and allowed to explore the objects. The animals were then placed in a separate holding cage for five minutes before finally being returned to the maze for five minutes with the same context and objects, allowing the rats to learn that the location of objects remained stable within a context.

In *Stage 4* animals received two trials in which they were placed in the E-maze for three minutes within one context for example X and two objects positioned in order A in the left arm of the maze and B in the right arm. They were then placed in a holding cage for a further three minutes, before being returned to the E-maze with the second context and the object order switched to the opposite locations in the E-maze arms (e.g. context Y: object order BA). Animals were allowed three minutes exploration of the objects before being removed to a holding cage for a further three minutes. Finally, the animals were returned to the maze for a further three minutes of exploration with one of the previous context-object combinations (i.e. context X: object order AB or context Y: object order BA, counterbalanced for order of presentation), allowing the animals to learn that the locations of objects remained stable within a context, even with the multiple number of context presentations.

Stage 5. was carried out identically to *(Stage 4.)*, except after the exposure to the second context animals were placed in the habituation chamber, the rats remained in the chamber for a duration of five minutes. During this time a copy of one of the objects was placed in the habituation chamber, and the animal was allowed to explore this object at will. Upon returning to the E-maze for the final ('test') phase, the animal's behaviour was monitored via video recording, to record the animal's choice of object, as well as noting the duration of object exploration. Animals were given sixteen trials at this stage, with counterbalancing of left-right positions of objects and order of presentation of contexts.

Stage 6. followed an identical set of protocols to that of *(Stage 5.)* with the exception that objects in the maze were now placed at the ends of the outer arms of the E-maze, so that they were not visible upon leaving the start-box (Figure 9). Rats were given 16 trials at this stage, with the same monitoring and counterbalancing strategy as that of *Stage 5.*

As was the case in *Stage 7* of the "what-where" task in after trials 8 and 17 of Stage 6, an identical procedure was followed to that of Stage 6, but no objects were present at the 'test' presentation of the E-maze. This was a probe trial designed to control for any possible extraneous cues which could aid animal's recollection in Stage 6 (i.e. odour).

b) Post experimental data collection phase

The rat behaviour was filmed and subsequently scored in the same manner as was described in Chapter 2. When the rat turned left or right, after leaving the 'start' box, and crossing the vector past which the hidden object had become visible, the rat was judged to have chosen an object. Turning toward the non-habituated object first was scored as remembering the location of the relative novelty.

When the object exploration was recorded, the rats were judged as exploring, when they directed their nose at a distance ≤ 1 cm from the object and/or touched it with their nose. Hence, the basic measures were the directions of preferential turning and the total time spent by the rats in the exploration of an object during the exposure

periods only.

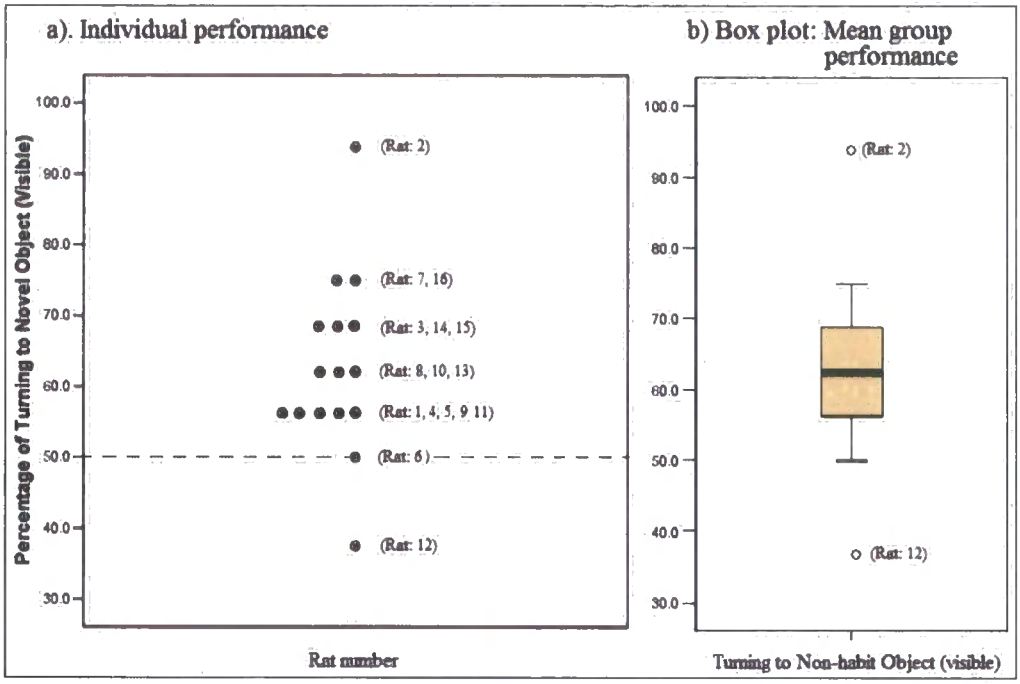
3.3 *Results*

3.3.1 Object habituation and preferential object exploration: objects *visible*

Rats turned toward the location of non-habituated objects, when the objects were immediately visible, more frequently than would be expected by chance (μ=62.9 percent). Over 16 days of experimental trials this preference for turning toward relatively novel object was significant ($t_{15} = 4.101$, $p<0.0005$, 1-tailed) (Figure.10a and Figure.11).

Figure.10

Percentage of turns toward *non-habituated* object over 16 trials of the object *visible* task.



Within this overall level of performance, there was wide variation between the performance of individual animals (Figure.10b). For example, Rat 2 performed with 94 percent accuracy, choosing the non-habituated object 17 out of 18 times, however Rat 12 had chosen novelty only 38 percent of the time, showing higher preference for the more familiar (habituated) objects, although not statistically significantly.

This performance was established irrespective of additional variables which are outlined in Figure 11. Such factors as the test day, test to context delay, context (X or Y) at 'test' stage and left or right tuning bias had no significant effect on choosing the non-habituated object at the 'test' stage of the experiment.

Although the object (A and B) exploration times did differ significantly between the individual rats, ($F_{1, 15} = 2.24$, $p<0.006$), no correlation ($r = 0.003$, $p=0.96$) existed between the rate of novelty recognition and the amount of time spent on total exploration of objects (A and B). In fact, none of the variables, controlled for in these trials, had a significant contribution to the animal turning preference other than the location of novelty in either context (X or Y).

Figure.11 F-value table (One-way ANOVA, given df=15); variables involved in turning choice toward objects A or B at 'test' stage, after 16 objects visible and 16 objects hidden trials.

	Test day	**Test to context delay**	**Context (X or Y) at 'text' stage**	**Pre-'test' stage exploration time**	**Left or Right turning bias**	**Non-habituated object preference**
Objects *visible*	0.030	0.069	2.958	0.002	0.002	*4.101***
Objects *hidden*	0.097	*3.904**	1.869	2.165	1.873	*5.580****

p<0.05 = *
p<0.001 = **
p<0.0001= ***

3.3.2 Context Dependent Object-in-Place Recollection: preference of novelty, objects *hidden*

The following is the analysis of rat recollection of "what" "where" "which" episodes, as manifested in their search for non-habituated objects, concealed in the peripheral arms of the E-maze.

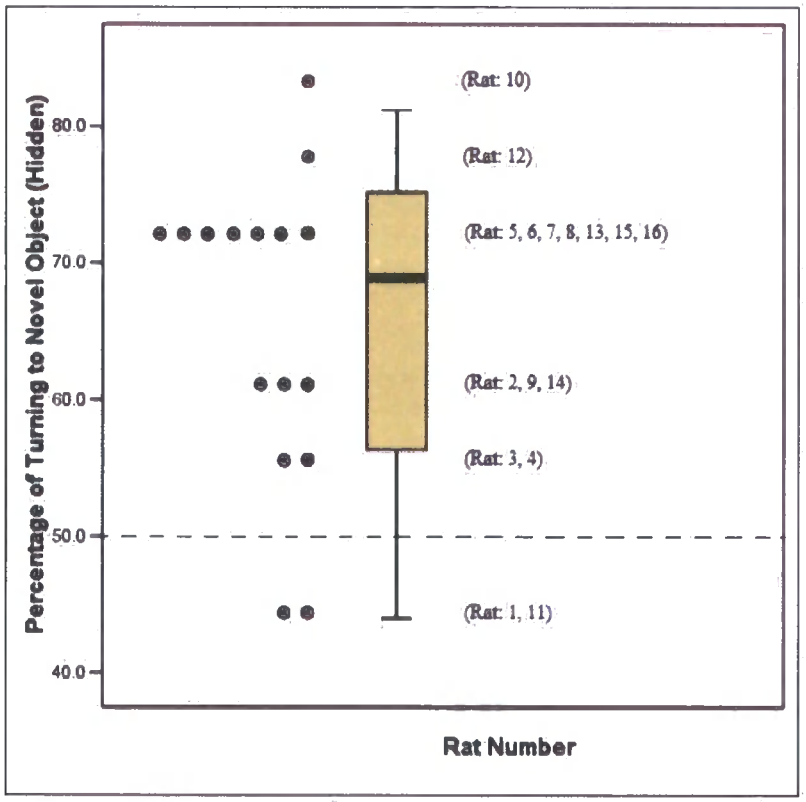


Figure.12

Percentage of turns toward non-habituated object over 18 trials of the object hidden task.

When the objects were hidden, in the left and right arms of the E-maze, the majority of rats preferred turning toward and exploring the novel objects (Figure.12). The average percentage of turns toward the relatively novel object after 18 experimental trials stood at 65.6 percent. This was a significant choice of turning toward the hidden non-habituated object, (t_{15} = 5.51, p<0.0001, 1-tailed) (Figure.11).

Within the 18 experimental days, there were two probe trials, days 9 and 18 (where objects were not presented in the test condition). The mean performance during the probe days did not

differ significantly from the overall performance, (t_{15} = 0.348, p=0.4) when the objects hidden and probe trial data was compared for the individual rats using a paired-sample test at a 2-tailed assumption (Figure.13). The rats demonstrated a significant preference for turning in the direction of the location where the more novel object would have otherwise been present, (t_{15} = 1.86, p=0.04 1-tailed). Otherwise in 16 object present trials the rats made significantly more turns towards the none-habituated object (t_{15} = 5.58, p<0.0001, 1-tailed).

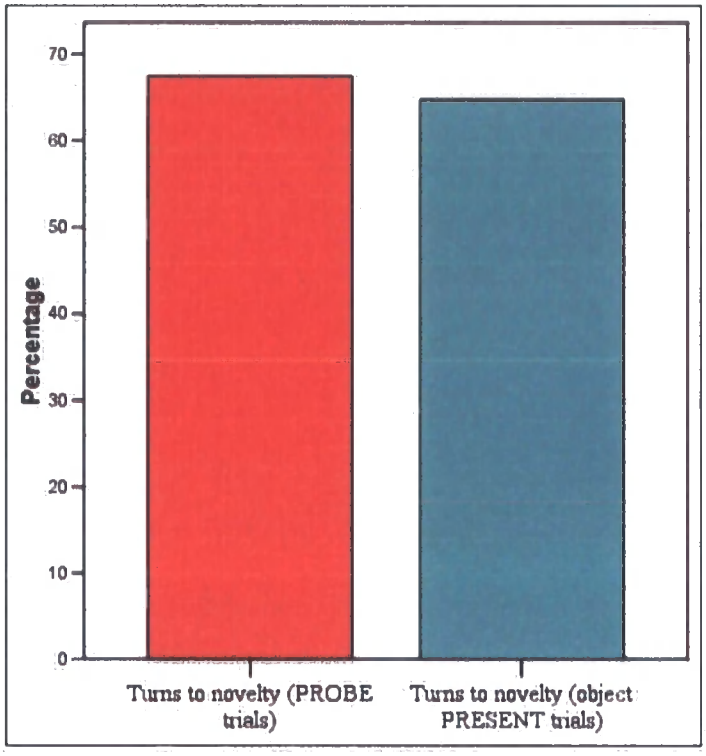


Figure.13

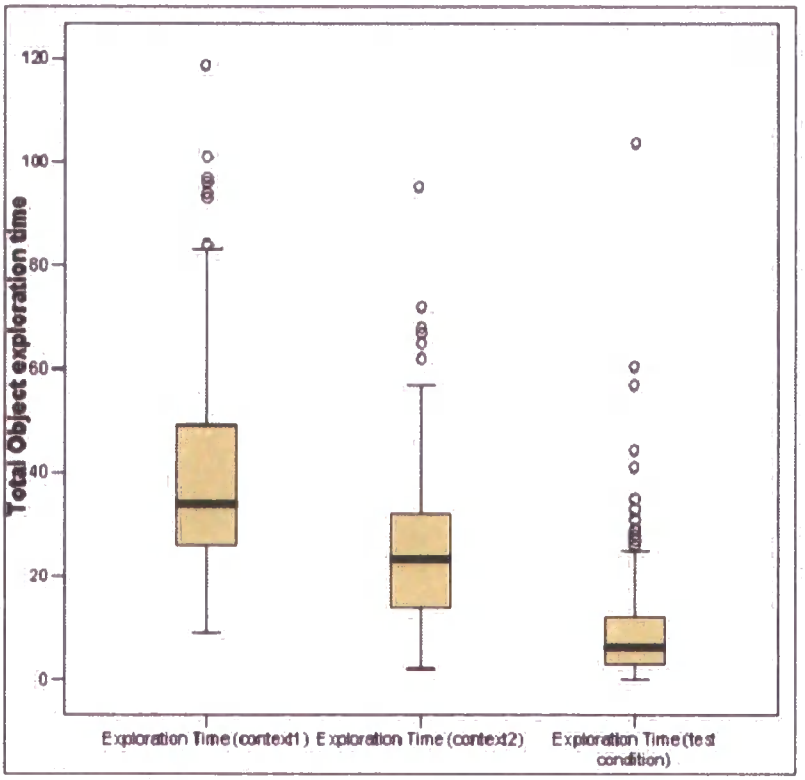
Percentage of turning to novelty in 16 object present trials, and days 9 and 18, probe trials.

3.3.3 Object exploration time as a measure of recollection

Interestingly, the exploration time of the objects decreased with every subsequent set of the object presentation. This decrease was indicated to be a statistically significant trend, as measured by the linear model of the repeated measures ANOVA ($F_{2, 15}$ = 59.1, p<0.001), demonstrating, that with an increased exposure to the objects the exploration rate progressively decreased (Figure.14). However, the object exploration time at the first E-maze exploration stages (context: X&Y, or Y&X) did not predict the choice of novelty at 'test' stage in line with the results of ANOVA ($F_{1, 15}$ = 2.165, p=0.1), in Figure.11.

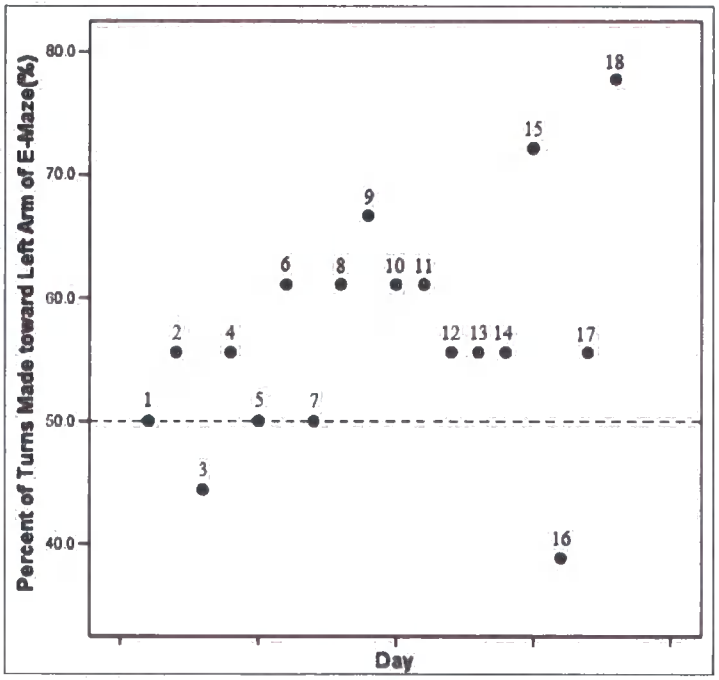
Figure.14

Relationship between the total object exploration time in contexts 1, 2 and the *test* context.



3.3.4 One-directional turning bias, objects hidden

During the objects hidden phase of testing a turning bias was noted. The data was analysed for the frequency of turns left as comparable to turning right from the 'start' arm. As the protocol was counterbalanced the number of left/right turns should have been equal.



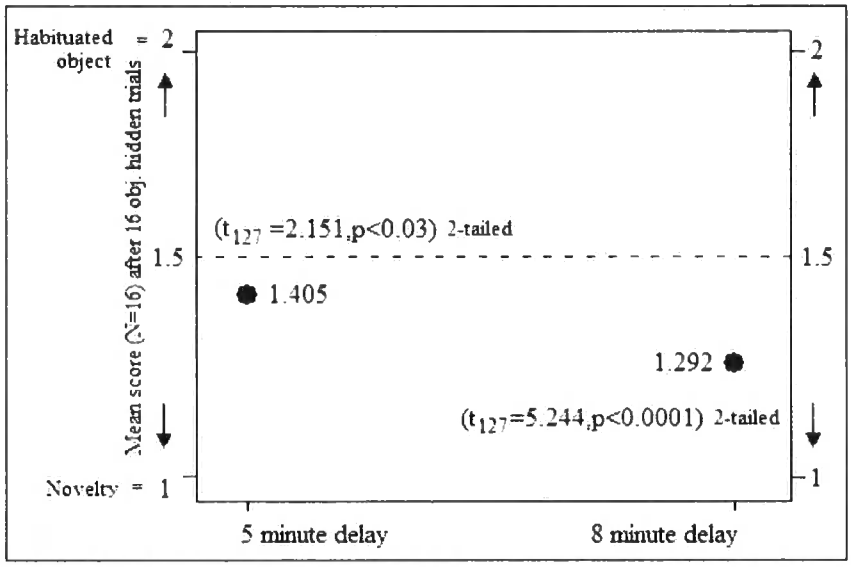
Percentage of left turns over the 18 days of the objects hidden experimental trials.

Figure.15

A significant preference of turning to the left arm (mean=57 percent) was observed over the 18 days of experimental trials (t_{15} = 3.239, p<0.005) (Figure.15). However, this bias did not have a significant effect on the choice of novelty ($F_{1, 15}$ = 1.873, p=0.2) in Figure.11.

3.3.5 Effect of delay duration between 'test' context and 'test' on recall accuracy.

Figure.11 indicated that the duration of delay (5 or 8 minute intervals) between the initial object exploration and 'test' stage affected event recollection ($F_{1, 255} = 3.904$, $p<0.05$). Both delays were followed by statistically reliable turning preference towards the location of the relatively novel object during the 'test' stage (Figure.16). However, it was of note that the difference in turning accuracy, as predicted by the delay length, was counter intuitive. Recall accuracy was higher at 8 minute delay interval than after the lesser 5 minute delay, possibly showing the evidence of the primacy effect. Fractional increase in the delay interval therefore, did not affect the accuracy of episodic-like recall tested in this experiment.



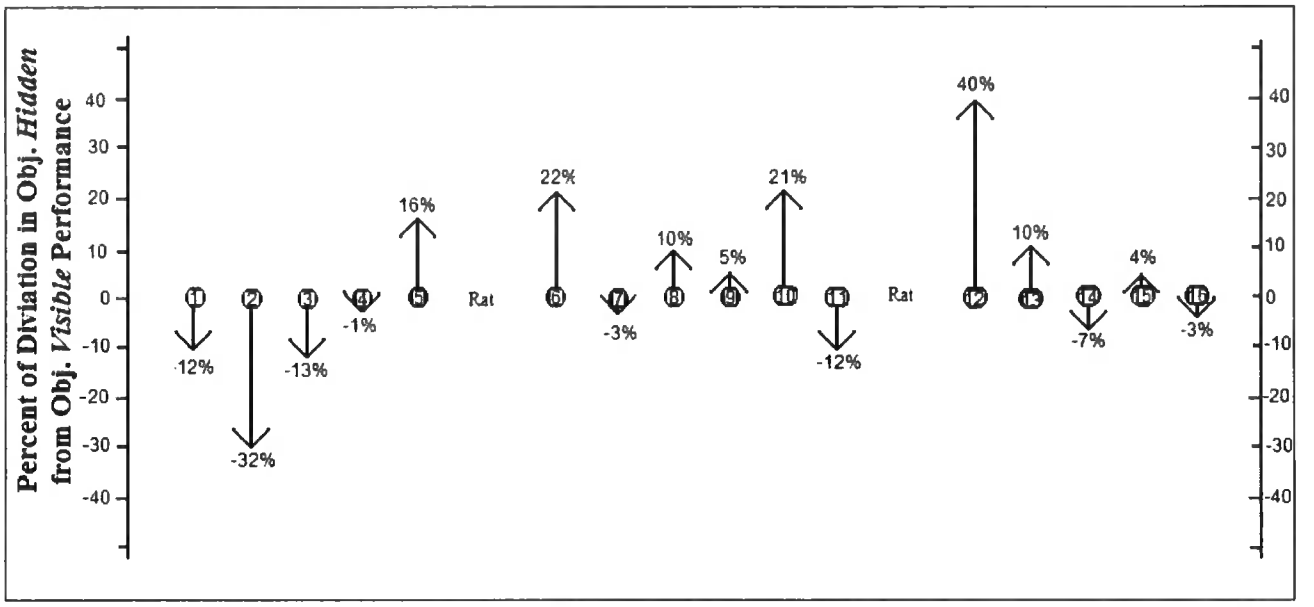
Difference between novelty preference at 5 and 8 minute delay between initial and subsequent 'test' object search.

Figure.16

3.3.6 Objects visible vs. objects hidden recollection; Comparison

Finally, in comparing individual rat performance on the objects visible and object hidden tests, no significant correspondence was observed between the two sets of data. Individual rat scores did not correlate significantly at ($r = -0.047$, $p=0.9$). In taking objects visible task performance as a baseline and comparing the objects hidden data the following distribution of residual percentages was attained, Figure.17.

Figure.17 Percentage change in turning toward the non-habituated objects during object hidden trials when compared to the same rat's performance in the objects visible trial.



There was no significant difference between the overall performance of the visible and hidden objects groups, (t_{15} = 0.621, p=0.5, 2-tailed). Performances, during both sets of trials, significantly favoured choosing novelty (Figure.18), however individual rat performances did not correspond between the two tests. This could have arisen due to such performance as of the rat 12, which showed a preference for familiar objects, in the objects visible tests, however performed in line with the rest of the sample when the objects were hidden and recollection was relied upon.

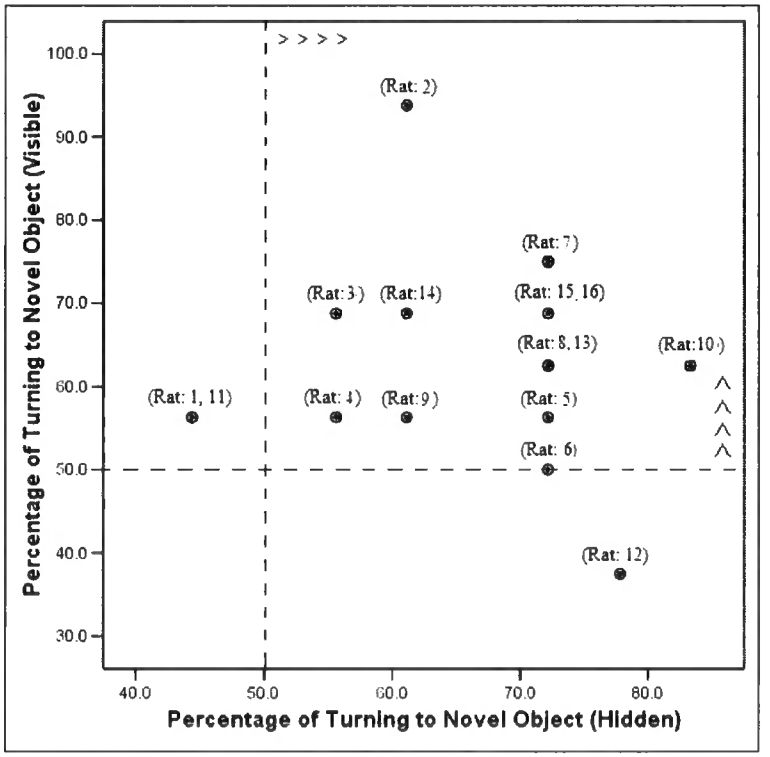


Figure.18

No correlation of novelty preference between objects visible and hidden tests.

3.4 Discussion

Object visible portion of this task provided evidence that object habituation was a reliable method of identifying memory for past experiences. It also provided evidence that the changes in

contextual background did not adversely affect object recollection, as reliable object recollection was established in contexts X and Y. This was further combined with findings of Chapter 2, which provided reliable evidence that habituation alone was sufficient to produce spontaneous E-maze search for non-habituated objects.

As Chapter 2 showed, when two different objects were hidden in the peripheral arms of the E-maze (one object in the left and a different one in the right arm) the rats were able to reliably recall the location of the non-habituated of the two objects. It was argued that this was achieved by recalling the location of the object from the earlier visit in the E-maze. The "objects hidden" condition introduced in this chapter demonstrated evidence for episodic-like recall. By hiding objects from immediate view, and introducing two contextually distinguishable episodes, where in one context object locations were the reverse of the other, rats were shown to be able to learn the association between the types of contexts (X or Y) and the location of the objects within these contexts. Having attained the "what" "where" association, and having connected this knowledge to the concept of "which" (context X or Y), the rats were able to predict the location of a specific object when they were reintroduced into either context (X or Y). Turning decisions of the animals were based on their ability to recognize "which" context they were in. Recalling the previous location of what was now a relatively novel object and turning towards the arm containing that object. This behaviour was demonstrated reliably in this particular rat population, providing evidence for episodic-like recall in the rat.

When novelty recognition was compared in objects visible and objects hidden tests no correspondence between individual performances was found. Perhaps when the objects were visible a decision to explore an object was determined by the object's immediate appearance, affecting the object preference by factors such as object complexity. In the object hidden condition, exact object appearance was not immediately viewed, therefore an ability to recall the correct novelty object-in-context location was more likely to determine test performance. Based on the evidence of the object hidden condition this task was proposed to be a reliable test of episodic-like recollection in the rat.

However, there were some criteria of the "what" "where" and "which" memory in the rat that was in need of more extensive investigation. Recollection accuracy was demonstrated to be reduced by the length of the delay interval between initial and test explorations (Eacott and Norman 2004, Squire et al., 2004). Therefore, the subsequent chapter will address the memory retention capabilities of rats used in testing the proposed episodic-like memory paradigm.

Chapter 4; Effect of delay on the rat episodic-like recall (*Tests 3-5*)

4.1 Introduction

Episodic-like memory ability was described in the previous chapter, where experimental animals could retain memories of "what" "where" "which" for up to eight minutes. However, although there is evidence that animals were capable of enduring information storage for a wide variety of events (Zentall, 1997) there is also evidence that such representation storage need not be perfect (Pearce, 1991). Therefore, to establish the precise criteria of episodic-like recollection in the rat the endurance for "what" "where" "which" paradigm, described in Chapter 3 was tested in the subsequent set of experiments.

A wide range of species were said to possess some level of ability to represent memories (Sherry at al., 1987). O'Keefe and Nadel (1978) suggested that the elaborate spatial representations are stored in the hippocampus. This region of the temporal lobe is thought to be involved in processing episodic memory in humans (Egan, 2003) and perhaps other mammals, such as rats (Ergorul and Eichenbaum, 2004). One outstanding feature of the human long term memory is its longevity. Human memory is capable of retaining accurate representation of some episodes over the duration of a life time, (Ebbinghaus, 1885). With some animals these principles, it seemed, are also true (Pearce, 1991). Hoarding birds are able to revisit more than three thousand cache sites months after the initial visit was performed (Vander Wall, 1982). This demonstrated the vast potential of avian memory storage of episodes, although these could have been retained as semantic information (Suddendorf and Busby, 2003). Furthermore, memories of visual scenes were demonstrated to last as long as two years in pigeons (Vaughan and Green, 1984).

To date there is ample evidence that rats are capable of memory storage for considerable periods of time, habituation (Whitlow, 1975), conditioned responses (Gleitman, 1971; Lieberman et al., 1985) and radial maze (Olton, 1978; Suzuki, 1980) studies, to name but a few. Moreover, animals like humans are prone to loss of information over long periods of time. For instance Thomas and Lopez (1962) demonstrated that the specificity of the stimulus employed during

training was forgotten over time. Introducing a gradually lengthening delay between the original event and the events' recollection was taken to test the effectiveness of Hebb's (1949) consolidation and retrieval (Lewis, 1979) interactive (long memory) models.

Previous rat memory studies showed that the rat ability to recall the configuration of a maze was handicapped by introducing a 24 hour delay between habituation and test trial phases (Deweer et al., 1980). Hence, it was expected that introducing a delay between the original episode and its subsequent recollection would hamper accuracy of memory performance. As Ennaceur and Delacour (1988) demonstrated, in an open-field rats were able to accurately remember object-place configurations following delays of up to 1 hour. 24 hour delays however affected rat memory sufficiently to diminish the event recollection accuracy. The abovementioned example of experimentally induced events-to-recall delays, provided evidence that rat memory of familiar events last between 1 hour and 1 day. These findings were supported by more complex open-field experiments of Eacott and Norman (2004). When interchanging contexts were added to the experimental design, which made the test environment event specific, rats were still able to display robust ability to remember after 15 minutes, 1 hour, but not 2 hour delays. Therefore, as episodic memory, was defined as a lasting memory (Tulving, 1972) it was reasonable to expect that in episodic-like recall rats would be capable of memory retention, despite delays between memory acquisition and retrieval.

In addition the duration of exploration time is proposed to be a likely determinant of the recall accuracy. Two studies evaluated the heritability of novelty seeking in great tits and demonstrated a presence of a large genetically based variation in tendency to explore and seek novel situations (McClearn, 1959). Similarly novelty exploration is a spontaneous, innate behaviour in rats (Ennaceur and Delacour, 1988). Given that it was argued that a stimulus must be fully attended to if it were to be readily learned about (Wagner, 1981), it was expected that rats which tended to partake in a heightened object exploration would also display a more accurate and durable event recollection.

4.2 Method (Test 3, 4 and 5) *(Behavioural Methodology)*

4.2.1 *Subjects*

The test subjects were twelve experimentally experienced animals, who were selected from Test 2 animal set, where rats 1, 3, 4 and 11 were removed from the tested group (as these animals failed to demonstrate evidence of recall at the minimum delay used in Chapter 3). Dark Agouti male rats aged 10 months, length 220-250mm and weights 350-400g, which were housed in sets of two and four in diurnal conditions (12h light/ 12h dark cycles) and tested during the light cycle. Throughout the study all animals had ad libitum access to food and water. Since the animals had recently completed similar experiments in the same apparatus they did not require further habituation.

4.2.2 *Apparatus*

The E-maze (with contexts, X and Y inserts) and the plexiglass lid, 12 trial-unique objects, video equipment and habituation chamber remained unchanged from the "what" "where" "which" task. The daily procedural protocols and the order of object presentation followed the protocol order of the first six days outlined in Chapter 3 (objects hidden task) (appendix c&d).

4.2.3 *Design*

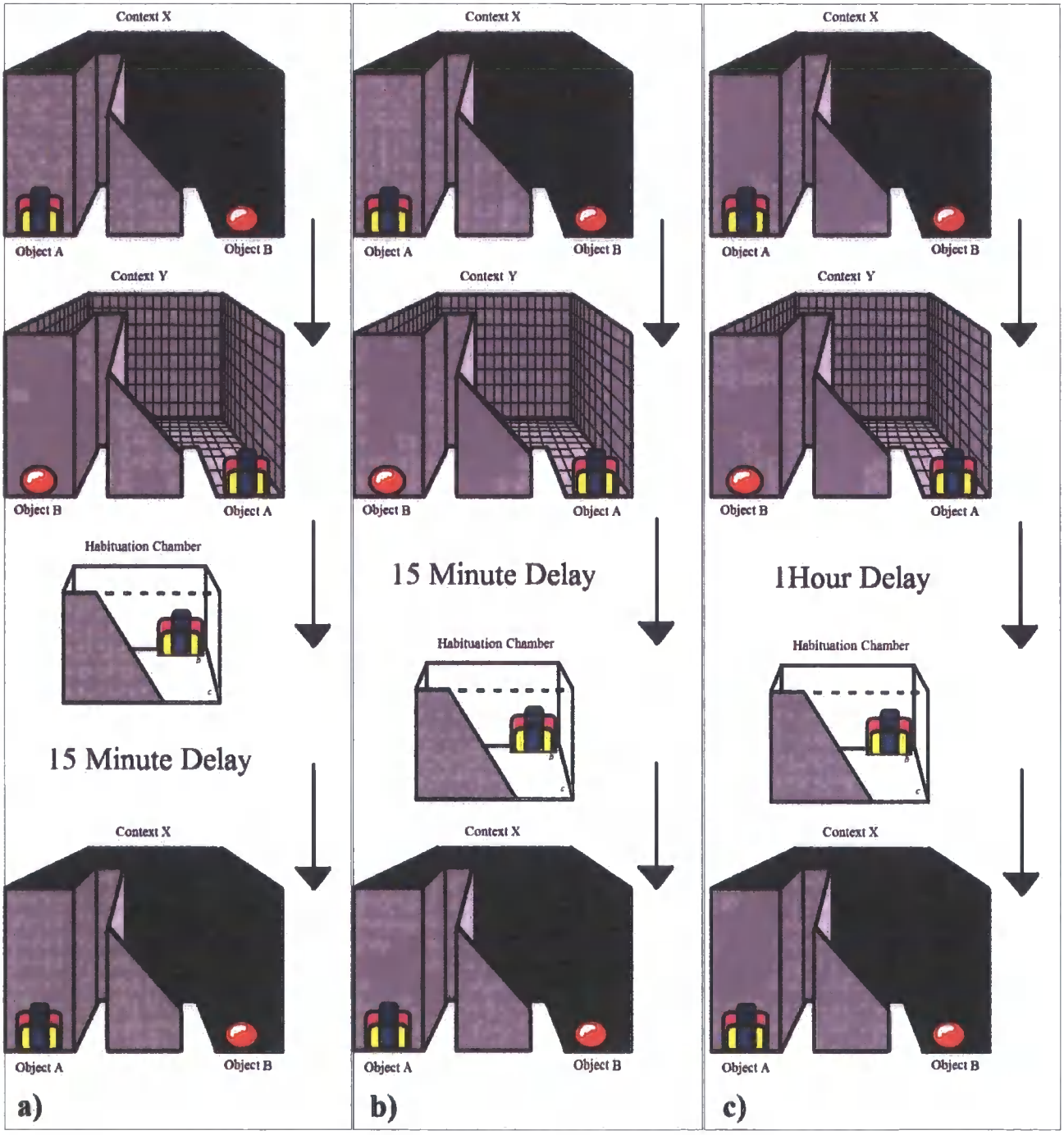
This study followed a similar design to that used in Chapter 3 (objects hidden test), with the addition of 15 minute post-habituation delay. As before, dependent variable assessed in this study was the direction of turning (towards the left or right arms of the E-maze which contained a novel object). The design criteria of Tests 4 and 5 were identical to Test 3, with exception to the conditions of the delay interval. Test 4 encompassed a 15 minute and Test 5 1 hour pre-habituation delays.

4.2.4 *Procedure*

a) *Test phase*

During this stage of testing habituation to the apparatus was no longer relevant, as the rats were already experienced test animals, familiar with the "what" "where" "which" task settings and

Figure.20a,b&c Order of experimental procedures in tests 3, 4 and 5.



the testing procedure. Therefore, the animals were introduced directly into *Stage 6* (see Chapter 3 procedure) where rats were placed into the E-maze for a two minute period and allowed to explore the objects in contexts X and Y, Figure.20a. Then, animals were habituated to one of the objects in the habituation chamber, where they remained for a duration of five minutes. During this time one of the objects was allowed to be explored at will. However, from here onward the procedure was altered in this version of the task.

After the period of habituation the animals were placed back into their home cage, where they remained for the subsequent 15 minutes. After the 15 minute delay the animal was placed back into the E-maze for the 'test' phase. Here the 'test' phase was identical to that of *Stage 6* (Chapter 3). The location of objects remained stable within either context (X or Y presented at 'test'). The animal's behaviour was monitored, via video recording, to assess the animal's choice of object exploration, as well as the duration of object exploration. Each animal was given six such trials, with counterbalancing of left-right positions of objects and order of presentation of contexts. Tests 4 and 5 followed an identical to that of Test 3 (Figure.20b&c), encompassing 9 and 6 trial days respectively.

b) Post experimental data collection phase

As was the case with the episodic-like task of Chapter 3 the data collection and scoring was the same in Tests 3, 4 and 5. Identical principles of video footage analysis were used as described in the Chapter 3 procedure.

4.3 Results (Rat Memory: Which Where When recollection, effects of delay)

4.3.1 15 minute delay *after* object habituation

When the 15 minute delay was introduced after the habituation stage of testing, the preferential turning toward the relative novel object described in Chapter 3 was no longer observed. During the 'test' phase the percentage of turns in the direction of novelty (non-habituated) or the habituated object was identical. No significant difference in attending to novelty was attained, (t_{11} = 0.0, p=1).

4.3.2 15 minute delay before object habitation

Reintroduction of the 15 minute delay before the object habituation stage affected the recollection of novelty location by decreasing recall accuracy. However, the rate of turning toward novelty remained significantly higher than chance (t_{11} = 1.935, p<0.04).

Contrast of preferential turning to novelty percentages between no delay and pre test 15 minute delay tests.

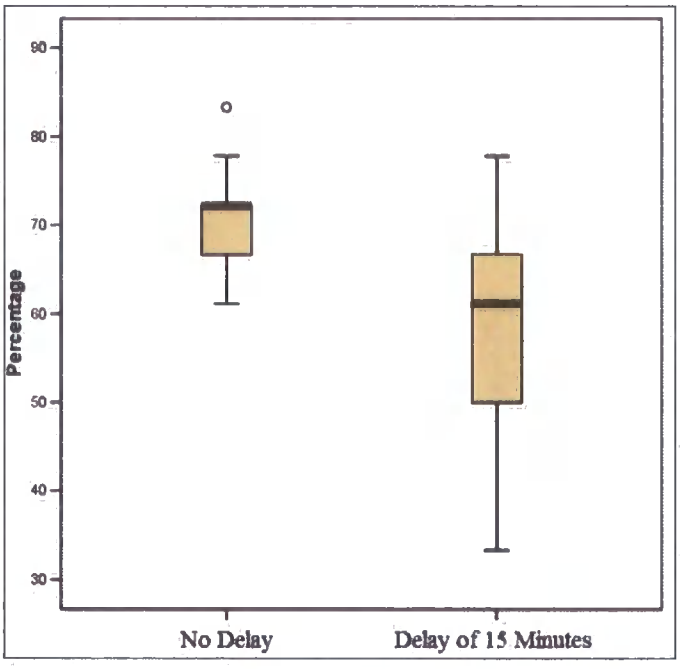


Figure.21

Performance accuracy at the 15 minute delay test did diminish, (Figure.21). The decline in performance was a statistically significant one as there was a significant difference between the 15 minute and no delay condition ($t_{11} = 3.072$, $p<0.01$). Recollection of novelty was less accurate after the 15 minute delay.

4.3.3 1 hour delay before object habituation

Lengthening of the pre-habituation delay interval further, resulted in impairment of episodic-like recall. There was no significant trend in turning preference ($t_{11} = 0.233$, $p=0.4$), hence after one hour delay rats did not display a statistically significant indication of the event recollection.

Cross comparison of turning to non-habituated objects rate with increasing delay before the test condition.

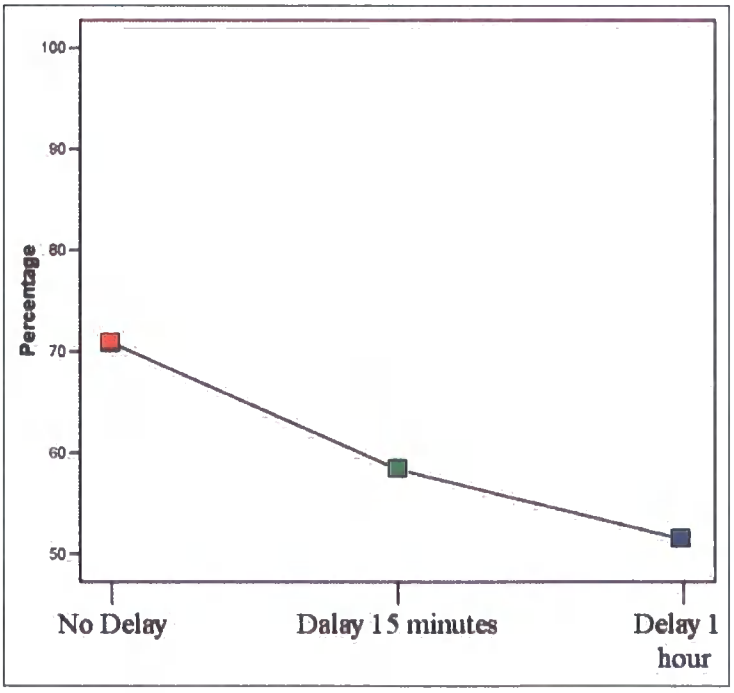


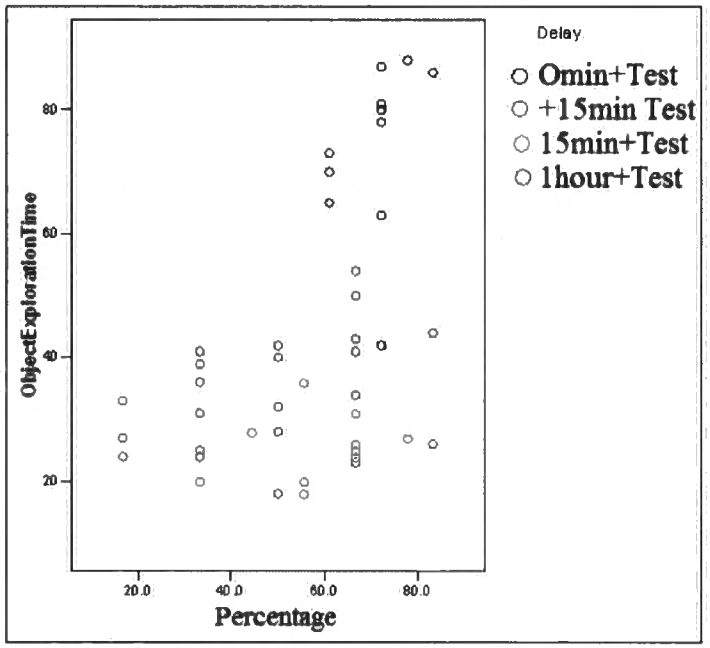
Figure.22

There was a significant decrease in the turning preference as the delay between exploration and habituation phases of testing had increased, illustrated by the linear regression in Figure.22. Repeated measures test showed this decrease in the performance to be a statistically significant one, given a correlation factor of Greenhouse-Geisser ($F_{2, 11} = 5.829$, $p<0.01$). However, the sphericity

assumption for this data was not met (χ^2= 2.937, df(2), p=0.2) showing the novelty preference in the individual animal performance not to correlate strictly between the three tests.

4.3.4 Novelty Preference and Exploration Time Continuity between Tests 2, 3, 4 and 5.

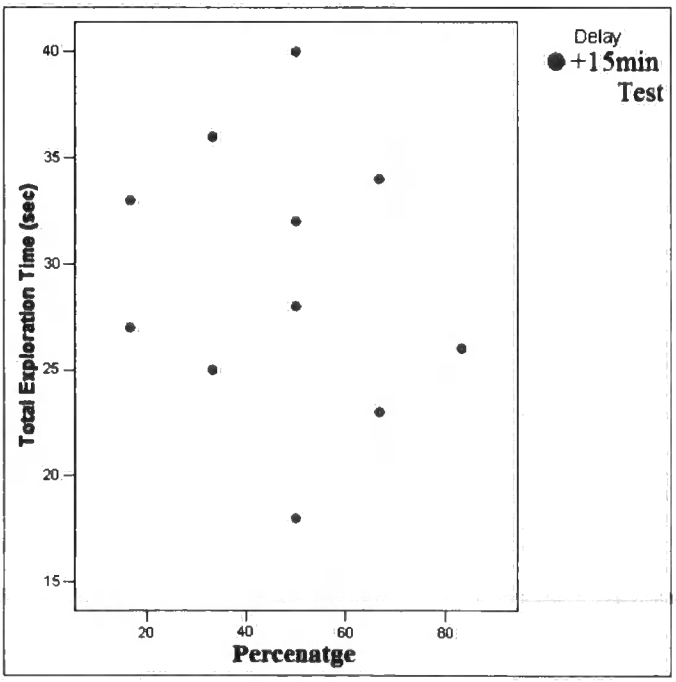
Lastly, in assessing the possible relationship between the total amount of time spent at exploration (during the first two exploration stages) and the preference of the non-habituated objects, a significant correlation between these two variables was observed.



Combined data scatter-plot of the total exploration time and percentage of turns to novelty, in no delay, post habituation 15 minute, pre habituation 15 minute and 1 hour delay tests.

Figure.23

When the data from all objects hidden tests (no delay, post habituation and pre habituation delays of 15 minutes and 1 hour) was computed a strong positive relationship was observed, (r = 0.472, p<0.001). As the magnitude of total exploration time increased so did the preferential turning in the direction of the relative novelty, (Figure.23).



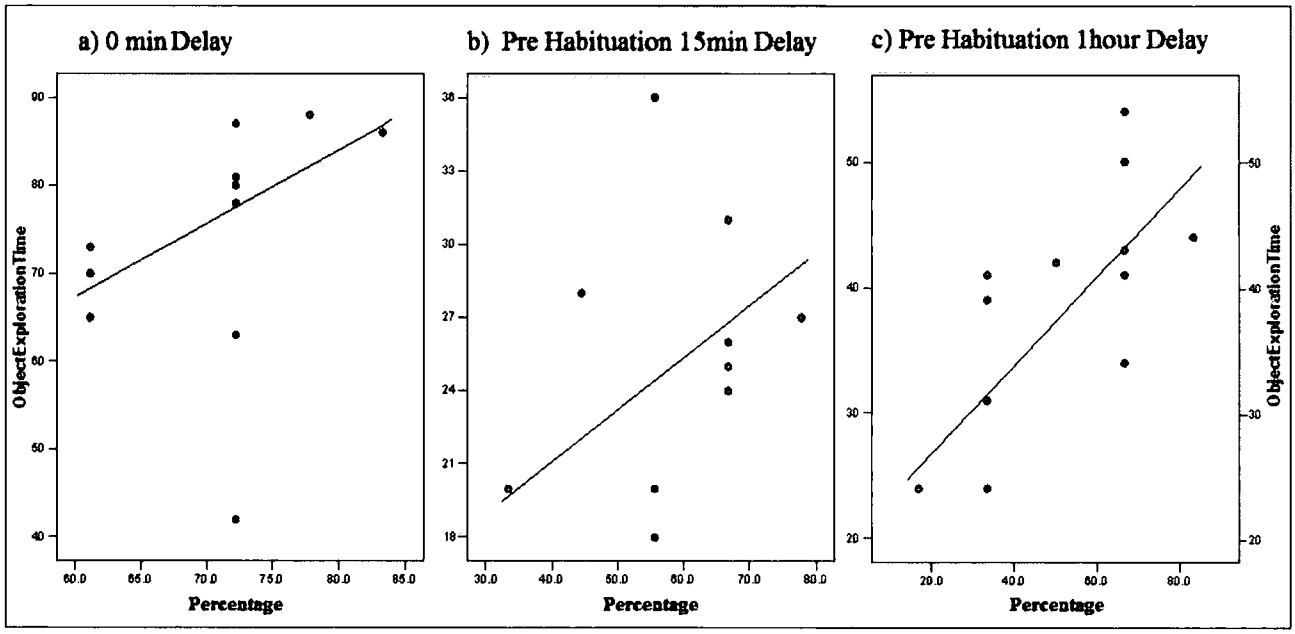
Scatter-plot of the total exploration time and percentage of turns to novelty, in post habituation 15 minute delay test.

Figure.24

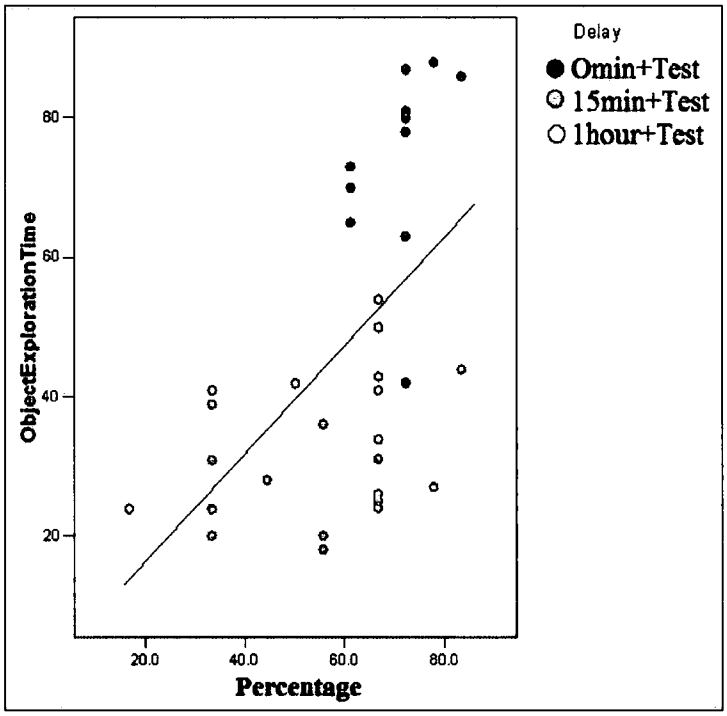
When all four tests were considered on their individual merit, only during the 15 minute post habituation test was there no correlation between preferential turning and total exploration time, N=12 (r = -0.026, p=0.8), (Figure.24).

Otherwise, in the test of "what" "when" "which"; no delay between habituation and 'test' stage, and all of the *pre* habituation delay tests, yielded a positive relationship between an event recollection and the total exploration time, Figure.25a,b&c. In the case of the 0 and 15 min delay there was no significant correlation, just a positive trend (r = 0.385, p=0.2) and (r = 0.252, p=0.4) respectively, however, at one hour delay the correlation was significant (r = 0.697, p<0.01).

Figure.25a,b&c Individual scatter-plots of the total exploration time and percentage of turns to novelty, in no delay, pre- habituation 15 minute and 1 hour delay tests.



Furthermore, when the data for the three stages of testing described in Figure.18 were analysed together a general relationship between the exploration time and preferential turning, a strong positive correlation was established, (r = 0.526, p<0.001), Figure.26. Thus, the length of object exploration (first two E-maze exploration phases) was directly related to the rat's ability to recall past episodes of the location of relative novelty.



Combined data scatter-plot of the total exploration time and percentage of turns to novelty, in no delay, pre habituation 15 minute and 1 hour delay tests.

Figure.26

4.4 *Discussion*

These experiments provided evidence that the post-habituation (+15min) stage delay interval provided a time gap sufficiently long to result in both habituated and non-habituated objects becoming relatively novel. Therefore, to test the effect of time passage on memory in episodic-like assignment, delay had to follow the original event exposure before the object habituation had occurred. Subsequent to this adjustment, as in Eacott and Norman (2004), after the delay of 15 minutes the rats were still able to demonstrate event recollection. The episodic-like memory was displayed after 15 minute but not after 1 hour delays. Although Eacott and Norman (2004) and Ennaceur and Delacour (1988) demonstrated recall or recognition after 1 hour delays this episodic-like task could have been too complex to maintain a high level of recollection accuracy after such a delay period. This task did show, however, that increasing the delay length resulted in a diminished episodic-like recollection. However, as our task required the animal to distinguish between two similar events the rat's inability to make the correct recall after 1 hour delay could occur due to confusion of the two episodes with one another. In the case of Eacott and Norman (2004) and Ennaceur and Delacour (1988) the tasks were simpler, which could explain why the performance diminished faster in our (more confusable) task than in the above mentioned ones.

Inability to recall episodic information after 1 hour delay could have arisen because the rats were unable to recall the experimental episode with a sufficient degree of accuracy. Delay length could have served to disrupt the precision of the animal's recall of the earlier episode. Perhaps an introduction of the pre-test visual prompts, such as those in Deweer et al.'s, (1980) maze completion tests, could have improved the accuracy of rat performance at 1 hour or even longer delay periods. Deweer et al. (1980) suggested that when rats were solving complex mazes, the encoded information was hindered during event recollection after the 24 hours delay. However, an introduction of the environmental cues improved the episode recollection. This perhaps could improve future research aimed at determining the capability of the "what" "where" "which" memory.

Interestingly, in these sets of experiments the exploration time served as a reliable predictor of recollection accuracy. With the exception of the post-habituation delay test, high rates of the initial object exploration predicted better event recollection in the rat. This correlation was significant test of the longest (1 hour) delay only. Here rats that explored the test objects the longest were able to display episodic-like recall with the greatest degree of accuracy. However, the problem of these findings stemmed from the fact that it was impossible to distinguish with any degree of certainty whether it was the higher levels of exploration which aided event recollection, or if perhaps those animals that had greater episodic-like memory capacity were also prone to investigate their environment at a heightened rate. It was however, an intuitively reached conclusion that an increased exposure to any one-trial episode should have aided the formation of "concrete representations" (Pearce, 1991 p.97) of that episode, strengthening potential ability to recall the event in question. It would be of great interest to devise a test which could disambiguate exploration rate and recollection of this episodic-like task.

The studies in chapters 2, 3 and 4 have established a reliable test of "what-where-which" (episodic like memory) which was demonstrated to last for up to 15 minute delays. Having established this it was of great use to understand the effect of neurological dysfunction on

recollection of episodes created in this test of "what" "where" "which". In subsequent Chapter 5 the effects of neurological damage (via the lesioning method) to the areas though to be implicated in episodic and episodic-like recall (the fornix) will be compromised, facilitating an assessment of the role performed by this compromised neural structure in the efficiency of the episodic-like memory function in the rat.

Chapter 5: Implication of fornix in episodic-like memory (*Test 6*)

5.1 Introduction

Episodic-like memory was first demonstrated in scrub jays by Clayton and Dickinson (1998) and later in the rat (Eacott and Norman, 2004). However, the neurology implicated in the episodic-like memory has not yet been universally agreed upon. The "extended hippocampal system" is central to the formation and processing of human episodic memory (Aggleton and Brown, 1999). Case studies demonstrated that focal bilateral damage to the hippocampal structures in humans (Vargha-Khadem et al., 1997, 2002) resulted in the loss of episodic but not semantic memory, whereas a recent case study of C.L. (Temple and Richardson, 2004) demonstrated intact episodic but impaired semantic memory. These studies provided evidence that episodic memory is an independently functioning neural system, as there was a double dissociation between episodic and semantic memory function (King et al., 2004). However, these case studies evaluated the results of the neurological damage sustained by humans in the early childhood. Such damage was present throughout the child's development, and therefore the effects of the neurological development was not equivalent to that of a pervasive neurological damage in a fully developed adult (Temple and Richardson, 2004).

Therefore, establishing a reliable test of episodic-like memory (Chapter 3) in the rat provided an opportunity to study the neural structures underlying event-specific memory. Preceding case studies and tests demonstrate that damage to the hippocampus causes impairments of episodic memory in humans, and impairments of spatial learning in animals (Gaffan and Hornak, 1997), and that compromising the fornix (an efferent of the hippocampus) resulted in a similar episodic memory deficits (Yasuno et al., 1999; Moudgil et al., 2000). The fornix is a major fibre bundle connecting the hippocampal formation with the septum and other sub-cortical structures (Eichenbaum et al., 1994), more specifically an afferent of the prefrontal cortex, mamillary bodies and the anterior thalamic nuclei (Aggleton and Brown, 1999). However, the role of fornix in the encoding and processing of episodic memory in the "extended hippocampal system" is still hotly

disputed (Squire et al., 2004). According to Clark et al., (2000) "fornix lesions need not mimic the effects of direct damage to the hippocampal tissue" (in Squire et al., 2004 p. 289).

Initially the fornix was implicated in the disruption of delayed mach-to-sample (DMS) memory, which was thought to be analogous to semantic memory (Gaffan, 1974). However, later DMS was shown to be unaffected by the fornix transection (Gaffan et al., 1984). Subsequent research demonstrated that spatial and episodic memories are dependent on the neural information relayed from the hippocampus through the fornix into the diencephalic and cortical structures (Aggleton and Brown, 1999). Eacott and Norman (2004) attempted to demonstrate the role of the fornix in the episodic-like memory by recreating Clayton and Dickinson (1998) episodic-like (what-where-when) memory task, whilst substituting occasion-specifying contexts "which" in place of "when" used by Clayton and Dickinson (1998). They demonstrated that severing the rat's fornix resulted in disrupting the memory for the trial-unique episodes whilst sparing the animal's ability to distinguish objects-place memories (semantic memory). However, it was argued that open-field tests assess event familiarity (Squire et al., 2004) and not episodic-like event recollection, as they fail to encapsulate all features of episodic-like memory (Hampton and Schwartz, 2004). However, the paradigm presented in this thesis (Chapter 3 and 4) provided an alternative test of the rat's episodic-like memory.

One advantage of studying the role of the fornix, rather than that of the hippocampus, in episodic-like memory lies in the consistency of the resulting damage. Although the role of the hippocampus in memory and navigation has been extensively studied, Squire et al., (2004) claimed that the size of the hippocampal lesion had a different effect on the recollection ability. Complete hippocampal ablation resulted in recognition and spatial learning problems, whereas partial lesions of the hippocampus diminished spatial learning alone. On a cellular level there is a well documented functional subdivision between the role of the CA1 and CA3 pyramidal cells within the hippocampus (Grön et al, 2001; Lee et al., 2004; Daumas et al., 2004). Therefore, to identify the function of the hippocampus, a complete hippocampal ablation was necessary, which was difficult

to achieve without damaging the surrounding cortical tissues. Alternatively, a fornix transection produced a significant disruption of information processing and outputs of the "hippocampal formation" whilst incurring a lower level of tissue damage (Aggleton and Brown, 1999). For this reason, fornix lesions were preferred to lesions of the hippocampus in the present study.

Therefore, in this Chapter we used the task described in Chapter 3 to assess the role of the "extended hippocampal system" in the episodic-like memory of a rat by way of bilateral surgical sectioning of the fornix. Memory impairment was expected to be evident in the rat's inability to find the location of the non-habituated objects with any degree of accuracy, whereas control animals should perform the episodic-like task, as accurately as did the intact animals described in Chapter 3. However, it was thought that object recognition (semantic memory) will be spared in both groups, so after finding the relatively novel object both groups would explore this object more extensively. Furthermore, during exploration, as in the earlier chapters, the animals are expected to be motivated by the relative novelty of the object, which is an innate exploratory behaviour (Ennaceur and Delacour, 1988). The accuracy of the episode recollection should be based on the object exploration time, given the results of Chapter 4. However, the animals with the fornix section would be expected to demonstrate poor recollection of the episodes regardless of their exploration time.

5.2 Method

5.2.1 *Subjects (Surgery)*

Sixteen naïve Dark Agouti male rats began the study. At the start of the study they were aged approximately 12 weeks and weighted 180-250g. They were housed in sets of four in diurnal conditions (12h light/ 12h dark cycles) and tested during the light cycle. However as detailed bellow (see surgery), two animals did not proceed to the experimental stage and so only 14 rats completed the study.

5.2.2 *Procedure (Surgery)*

The surgery was performed on 16 animals. Two animals subsequently died due to post-operative respiratory complications. Of the remaining 14 rats, 7 rats received bilateral fornix lesions and 7 animals formed a sham-operated control group.

a) Fornix lesion: the target lesions were based on stereotaxic coordinates delineated by Paxinos and Watson (1986) used in the predating experiments by Eacott and Norman (2004). Each rat was anaesthetised using isophorone (3-3.5 percent), given a 0.5 O_2 flow rate; the animal's head was shaved and positioned in a stereotaxic head holder angled parallel to the operating table surface. The scalp was incised along the line of the midline and bregma was located and measured. The first lesion site was calculated as 5.3 mm anterior and 0.7 mm lateral to ear bar zero and 0.4 mm posterior and 0.7 mm lateral to bregma. Where the anterior/posterior positions differed between the two calculations a mean of the two measurements was used. The second target site was calculated in the same way using mediolateral measurements of 1.7 mm lateral to both bregma and ear bar zero, (Paxinos and Watson, 1986).

Using a dental drill a portion of the skull surface overlying the abovementioned locations was removed on each side of the midline in a single section to expose the dura. The top of the dura was measured at each of the four target sites. The dura was cut at each site to allow the insertion of the electrode needle into the brain. Lesions were made using RFG4-A RF lesion generator. The electrode (0.3 mm tip length, 0.25 mm diameter) was lowered along the vertical axis into the exposed brain tissue.

At the first lesion site a depth of 4.5 mm relative to the top of the skull measured at bregma and a depth of 3.7mm relative to the top of the dura was used (if there was a discrepancy between the two measurements the measurement from top of dura was always taken as the correct one). In the case of the second site, the depth of 4.6 mm relative to the top of the skull at bregma and a depth of 3.8 mm relative to the top of the dura. Current was passed through the fornix fibres at such power as to achieve an approximate temperature of 75°C, this procedure lasted for one minute. The same

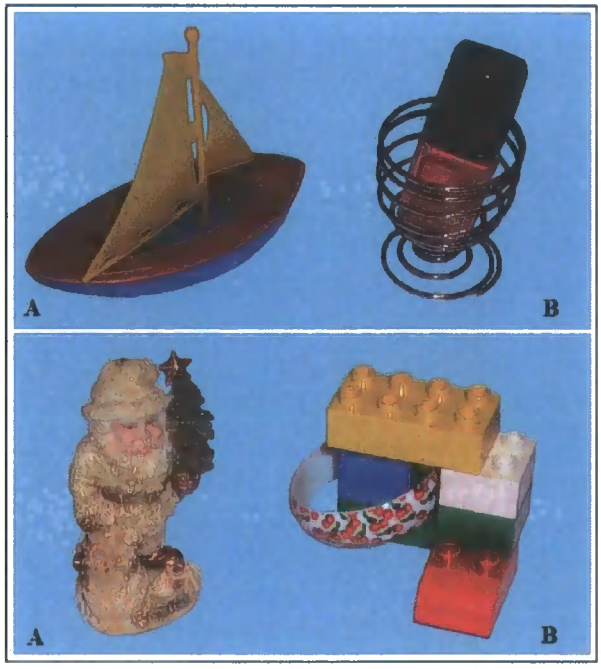
formula was followed on the contralateral side. Subsequently, the bone matter was not replaced and the scalp closed.

b) Sham surgery (Control): the surgery was identical in the sham animals as the one performed on the fornix group, with the exception that the dura was not cut and the electrode needle was not lowered into the brain. As a result, there was a decrease in length of the surgical procedure for the sham groups in comparison to the operated group.

All surgeries were performed as expected and will be histologically examined for the completeness and the presence of unexpected damage. However, due to the limitations of time, this will occur after the completion of this thesis and therefore the histological analysis will not be described here.

5.2.3 *Apparatus (Test 6)*

The apparatus and procedures used here were the same as those used in Chapter 3 (appendix a, c&d).



Sample of experimental objects A&B form object hidden trials; testing days 1 and 2

Figure.27

5.2.4 *Design(Test 6)*

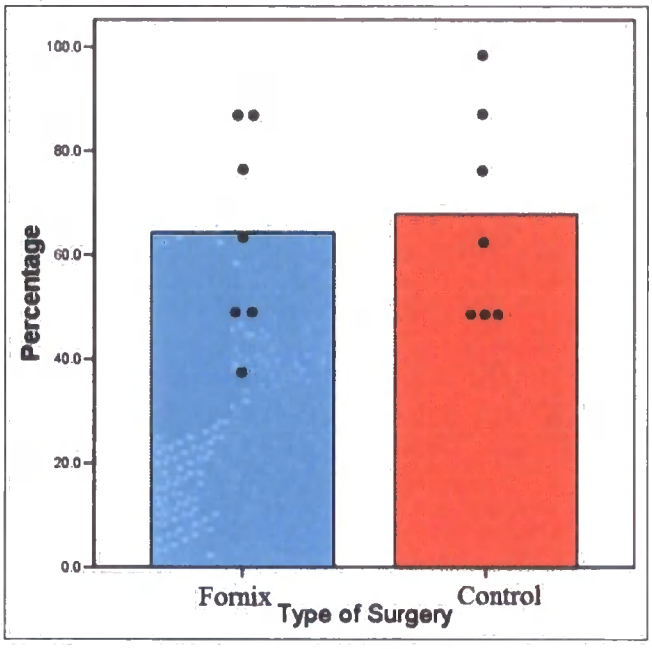
These sets of tests followed a between-subjects design. All animals were exposed to the same 34 experimental procedure trials. During testing the experimenter was 'blind' to the type of surgery sustained by the individual experimental animals. Animal behaviour was also scored 'blind' form the video-recording.

In this study the context (either X or Y), object location (either in the left or the right arm of the maze) and the actual object used in the habituation procedure, the test procedure insured that the

object visible condition. Both the control ($t_6 = 2.335$, $p<0.03$) and fornix ($t_6 = 1.922$, $p<0.05$) operated groups turned toward the unhabituated object significantly above chance levels.

The turning preference scores of the fornix and control groups in the object visible test

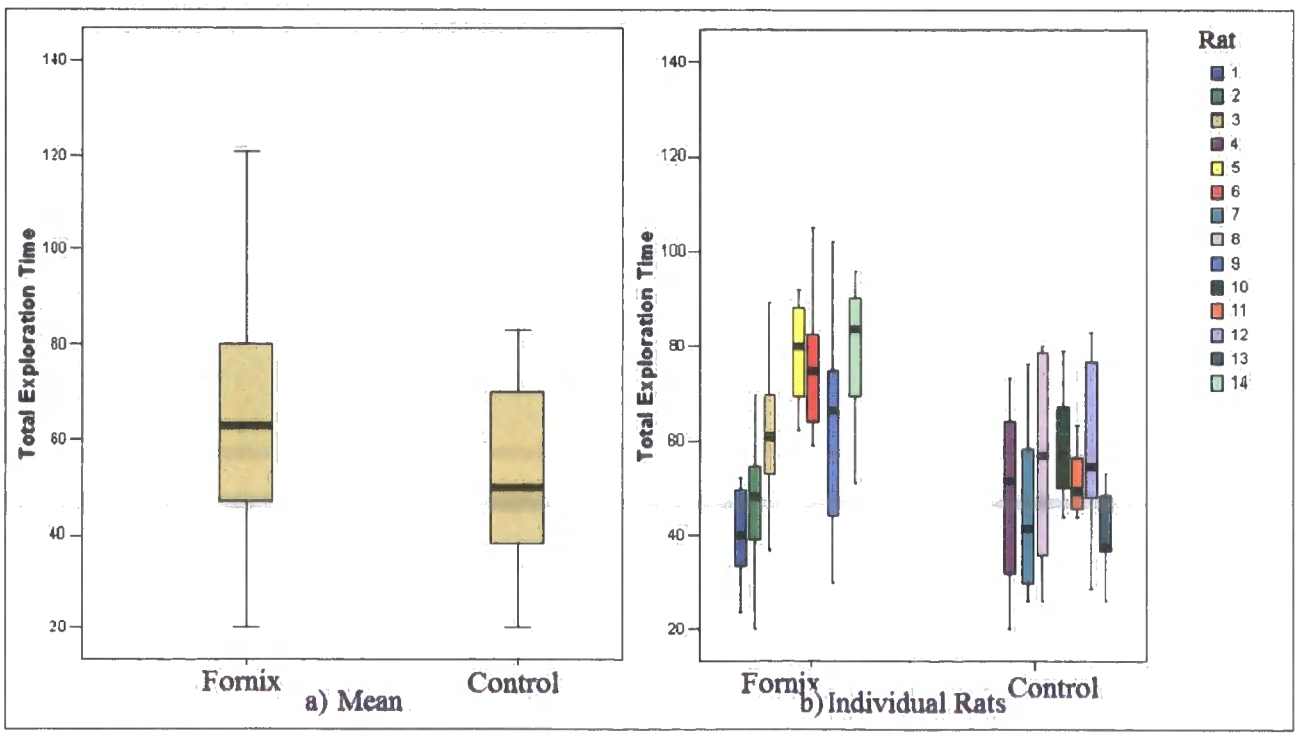
Figure.28



Moreover, when the turning preference of the two groups was compared no significant difference in performance was observed ($t_{12} = 0.281$, $p=0.8$) between fornix and control groups.

The two groups were also matched for performance on variables, such as preference of left or right sides of the E-maze ($F_{1, 13} = 2.704$, $p=0.1$) and delay between test context and test phase ($F_{1, 13} = 0.361$, $p=0.6$). However, fornix and control groups did differ on their object exploration time ($F_{1, 13} = 7.205$, $p<0.007$), individual animals of the fornix group explored objects longer, and also the exploration of the fornix group was more varied than the controls, (Figure.29).

Figure.29 Total object exploration time of the fornix and control groups in the objects visible test



5.3.2 Turning preference, objects hidden

When the objects A and B were hidden in the peripheral arms of the E-maze, given 16 experimental trials, only the control group turned significantly above chance in the direction of the non-habituated objects, (t_6 = 2.902, p<0.02). The fornix group showed no significant turning preference (t_6 = 1.373, p=0.2), (Figure.30a). The objects hidden test incorporated two probe days (where at test stage the hidden objects were removed from the E-maze). The control group mean (67 percent) was similar to the object present performance (64 percent), though not statistically above change as the sample sizes was only two trials, (t_6 = 1.0, p=0.4). The Fornix group performed marginally, although not significantly, better during probe than the 16 test trials, (t_6 = 1.441, p=0.2) (Figure.30b). Importantly, the independent sample t-test demonstrated that, when compared, the control and the fornix groups were not statistically different, despite the difference between the two groups approaching significance at (t_{12} = 1.488, p<0.08, 1-tailed), Figure.30a&b.

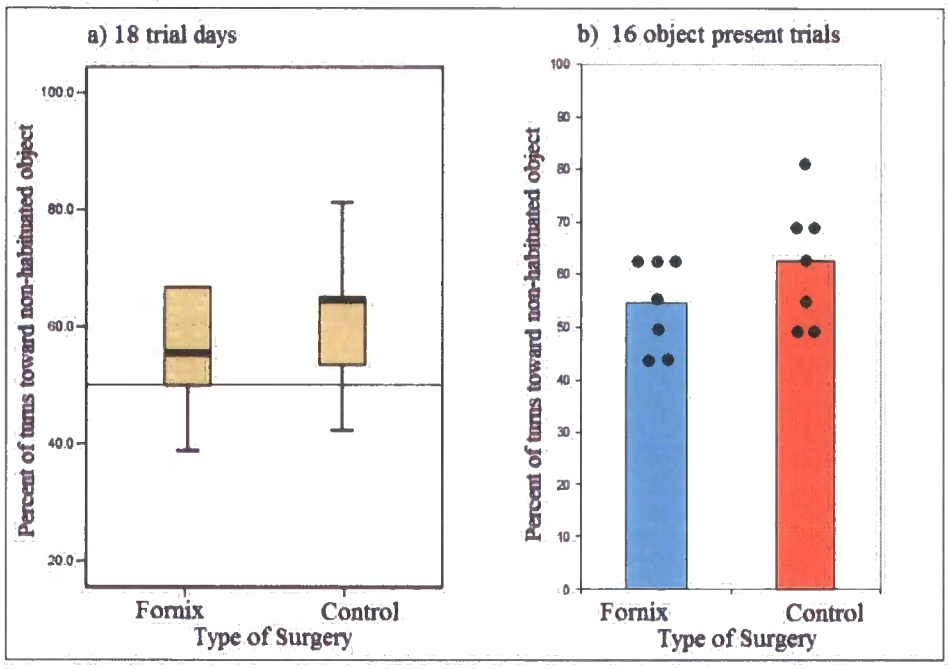


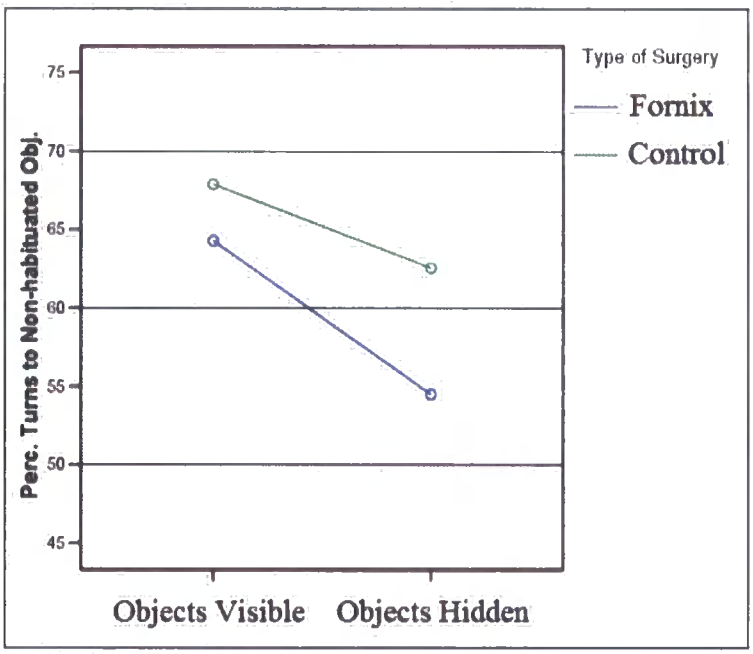
Figure.30a&b

Preferential turning of the fornix and control groups in the objects hidden test

Throughout the objects hidden trials the turning preference of the control and fornix groups were not affected by any of the test variables including E-maze side preference ($F_{1, 13}$ = 1.648, p=0.2), delay between test context and test phase ($F_{1, 3}$ = 0.045, p=0.8), or the total exploration time ($F_{1, 13}$ = 0.100, p=0.8).

5.3.3 Comparison of objects visible and hidden (Univariate ANOVA).

When the event recollection of the two groups was compared to one another, and not the chance of turning in the direction of the non-habituated object however, turning in the direction of the non-habituated object was similar in both control and fornix groups. There was no significant difference between the overall object visible and hidden performance ($F_{1, 12} = 2.909$, $p=0.1$). The control group turned in the direction of non-habituated objects more consistently, but there was no statistical difference in choosing the non-habituated object between the control and the fornix groups ($F_{1, 12} = 0.651$, $p=0.4$). Furthermore, the two groups were similarly affected by the position of the objects in the E-maze. Performance was higher in the objects visible condition and declined in the objects hidden similarly in both groups as there was no difference in performance, therefore there was no interaction between the groups ($F_{1, 12} = 0.254$, $p=0.623$), (Figure.31).



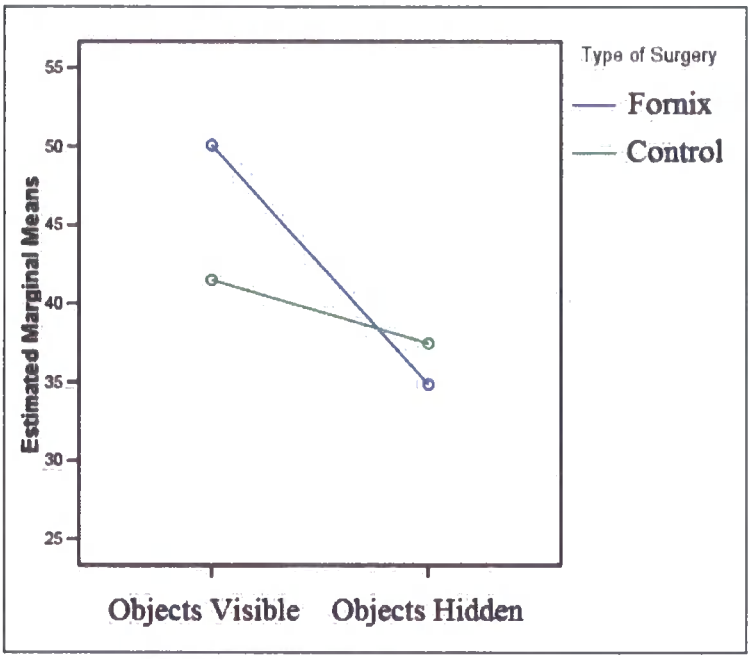
Comparable novelty preference performance on objects visible and objects hidden test, by fornix and control groups.

Figure.31

5.3.4 Exploration time at objects visible and hidden tests.

Object exploration time during the first two stages of exploration differed significantly between objects visible and hidden conditions ($F_{1, 12} = 10.318$, $p<0.007$) and the interaction between the fornix and control groups was approaching significance ($F_{1, 12} = 3.486$, $p<0.09$). Objects visible condition yielded much longer object exploration times. When the individual performances were analysed the object exploration reduced significantly when objects were hidden from view in the

fornix ($F_{1, 6}$ = 9.853, p<0.02) but not the control group ($F_{1, 6}$ =1.309, p=0.3). Also, despite a dramatic reduction of the exploration time in the fornix group, in the hidden compared to visible conditions, there was no overall difference between performances of the two groups ($F_{1, 12}$= 0.749, p=0.4), (Figure.32). However, as mentioned in 5.3.1 the fornix groups explored objects at a much higher rate than the controls, which was not the case in the object hidden test ($F_{1, 13}$ =2.021, p=0.4).



Comparable total exploration time performance over the objects visible and objects hidden test, by fornix and control groups.

Figure.32

5.4 Discussion

In this study when the objects were visible, turning toward and exploring the relatively novel object, was markedly above chance in both the control and fornix groups. This demonstrated that the fornix damage did not affect the object recognition memory. It was worth noting that despite elevated tendency to explore in general, fornix lesioned rat's object recognition was similar to that of the control group. Hence, the heightened object exploration did not aid the performance of the fornix group.

When the objects were hidden performance of both groups diminished. Although the episodic-like recollection of the control animals was slightly closer to the rate of recall demonstrated by the intact animals in Chapter 3, their recollection did not differ significantly from that of the fornix group. Fornix lesioned animals performed less reliably at episodic-like recollection but not significantly worse than the control group. It was therefore concluded that there was insufficient evidence that the fornix section disrupted the accuracy of episodic-like memory in

this sample of rats. These results therefore do not provide enough evidence to back the proposed neural circuitry of the recall-familiarity model of the Aggleton and Brown (1999). Interestingly, during the objects hidden test the object exploration rate of the fornix group dropped dramatically and control exploration remained the same. The findings suggested that the fornix severed animals were pone to heightened object exploration when objects were immediately present and not if they had to explore the E-maze environment in order to find the objects, perhaps providing evidence of memory or spatial navigation impairments.

When the recollection accuracy was measured against chance the fornix animals did not show a significant episodic-like recall and the control animals did, but the two groups performed similarly when the two group were compared with one another. It is possible that the performance of the two groups could have been statistically similar because of the relatively low group sizes, as the statistics did indicate that the control group was more accurate at the episodic-like recollection than the fornix group, although not significantly so. Therefore, in future it would be useful to use a greater number of experimental animals; hence improving the power of data analysis, also higher number of experimental trials could further aid the statistical accuracy. The evidence attained in Test 6 stand to suggest that a more extensive study of the effect of the fornix section on recollection is necessary to provide evidence for the role of this neural structure in the function of the episodic-like memory. The current data is insufficient to prove or disprove the function of the fornix in the Aggleton and Brown's (1999) model of the episodic memory.

Chapter 6

6.1 Discussion

The set of experiments presented in Chapters 2-5 provided evidence that episodic-like memory could be behaviourally demonstrated in rats, which supports the findings of preceding bird (Clayton and Dickinson, 1998; Clayton et al., 2001), monkey (Gaffan, 1994) and rat (Eacott and Norman, 2004; Ergorul and Eichenbaum, 2004; Fortin and Eichenbaum, 2004) studies. This was shown to be a lasting memory, reliably retained for up to 15 minutes, supporting the earlier findings of the Eacott and Norman (2004) study. However, there was insufficient evidence to show that the uncompromised function of the fornix, an efferent of the hippocampus, was necessary for the retention of the episodic-like information. This thesis does not provide enough reliable evidence to support the neural structures of the episodic memory model proposed by Aggleton and Brown (1999), which does not stand to undermined the model, but merely to state that a more extensive study of the fornix transection is necessary to fully evaluate the importance of the 'hippocampal formation' in the episodic-like memory system.

Our initial experimental findings demonstrated that the rats were able to retain memories of objects ("what") and their locations ("where"), as in the earlier study of Day et al., (2003). In the object habituation experiment, animals were motivated enough by relative novelty to search for the objects they were not habituated to, confirming that the object habituation paradigm was a reliable method of identifying object recognition and preference. Therefore, it was concluded that it was possible to use this behavioural methodology and E-maze to test more complex memory, as the rats were able to retain memories for the previously experienced episodes and predict the location of the hidden object.

However, the initial set of test trials did not encompass one concept vital to episodic-like definition, the ability to distinguish between past experiences; the notion of "when" (Clayton and Dickinson,1998) or "which" (Eacott and Norman, 2004). Therefore, the memory test was further complicated to incorporate the criteria of "which" by providing the contextual association with the

trial unique event. Additional changes of the background did not adversely affect object recollection. Reliable object recollection was established in contexts X and Y, as when the object search was unnecessary, as they were immediately visible, the objects were remembered regardless of which context they were in.

Further complicating the experimental design by hiding the objects from immediate view, and introducing two contextually distinguishable episodes, provided evidence that rats were able to learn the association between the types of contexts (X or Y) and the location of the objects within these contexts. Rats attained the "what" "where" association, and having connected this knowledge to the concept of "which" (context X or Y) were able to predict the location of a specific object when they were reintroduced into either context (X or Y), which was similar to the episodic-like ability demonstrated by the scrub jays in the Clayton and Dickinson's (1998) experiment. Animals based their turning decisions on their ability to recognize "which" context they were in recalling the previous location of what was now a relatively novel object and turning towards the arm containing that object. The evidence of this behaviour was demonstrated at a very high level of statistical significance, providing the evidence for an episodic-like recall in the rat. Therefore this experiment demonstrated that the non-human animals are capable of episodic-like recollection, thus contradicting the propositions that the episodic recollection is unique to humans, (Tulving, 2002). This study also offers evidence that episodic-like ability could de demonstrated in mammals as well as birds (Clayton and Dickinson, 1998; Clayton et al., 2001) and could therefore be subject to comparison between different animal species.

As it was previously claimed that the recollection accuracy was reduced by the delay length between the original event and an episode recollection (Eacott and Norman 2004) we also addressed the capabilities of the rat's memory in retention of the episodic-like memory. We became aware that introducing a delay gap (in the case of our experiment of 15 minutes) after the object habituation phase caused the rats to perceive the habituated objects as relatively novel again. This was thought to be the case because introducing a delay after habituating an animal to a given object provided a

sizable passage of time. Making the animals perceive the habituated object as relatively novel. Hence, at the test phase both objects were perceived as of similar interest for general exploration. Therefore, it was ascertained that to test the effect of time passage on episodic-like memory the delay had to follow the original episode, and the object habituation administered after the delay interval.

After the delay introduction was adjusted, as in Eacott and Norman (2004), the rats were able to recall the events following the delay of 15 minutes reliably, however after the delays were increased to 1 hour rat recollection diminished. Although Eacott and Norman (2004) and Ennaceur and Delacour (1988) demonstrated recall or recognition after 1 hour delays this task was more complex, which could explain why the accuracy of the event recollection could have been lower in this study. Also the sample size used in the delay experiments was reduced from the original non-delay experiment, which reduced the power of the subsequent statistical analysis. Larger population size might have yielded positive results at the longer delay intervals. This task did show, however, that increasing the delay between event and its subsequent recall resulted in the diminished recollection accuracy. These results followed a similar pattern to the one established in the human based studies, showing that the episodic memory is particularly prone to alteration and inaccuracy (Schacter, 1995).

In the test of episodic-like recall high rates of object exploration correlated with more reliable event recollection. This relationship was particularly significant when pre-recollection delay was the longest (1 hour), so the rats that explored objects the longest displayed the episodic-like recall with the greatest degree of accuracy. It was concluded that an increased exposure to any episode should aided the formation of that episode (Pearce, 1991), strengthening the potential recall of the event.

Having established a reliable test of the episodic-like memory we used this task to understand the effect of neurological dysfunction in the episodic-like recollection. As the fornix transection is thought to provide an alternative to a hippocampal disconnection (Olten et al., 1982;

Aggleton et al., 1986b, 1992) the role of the 'hippocampal formation' was investigated by administering bilateral lesioning of the fornix. The fornix section did not affect the rat's ability to identify experimental objects, sparing object recognition (semantic) memory, as was the case in the Eacott and Norman (2004). It was also noted that despite elevated tendency to explore in general the ability to recognise objects in fornix severed rat's was similar to that of the control group. Therefore, the evidence of this study support the model proposed by Aggleton and Brown (1999) that stipulated that the hippocampal formation is not involved in the memory responsible for the object recognition.

During the episodic-like memory task recall accuracy of control and fornix severed groups diminished. Although the episodic-like recollection of the control animals was still similar to the rate of recall demonstrated by the intact animals in the earlier studies and the performance of the fornix animals was less reliable. Overall there was no significant difference between the performances of the two groups. Therefore, unlike the proposed model of the episodic memory (Aggleton and Brown, 1999) this test had not yielded sufficient evidence that the hippocampal formation, thought the function of the fornix, was involved in the episodic-like memory paradigm described in this thesis.

It is possible that the performance of the two groups could have been statistically similar because of the relatively low group sizes, as the statistics did indicate that the control group was more accurate at the episodic-like recollection than the fornix group, although not significantly so. Furthermore, as the two groups were not tested on their recollection accuracy over the lengthened (15 min and 1 hour) delay intervals. Testing the affects of delay may aid our understanding of the role of the fornix in the episodic-like memory. The current data therefore, is insufficient to prove or disprove the function of the fornix in the Aggleton and Brown's (1999) model of the episodic memory. Interestingly the fornix group also explored the objects during the initial exploration phase of the test at a reduced rate when the objects were hidden, which could have been symptomatic of

some memory impairments or perhaps suggesting that the fornix severed animals sustained some additional problems of spatial navigation.

The abovementioned tests demonstrated reliable episodic-like recollection in the rat. However, the ability to perform this task was not attained by all animals. Some rats were unable to locate the non-habituated object based on the contextual cue. Some rats adopted a strategy of turning at random or turning in one direction only (e.g. left), as opposed to using event recollection to locate the non-habituated object. This suggested that some rats failed this task because they were unable to comprehend the E-maze test. To address this problem of task learning, in future testing, it would be useful to test a larger population sample and later remove individuals which were unable to learn the task. This could improve overall group performance over the no and delay episodic-like recollection task.

Longer pre-recollection delay of 1 hour resulted in rat's inability to distinguish accurately between the test episodes. As mentioned above the concept of "which" is not a direct substitute for the original "when" used by Clayton and Dickinson (1998), and that could have affected the general performance accuracy. As the two episodes were highly similar the delay length could have served to confuse the two very similar episodes and not necessarily forget them, as the outcome of the task had suggested. Pre-test visual prompts, such as those used in Deweer et al.'s, (1980) could improve the accuracy of rat performance after longer delay. An introduction of the pre-test environmental cues could therefore, improve episodic-like recollection after longer delay intervals in future research of this type of memory.

When the effects of fornix section were tested the two groups (fornix and control) did not differ significantly in their ability to perform the episodic-like memory task, despite the fact that overall fornix animals did not turn toward the non-habituated object at the above chance rate and the control animals did. The statistical similarity between the two groups could have arisen because of the relatively low group sizes, despite the controls approaching recollection superiority in

episodic-like memory over the fornix group. In future research higher numbers of experimental animals or a greater number of experimental trials should be used to improving the statistical power of data analysis (Howell, 1997).

Finally, there are a number of control tests necessary to further understand the function of the episodic-like memory in the rat. For instance it is essential to demonstrate that the rats' ability to navigate within the E-maze was not affected by the fornix section, and that fornix section disrupted the episodic-like recall only. Therefore, as the normal rats described in Chapter 2, fornix severed animal should be able to demonstrate intact memories of "what-where". Also, the implication of hippocampal neurology needs to be explored, where perhaps reversible fornix or hippocampal deactivation could provide useful evidence for the role of the consolidation and retrieval in the episodic-like event recollection.

6.2 Conclusions

In following an ever evolving concept of episodic memory, this thesis undergone an investigation of the possible model of the animal episodic-like memory. Here I described a behavioural task which was able to demonstrate a memory for specific objects, placed in a given location depending on a specific past occasion, otherwise branded as the "what" "where" and "which" episodic-like recall in the rat. Not only did this work show that the rats were capable of recalling episodes, there were also able to retain such memories for at least fifteen minutes before accurately recalling past events. However, there was insufficient evidence that the fornix was involved in the episodic-like memory of the "what" "where" and "which" task, and a more detailed study is necessary to demonstrate the affect of fornix transection on animal recollection ability, to further develop our understanding of the neural bases for the episodic-like model.

These findings are very useful for prospective research, as a rat model of episodic-like memory stands to show that the animal's ability to recall trial unique episodes is indicative of the fact that perhaps the non-human memory is not as far removed from the realms of the human one as

was previously thought. This investigation therefore, provides additional grounds for understanding and research in the general field of episodic and/or episodic-like memory.

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Appendix

a. Test Score Sheet

b. "Object-Place" Test and Probe Protocols

c. "What-where-which" trial protocol

d. Probe trial protocol

Appendix a): *Test Score Sheet*

Test [ ] Day [ ] Rat No. [ ]					Habituation		
	Context [ ]		Context [ ]			Test Context [ ]	
Object							
	Explored YES / NO		Explored YES / NO			Explored YES / NO	
Object Exploration Time (sec)					YES NO		
Rat No. [ ]					Habituation		
	Context [ ]		Context [ ]			Test Context [ ]	
Object							
	Explored YES / NO		Explored YES / NO			Explored YES / NO	
Object Exploration Time (sec)					YES NO		
Rat No. [ ]					Habituation		
	Context [ ]		Context [ ]			Test Context [ ]	
Object							
	Explored YES / NO		Explored YES / NO			Explored YES / NO	
Object Exploration Time (sec)					YES NO		
Rat No. [ ]					Habituation		
	Context [ ]		Context [ ]			Test Context [ ]	
Object							
	Explored YES / NO		Explored YES / NO			Explored YES / NO	
Object Exploration Time (sec)					YES NO		

Appendix b): *"Object-Place" Test and Probe Protocols*

Days 6-17

Day 6	**Day 7**	**Day 8**	**Day 9**
Objects Exploration (right)**A B**(left)	Objects Exploration (right)**A B**(left)	Objects Exploration (right)**B A**(left)	Objects Exploration (right)**A B**(left)
Habituate **B**	Habituate **A**	Habituate **B**	Habituate **A**
Test (right)**A B**(left)	Test (right)**A B**(left)	Test (right)**B A**(left)	Test (right)**A B**(left)
Day 10	**Day 11**	**Day 12**	**Day 13**
Objects Exploration (right)**A B**(left)	Objects Exploration (right)**B A**(left)	Objects Exploration (right)**B A**(left)	Objects Exploration (right)**A B**(left)
Habituate **B**	Habituate **A**	Habituate **B**	Habituate **A**
Test (right)**A B**(left)	Test (right)**B A**(left)	Test (right)**B A**(left)	Test (right)**A B**(left)
Day 14	**Day 15**	**Day 16**	**Day 17**
Objects Exploration (right)**A B**(left)	Objects Exploration (right)**B A**(left)	Objects Exploration (right)**B A**(left)	Objects Exploration (right)**A B**(left)
Habituate **B**	Habituate **A**	Habituate **A**	Habituate **A**
Test (right)**A B**(left)	Test (right)**B A**(left)	Test (right)**B A**(left)	Test (right)**A B**(left)

Day 18 (Probe trial)

Day 18
Objects Exploration (right)**A B**(left)
Habituate **B**
Probe test (No objects)

Appendix c: *"What-where-which" trial protocol*

DAY ONE

Minute	RAT1	RAT 2
	X(AB)	
1	Expose rat 1	
2	to X(AB)	
3	Y(BA)	
4	Expose rat 1	
5	to Y(BA)	
6	Habituate rat	Y(BA)
7	1 to object A	Expose rat 2
8		to Y(BA)
9		X(AB)
10		Expose rat 2
11		to X(AB)
12	X(AB)	Habituate rat
13	Test rat 1	2 to object B
14	X(AB)	
15		
16		
17		
18		Y(BA)
19		Test rat 2
20		Y(BA)

Minute	RAT 5	RAT 6
	X(BA)	
1	Expose rat 5	
2	to X(BA)	
3	Y(AB)	
4	Expose rat 5	
5	to Y(AB)	
6	Habituate	X(AB)
7	rat 5 to	Expose rat
8	object B	6 to X(AB)
9		Y(BA)
10		Expose rat 6
11		to Y(BA)
12	X(BA)	Habituate
13	Test rat 5	rat 6 to
14	X(BA)	object A
15		
16		
17		
18		Y(BA)
19		Test rat 6
20		Y(BA)

Minute	RAT 3	RAT 4
	X(BA)	
1	Expose rat 3	
2	to X(BA)	
3	Y(AB)	
4	Expose rat 3	
5	to Y(AB)	
6	Habituate rat	Y(AB)
7	3 to object B	Expose rat 4
8		to Y(AB)
9		X(BA)
10		Expose rat 4
11		to X(BA)
12	Y(AB)	Habituate rat
13	Test rat 3	4 to object A
14	Y(AB)	
15		
16		
17		
18		X(BA)
19		Test rat 4
20		X(BA)

Minute	RAT 7	RAT 8
	Y(BA)	
1	Expose rat	
2	7 to Y(BA)	
3	X(AB)	
4	Expose rat	
5	7 to X(AB)	
6	Habituate	Y(AB)
7	rat 7 to	Expose rat 8
8	object A	to Y(AB)
9		X(BA)
10		Expose rat 8
11		to X(BA)
12	X(AB)	Habituate rat
13	Test rat 7	8 to object B
14	X(AB)	
15		
16		
17		
18		Y(AB)
19		Test rat 8
20		Y(AB)

Minute	RAT 9	RAT 10
	X(BA)	
1	Expose rat 9	
2	to X(BA)	
3	Y(AB)	
4	Expose rat 9	
5	to Y(AB)	
6	Habituate rat	Y(AB)
7	9 to object A	Expose rat
8		10 to Y(AB)
9		X(BA)
10		Expose rat
11		10 to X(BA)
12	X(BA)	Habituate rat
13	Test rat 9	10 to object
14	X(BA)	B
15		
16		
17		
18		X(BA)
19		Test rat 10
20		X(BA)

Minute	RAT 13	RAT 14
	X(AB)	
1	Expose rat	
2	13 to X(AB)	
3	Y(BA)	
4	Expose rat	
5	13 to Y(BA)	
6	Habituate	X(BA)
7	rat 13 to	Expose rat
8	object B	14 to X(BA)
9		Y(AB)
10		Expose rat
11		14 to Y(AB)
12	X(AB)	Habituate
13	Test rat 13	rat 14 to
14	X(AB)	object A
15		
16		
17		
18		Y(AB)
19		Test rat 14
20		Y(AB)

Minute	RAT 11	RAT 12
	X(AB)	
1	Expose rat	
2	11 to X(AB)	
3	Y(BA)	
4	Expose rat	
5	11 to Y(BA)	
6	Habituate rat	Y(BA)
7	11 to object	Expose rat
8	B	12 to Y(BA)
9		X(AB)
10		Expose rat
11		12 to X(AB)
12	Y(BA)	Habituate rat
13	Test rat 11	12 to object
14	Y(BA)	A
15		
16		
17		
18		Y(BA)
19		Test rat 12
20		Y(BA)

Minute	RAT 15	RAT 16
	Y(AB)	
1	Expose rat	
2	15 to Y(AB)	
3	X(BA)	
4	Expose rat	
5	15 to X(BA)	
6	Habituate rat	Y(BA)
7	15 to object	Expose rat
8	A	16 to Y(BA)
9		X(AB)
10		Expose rat
11		16 to X(AB)
12	Y(AB)	Habituate rat
13	Test rat 15	16 to object
14	Y(AB)	B
15		
16		
17		
18		X(AB)
19		Test rat 16
20		X(AB)

Appendix d): *Probe trial protocol*

DAY EIGHTEEN – no objects on test!

Minute	RAT1	RAT 2
	X(J1 I1)	
1	Expose rat 1	
2	to X(J1 I1)	
3	Y(I1 J1)	
4	Expose rat 1	
5	to Y(I1 J1)	
6	Habituate rat	Y(I1 J1)
7	1 to object I1	Expose rat 2
8		to Y(I1 J1)
9		X(J1 I1)
10		Expose rat 2
11		to X(J1 I1)
12	X()	Habituate rat
13	Test rat 1 X()	2 to object J1
14		
15		
16		
17		
18		X()
19		Test rat 2 X()
20		

Minute	RAT 5	RAT 6
	X(I1 J1)	
1	Expose rat 5	
2	to X(I1 J1)	
3	Y(J1 I1)	
4	Expose rat 5	
5	to Y(J1 I1)	
6	Habituate rat	X(J1 I1)
7	5 to object J1	Expose rat 6
8		to X(J1 I1)
9		Y(I1 J1)
10		Expose rat 6
11		to Y(I1 J1)
12	X()	Habituate rat
13	Test rat 5 X()	6 to object I1
14		
15		
16		
17		
18		Y()
19		Test rat 6 Y()
20		

Minute	RAT 3	RAT 4
	X(I1 J1)	
1	Expose rat 3	
2	to X(I1 J1)	
3	Y(J1 I1)	
4	Expose rat 3	
5	to Y(J1 I1)	
6	Habituate rat	Y(J1 I1)
7	3 to object J1	Expose rat 4
8		to Y(J1 I1)
9		X(I1 J1)
10		Expose rat 4
11		to X(I1 J1)
12	Y()	Habituate rat
13	Test rat 3 Y()	4 to object I1
14		
15		
16		
17		
18		Y()
19		Test rat 4 Y()
20		

Minute	RAT 7	RAT 8
	Y(I1 J1)	
1	Expose rat 7	
2	to Y(I1 J1)	
3	X(J1 I1)	
4	Expose rat 7	
5	to X(J1 I1)	
6	Habituate rat	Y(J1 I1)
7	7 to object I1	Expose rat 8
8		to Y(J1 I1)
9		X(I1 J1)
10		Expose rat 8
11		to X(I1 J1)
12	Y()	Habituate rat
13	Test rat 7 Y()	8 to object J1
14		
15		
16		
17		
18		X()
19		Test rat 8 X()
20		

Minute	RAT 9	RAT 10
	X(I1 J1)	
1	Expose rat 9	
2	to X(I1 J1)	
3	Y(J1 I1)	
4	Expose rat 9	
5	to Y(J1 I1)	
6	Habituate rat	Y(J1 I1)
7	9 to object I1	Expose rat 10
8		to Y(J1 I1)
9		X(I1 J1)
10		Expose rat 10
11		to X(I1 J1)
12	X()	Habituate rat
13	Test rat 9 X()	10 to object
14		J1
15		
16		
17		
18		Y()
19		Test rat 10
20		Y()

Minute	RAT 13	RAT 14
	X(J1 I1)	
1	Expose rat 13	
2	to X(J1 I1)	
3	Y(I1 J1)	
4	Expose rat 13	
5	to Y(I1 J1)	
6	Habituate rat	X(I1 J1)
7	13 to object	Expose rat 14
8	J1	to X(I1 J1)
9		Y(J1 I1)
10		Expose rat 14
11		to Y(J1 I1)
12	X()	Habituate rat
13	Test rat 13	14 to object
14	X()	I1
15		
16		
17		
18		Y()
19		Test rat 14
20		Y()

Minute	RAT 11	RAT 12
	X(J1 I1)	
1	Expose rat 11	
2	to X(J1 I1)	
3	Y(I1 J1)	
4	Expose rat 11	
5	to Y(I1 J1)	
6	Habituate rat	Y(I1 J1)
7	11 to object	Expose rat 12
8	J1	to Y(I1 J1)
9		X(J1 I1)
10		Expose rat 12
11		to X(J1 I1)
12	Y()	Habituate rat
13	Test rat 11	12 to object
14	Y()	I1
15		
16		
17		
18		X()
19		Test rat 12
20		X()

Minute	RAT 15	RAT 16
	Y(J1 I1)	
1	Expose rat 15	
2	to Y(J1 I1)	
3	X(I1 J1)	
4	Expose rat 15	
5	to X(I1 J1)	
6	Habituate rat	Y(I1 J1)
7	15 to object	Expose rat 16
8	I1	to Y(I1 J1)
9		X(J1 I1)
10		Expose rat 16
11		to X(J1 I1)
12	X()	Habituate rat
13	Test rat 15	16 to object
14	X()	J1
15		
16		
17		
18		Y()
19		Test rat 16
20		Y()