Further development of an episodic-like task in rats.

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FURTHER DEVELOPMENT OF AN EPISODIC-LIKE TASK IN RATS

Abstract: Further development of an episodic-like task in rats.

Mónica Jiménez Rodríguez

This thesis aimed to study the impairment observed in rats with fornix damage when performing an episodic memory task that requires recall of the location of objects in a context. To test whether the impairment may have been due to a spatial memory dysfunction, the same group of animals was tested in a spatial control task which is the same in all aspects to the episodic memory task except that there is no role of context. Animals without damage on the fornix gave evidence that the task was not working. This poor performance may have been caused by the experience or the age of the animals or slightly different procedures compared to previous published work. To explore this aspect, a group of young and non-experienced animals was taken to study their performance on the task. Still poor results were obtained which led to study slightly modifications on the procedures which may improve those. There was some evidence that procedural differences may have contributed to different results compared to previously published work. With the aim of improving the task, some modifications were studied. In this sense, the absence of the habituation object did not improve the preference to explore the more novel one but a preference for the location whereby the habituation object used to be was found. The presence of a completely novel object showed good results in the sense of animals having a preference to explore this object. In addition, a novel version of the task showing a cue object to the rat associated to the location where the complete novel object would be, also showed good results. On this basis, the task was found to be a reliable task to study cued recall in the rat. The study of some conditions on this cued recall task showed that animals were able to remember after 20 minutes delay when the cue object was only explored for 1 minute. To investigate whether animals may have been used cues to solve the task, a test was performed which showed that the performance of the animals on the task may have been a result of odour detection rather than memory.

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Chapter 3: Factors in the E maze

Chapter 4: The effect of certain modifications on the what-where-which task and the role of the olfactory cues

Chapter 1: Introduction

This thesis aims to investigate the performance on the episodic memory tasks described in the E maze (Eacott et al, 2005) in the rat by studying what-where and what-where-which memories. In doing so, chapter 2 studies the performance on these tasks in a group of animals (Zinkivskay, 2006) that have either a lesion of the fornix or a sham lesion. Following the results on that chapter, chapter 3 studies possible aspects that might have an effect on the performance. In chapter 4 some variations of the what-where-which task (episodic memory task) are investigated and the hypothesis that animals may use odour cues in the E maze when performing the task is tested.

Episodic memory corresponds to the system that "receives and stores information about temporally dated episodes or events, and temporal-spatial relations among these events" (Tulving, 1972), whereas semantic memory involves the knowledge about words and other verbal symbols, their meaning and references, relations among them and more broadly, about concepts. In *Elements of Episodic Memory,* Tulving (1983) suggested that episodic memory and semantic memory corresponded to two functionally different but interacting systems. He proposed two states of awareness; autonoetic ("self-knowing") consciousness and noetic ("knowing") consciousness. Episodic memory involves the autonoetic consciousness as he named "remembering", whereas semantic memory is identified with noetic consciousness. Episodic memory entails two underlying processes, recollection ("remembering") or retrieval of experienced items and familiarity ("knowing") resulting when the familiar items are accepted as having been explored. On the other hand, recognition involves familiarity to the stimulus and the recollection of the stimulus. These two processes were differentiated based on the receiver operating characteristics (ROC) curves of recognition (Yonelinas, 1994; Yonelinas, 2001). The analysis of

ROC functions shows the probability of "hits" or corrects identifications of old stimuli, against "false alarms" or incorrect identification of new stimuli as if they were old. The ROC curve includes the recollection component as a linear function and the familiarity component as a symmetrical curvilinear function, indicating the existence of both processes.

These processes have become integrated into the taxonomy of memory. Briefly, episodic memory and semantic memory are included in the group of declarative (explicit) memory, referring to the conscious recollection or awareness of events (episodic) and facts (semantic) (Tulving, 1983; Tulving, 1987; Nyberg et al, 1998; Squire and Zola, 1998). This form of memory is different from non-declarative (implicit) memory that constitutes the non-conscious recollection of learned abilities such as skills and habits, priming, simple and classical conditioning, non-associative learning, etc (Jacoby and Witherspoon, 1982; Tulving, 1983; Graf and Schacter, 1985; Rugg et al, 1998). The anatomy of explicit memory is known to be related to the medial temporal lobe and medial thalamic regions while the implicit memories were found not to be impaired by damage on these regions and would be dependent on other regions such as the basal ganglia and the cerebellum (Daum and Ackermann, 1997). Ascending in this scale, both declarative and non-declarative memory entail the permanent or stable storage of the information received that has been defined as long-term memory.

1.1: The hippocampus and memory

The medial temporal lobe is constituted by the hippocampal formation, entorhinal, perirhinal and parahippocampal cortices (Squire and Zola-Morgan, 1991). The hippocampal formation together with adjacent cortex including the entorhinal, perirhinal and parahippocampal cortices were identified as being important for declarative memory (Squire and Zola-Morgan, 1991; Squire, 1992;

Squire and Zola, 1996). A model was proposed whereby episodic memory would be dependent on the hippocampus whereas the acquisition of factual knowledge (semantic memory) would not rely upon the hippocampus (Vargha-Khadem et al, 1997). Supporting the model, Tulving and Markowitsch (1998) suggested that declarative memory would be characterized by shared features common to both episodic and semantic memories. In this view, the perihippocampal cortical regions would be related to declarative memory, whereas episodic memory relies on the hippocampus. This model provides evidence supporting the notion that the acquisition of factual knowledge (semantic memory) can occur independently from episodic memory and that episodic memory may be more dramatically impaired that the semantic memory in anterograde amnesia. It is worth noting that along with this model or view, it was proposed another view that suggested both semantic and episodic memory processes are dependent on the medial temporal lobe and diencephalic structures and that both processes would have a similar impairment in amnesia. It was also argued that episodic and semantic memories would be dissociable in amnesic patients who had severe frontal lobe damage in addition to a medial temporal lobe and diencephalic damage (Squire and Zola, 1998).

The information obtained from behavioural studies, human clinical studies, single-unit recording studies and brain activation studies have provided support to a model of episodic memory that involves medial temporal and medial diencephalic interactions (Aggleton and Brown, 1999). A common feature of the anterograde amnesia is damage to part of an extended hippocampal system comprising the hippocampus, the fornix, the mamillary bodies and the anterior thalamic nuclei (Aggleton and Saunders, 1997; Aggleton and Brown, 1999). The model is based on the view proposed previously by Delay and Brion (1969). It proposes that the anatomical basis of the anterograde amnesia is formed by the connections between the hippocampus, the mamillary bodies, and the medial thalamus (figure 1.1).

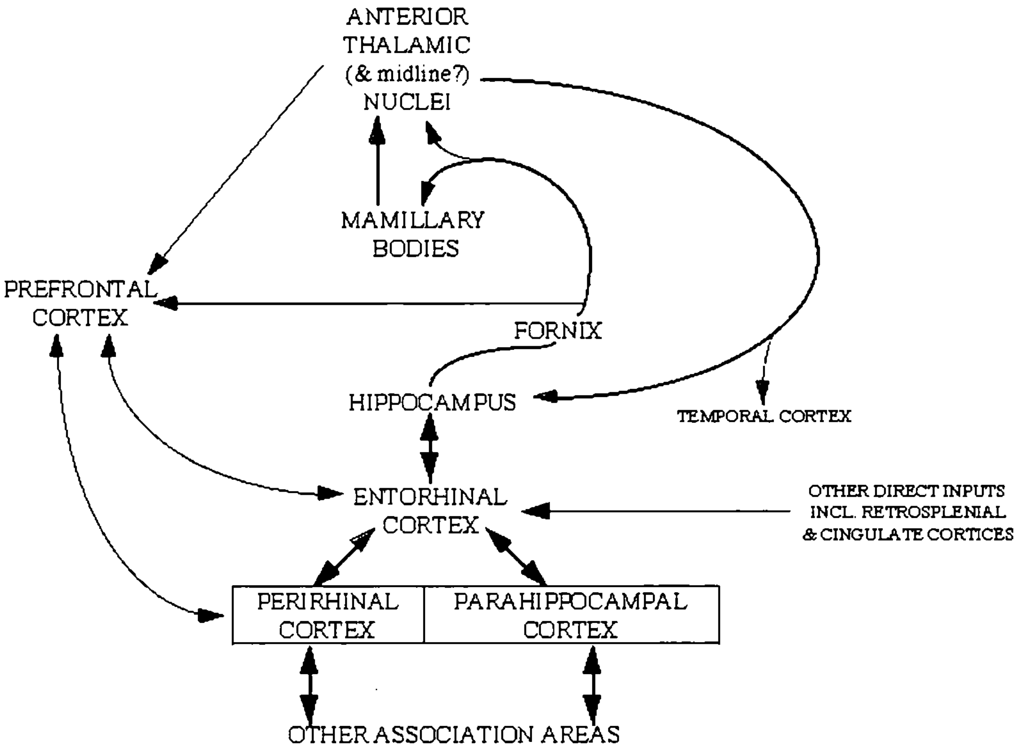


Figure 1.1: Schematic diagram of the main pathways involved in the encoding of episodic memory and underlying recollective aspects of recognition. The thickness of the connective lines gives information of the relevance of those connections. This diagram is taken from Aggleton and Brown (1999).

The efferents from the hippocampus to the medial diencephalon are essential for normal hippocampus functioning and therefore they can be considered as functional extensions of the hippocampus. The main thalamic targets are the anterior thalamic nuclei, which receive direct hippocampal projections via the fornix, and indirect hippocampal projections via the mamillary bodies and the mamillothalamic tract. There are other thalamic nuclei that might participate in this system as the rostral midline nuclei and the lateral dorsal nucleus. One component from the anterior thalamic nuclei projects back to the hippocampus and to the adjacent temporal cortical regions using mainly the cingulum bundle, which form part of a system that influences the temporal lobe processing. Any damage to the different parts of the system generates similar memory impairments. There are other relevant outputs of the system that include the cingulate and prefrontal cortices, but the system beyond the anterior thalamic nuclei becomes more diffuse and those structures are not so relevant for episodic memory. The prefrontal cortex is suggested to have a role in engaging efficient encoding strategies for subsequent recall. The hippocampus and perirhinal cortex are connected anatomically but also they have independent projections to other association cortical areas and therefore it has been suggested that they would form two independent parallel temporal-thalamic systems having distinct contributions to learning and memory. Whereas the hippocampus is connected to the anterior thalamic nuclei, the perirhinal cortex is connected with the medial dorsal thalamic nucleus.

Specifically, the perirhinal cortex is related to the multifeature information about the properties of visual objects providing the necessary associations of visual information of an object but not any visual association between objects (Gaffan et al, 2000; Eacott et al, 2001). Together with the perirhinal cortex, the postrhinal cortex form highly interconnected areas with the hippocampus, but they would have different functions on learning and memory as it has been shown in several studies both in non-human primates and rats (Gaffan et al, 2000; Eacott et al, 2003; Norman and Eacott 2004; Gaffan et al, 2004; Eacott, 2005; Norman and Eacott, 2005). Thus, the postrhinal cortex would be related to the encoding of objects within a scene and context whereas the fornix would be important in learning the combination of objects, their positions and the context whereby they are.

It has been reported that the hippocampus is directly involved in recollection (Fortin et al, 2004; Cipolotti et al, 2006; Daselaar et al, 2006; Gilboa et al, 2006; Eichenbaum et al, 2007; Sauvage et al, 2008) whereas familiarity did not require the hippocampus but the perirhinal cortex (Bogacz et al, 2001). The disruption of the extended hippocampal system including the hippocampal formation, fornix, mammillary bodies and anterior thalamic nuclei has been shown to impair the conscious recollection in the anterograde amnesia whereas the familiarity-based memory was intact (Gilboa et al, 2006; Carlesimo et al, 2007). This constitutes the dual-model of recognition whereby recollection and familiarity would form two independent processes. In support to this view, a dissociation between recollection and familiarity have been proposed on the basis of the behavioural account, electrophysiological and anatomical studies (Yonelinas, 2001; Yonelinas, 2005; Woodruff et al, 2006; Skinner and Fernandes, 2007) and the study of this aspect is further analyzed (Eacott and Easton, 2007).

In contrast to this view, a single-model suggests that both processes may rely on the same neural substrate and it has been reported the hippocampus as a structure implicated in the two functions (Cipolotti et al, 2006; Wais et al, 2006; Bird et al, 2007). The two models are a matter of controversy and the stimuli-based processes have been studied differentially depending on distinct stimuli. Recently, it has been also argued that the view that the hippocampus amnesia is characterized by a strong deficit in recall memory is incorrect and an alternative view has been suggested on the basis of evidence found in humans, monkeys and rodents which shows familiarity processes in the hippocampus as retrieval of experiences in the perirhinal cortex (Kopelman et al, 2007; Squire et al, 2007). Therefore, this alternative perspective proposes that the hippocampus and the adjacent perirhinal cortex would function together in a cooperative and complementary way.

Episodic memory is also defined by its content (i.e. spatio-temporal; context). The relation of the hippocampus to scene learning and the contribution of this structure with memory for the location of objects in a contextual scene have been studied. Gaffan (1994) introduced the term "scene memory", analogous to that of episodic memory based on the observation that object and context memory were impaired when the memory system was interrupted in human, monkeys and rats. What defines the scene is the disposition of the objects in specific places and within a specific background or context. Specifically, scene learning is dependent on the hippocampus (Murray et al, 1998), the entorhinal cortex (Charles et al, 2004), the fornix (Gaffan and Harrison 1989) and diencephalic structures related to the hippocampus (anterior thalamus and mamillary bodies) (Gaffan et al, 2001).

There are several tasks that use spatial information such as the delayed non-matching-to-place (DNMTP), radial maze, water maze and its versions (oasis maze, annular water maze). The hippocampus has proved to be essential in spatial memory (Olton et al, 1978; Olton et al, 1979; Smith and Milner, 1981; Wilson and McNaughton, 1993; Hannesson and Skelton, 1998).

In this regard, the existence of place cells within the hippocampus whose firing is location specific was found (O'keefe and Dostrovs, 1971; O'keefe, 1976; O'keefe, 1979; Breese, 1989; Lenck-Santini et al, 2001). It is still a matter of question though, how these place cells may contribute to the animal's spatial performance forming a specialized navigational system, what are the qualitative and quantitative characteristics of sets of these cells in specialized areas within the hippocampus and how other brain regions would interact with the firing patterns of those cells. The particularities of the contribution of other structures such as the fimbria or fornix (O'keefe et al, 1975) have been referred to the participation in the long-term potentiation in the hippocampus

(Hanesson and Skelton, 1998). The anatomy of the hippocampus together with lesion studies suggested that the two main afferents of CA3 might participate in the encoding and recollection of spatial memory differentially (Lee and Kesner, 2004). In particular, it was reported that dentate gyrus-mediated mossy fibers would contribute to the encoding whereas the perforant path would be involved in the retrieval of spatial memories. Other related structures to the hippocampus such as the enthorhinal cortex were proposed to collaborate also in spatial memory by a non redundant but complementary interaction (Hebert and Dash, 2004).

Not only the hippocampus has been considered as a key for spatial learning and navigation but also it has been studied its role in non spatial tasks such as the self-motion representation. In that respect, there is evidence of a cell type in the CA1 region of the rat hippocampus that codes for the directional heading independent of location information denominated the head direction cells (Leutgeb et al, 2000). Both types of information that is, head direction and location would form part of an integrated system modulating the navigational behavioural patterns controlled by external cues.

The hippocampus would not be considered to be involved exclusively in spatial memory (Day, 2003). It was shown that the hippocampus and its related areas were essential for non spatial memory having a temporal function for long-term memory (Clark et al, 2002). The hippocampus is well-known to be involved in anterograde amnesia (Scoville and Milner, 1957) but it also may be relevant when retrograde amnesia (Cipolotti et al, 2001; Clark et al, 2005) playing a role in recent and remote memories (Broadbent et al, 2006). The hippocampus has also been related to memory for when an event happened and temporal order memories. In this sense, it has been found that the parahippocampal areas would be greater related to spatial memory than the hippocampus in contrast to temporal tasks which would rely more strongly on the hippocampus (Ekstrom and Bookheimer, 2007). The "Sequential order and recognition task" (Fortin et al, 2002) demonstrates the capacity in rats for encoding temporal sequences of items distinguished by their olfactory information and the remembering of prior items using a positive reinforcement or reward. These findings support the capacity that animals have for remembering the order of events and that hippocampus has and essential contribution to this type of memory processing. One characteristic of episodic memory refers to our capacity for differentiating one memory from the other although they share common elements. This particular feature has been studied in "The sequence disambiguation task" (Agster et al, 2002), which presents two odour sequences of a total of six elements sharing two intermediate elements in order to guide the correct selection of consecutive odours for one sequence. The performance of rats reveals the ability to disambiguate overlapping odour sequences and the implication of the hippocampus for the execution of this function.

It has been reviewed that the hippocampus is critical to episodic memory by virtue of its roles in spatial and/or temporal memory. How the neural substrates within the hippocampus carry out these activities is a matter of research. Recent observations suggest that interleaving CA3 sequences bind CA1 assemblies representing overlapping past, present, and future locations into single episodes (Dragoi and Buzsaki 2006). It has been proposed that spatial and temporal processing are performed by parallel homologous circuits within the entorhinal cortex, dentate gyrus and CA3 (Gorchetchnikov and Grossberg, 2007) which may explain how these two aspects are integrated into episodic memory.

1.2: Episodic memory in animals

Understanding episodic memory in animals has proved essential. The development of a model of episodic-like memory in animals allows understanding animal's cognitive capabilities. A mammalian model of episodic memory is important to comprehend the neural basis, molecular mechanisms and behavioural attributes of this type of memory in mammals. In this sense, it contributes to the development adequate therapies using animal models of human neurodegenerative diseases where episodic memory is significantly affected. The major problems for the application of Tulving's and other similar taxonomies to animals are the distinction of implicit memory and explicit memory on one hand and whether the concepts of semantic memory and episodic memory are important to the animal memory. The attribute of conscious awareness appears to be hardly demonstrable without the use of language, however, there is no claim that language is essential in representing and remembering events (Morris, 2001). It has been proposed that animals would have episodic-like memory partly because a number of tasks studying this form of memory have been developed in animals that although they do not strictly fulfill the criteria for episodic memory, they do have an episodic-like character.

There are various learning and memory tasks studying this form of memory that potentially has an "episodic" character in animals. Clayton and Dickinson (1998), named this category of memory "episodic-like" in which the animal recall "what, where and when" (Griffiths and Clayton, 2001). This work presents an ingenious food-caching paradigm in which scrub jays are exposed to perishable favored food (worms) and non-perishable food (peanuts). The birds learn that after a long interval its favored food (worms) decay and become unpalatable. Then, the scrub jays are allowed to recover the perishable worms or the non-perishable peanuts that they have previously cached in visuospatially differentiated locations and contexts, showing a preference to recover the fresh worms after short time periods but not after longer intervals during which the worms have decayed. This paradigm demonstrates memory for where and when the food items (what) were cached but relies on the food storing habits of scrub jays and therefore it would be useful to have adapted behavioural tasks to other laboratory animal species.

There are several studies focusing on the analysis of memory for "what, where and when" in other species. These experiments tried to replicate the Clayton and Dickinson's results in monkeys and rats, but they failed on the basis of a poor temporal component. Bird et al (2003) studied this type of memory in rats in an 8-arm radial maze during 6 experiments. Broadly, this analysis shows how the rats remember what type of food they stored where but not when. In particular, the rats could remember in which arm their favoured type of food where, but they were not able to keep record of the time delays (25 hrs.) by when their favoured food were degraded showing still a preference for these sites where their favoured food was already decomposed. In summary, they could not remember when they stored their favoured food where that is, how long ago they stored it and where. Hampton et al (2005) showed how the monkeys remembered the locations of either preferred or non preferred food at delays of 1 hour and 25 hours, but they did not learn that their preferred food was only available after the shorter period of time. Those paradigms, although having a poor temporal element, they use recall and therefore can provide useful information about human episodic memory.

Recently, Babb and Crystal (2006) have studied a similar version of the Clayton and Dickinson's experiment in other specie such the rat. In this sense, the rats explored a radial maze with different flavours of food in the arms of the maze. Some arms contained a non-distinctive flavour (regular chow pellets) whereas other contained distinctive flavours (raspberry or grape). Then, the rats were returned to the maze after short or prolonged delays. The rats learned that at shorter delays none of the previously baited arms contained food any longer and therefore they learned that they should go towards those arms they had not visited before or those that did not contain food. After long delays, the distinctive flavours were available in the arms again and the previously unvisited arms of the maze were baited with chow pellets but the arms previously baited with chow were now empty. It was demonstrated that the rats visited the replenished arms that were not visited at the shorter delays. Therefore, it was shown memory for where (which arm) and when (short or long delay) but it does not include what component since they do not need to differentiate distinctive flavours. Nevertheless, this aspect was studied by including an aversive distinctive flavour by pairing the flavour with Lithium Chloride. At long delays the rats visited the replenished non-devalued food but did not go to the replenished arms baited with the aversive flavour. Therefore, memory was demonstrated for what flavour, where it was in which arm and although this part of the study used long delays it was shown previously memory for when or how long ago they visited the arms.

Ergorul and Eichenbaum (2004) have studied memory for what, where and when in rats. In essence, this study presented the rat to a series of four odours allocated in cups, each at a different location on a platform. To examine the contribution of olfactory and spatial information, there were probe tests where either the spatial information was absent (odour probe) or the odour cue was not present (spatial probe). In other words, the odour probe shows two cups with two odours from the familiar sequence that are located in a right-left position with respect to a referential axis that did not correspond to their location in the initial sequence whereas the spatial probe shows two cups without the olfactory stimulus in the same positions as in the sequence. The rats with hippocampal damage perform well above chance in the odour probes, so they can remember the sequence of odours even without spatial associations. However, in the spatial probes, the performance is maintained at the same level as expected by chance. On the final what-where-when trial, both olfactory and spatial information are presented. Therefore, the study showed that the rats were able to integrate the what, where and when information to remember order of sequence of events and that the hippocampus was essential for this type of memory. Other tasks such as "Sequential order and recognition task" (Fortin et al, 2002) demonstrate the capacity in rats for encoding temporal sequences of items distinguished by their olfactory information and remembering prior items using a positive reinforcement or reward. Normal rats perform sequential order judgements above chance, being easier for more widely separated items. Nonetheless, hippocampal lesion groups performed near chance level. These findings support the capacity that animals have for remembering the order of events and that hippocampus has an essential contribution to this type of memory processing. One characteristic of episodic memory refers to our capacity for differentiating one memory from the other although they share common elements. This particular feature has been studied in "The sequence disambiguation task" (Agster et al, 2002), which presents two odour sequences of a total of six elements sharing two intermediate elements in order to guide the correct selection of consecutive odours for one sequence. The performance of rats reveals the ability to disambiguate overlapping odour sequences and the implication of the hippocampus for the execution of this function.

Dere et al have designed a task adapted for mice and rats to study episodic-like memory for "what, where and when" (Dere et al, 2005; Kart-Teke et al, 2006). The task consists of a three-trial object exploration paradigm in which different versions based on the novelty paradigm were combined to study object recognition memory (what), the memory for locations whereby objects were located initially (where) and the temporal order in which the objects are presented (when). For this purpose, they included three trials of 10 minutes separated by a delay of 50 min. The first trial shows four objects in an open field with particular locations, the second trial presents other four different objects in specific locations. The test trial includes two objects from the first trial, one stationary or positioned as it was in the first trial and the other displaced from its initial position. The other two objects correspond to those from more recent trial or second one in their stationary positions. The results show that the rodents explore for longer times the objects that have been presented the furthest ago in time (first trial) and within this type that one that is positioned in a novel configuration of place (displaced). Although the task does not rely on recall, it is based on the natural exploratory behaviour which would be an important component of episodic memory (Easton and Eacott, in press).

The term "scene memory" (Gaffan, 1994) has been used to describe a type of memory that is analogous to episodic memory based on the observation that object and context memory were impaired when the memory system was interrupted in human, monkeys and rats. Specifically, scene learning was found to be dependent on the fornix (Gaffan and Harrison 1989) and diencephalic structures related to the hippocampus such as the anterior thalamus and mamillary bodies (Parker and Gaffan, 1997a; Parker and Gaffan, 1997b). What defines the scene is the disposition of the objects in specific places and within a specific background or context. It was suggested that the criteria for episodic-like memory may be redefined to include any occasion-specifying characteristic of the memory for the event in substitution of the temporal aspect when. Moreover, in humans the memory for the temporal context of the event is very poor and can be dissociable from episodic memory itself (Friedman, 1993). In this sense, scene memory involves a redefined triad of episodic-like memory that entail what, where and an occasion-specifying context or which.

Following these studies, Eacott and Norman (2004) adapted Gaffan's task of scene memory in monkeys (Gaffan, 1994) by developing a simple and novel task in rats to analyze recognition memory for objects, their spatial location and the context within they were situated. This task is based on the one-trial object recognition task (Ennaceur and Delacour, 1988), which shows the natural tendency that rats have to explore the novel aspects of their environment. The paradigm is performed in an open field and it associates a right or left position to two distinct objects with a specific context. Both the objects and the context are clearly identifiable by visual and tactile cues. There are two presentation events distinguished by its context, which acts as an "occasion specifier" differentiating the two occasions or episodes. The paradigm shows a new position of one of the objects within a context generating a novel object-place-context composition or a novel what-where-which combination and a novel what-where combination when only one context has been shown. Since this task constitutes an episodic memory task, it would be predicted that it should be severely impaired by lesions within the hippocampal system. This study focuses on the effects of perirhinal, postrhinal and fornix lesions on the performance of the task, demonstrating that the recognition memory for the combination of object, place and context was substantially impaired in fornix lesion animals.

Therefore, it has been proposed that an alternative to study episodic memory would include "which" or the contextual background for an event. In this sense, this alternative can involve temporal aspects (Easton and Eacott, in press).

There are other tasks that require recall such as the paired associate task developed by Day et al (2003). Although not necessarily episodic-like according to strict criteria, such tasks incorporate recall and so are useful for exploring particular aspects of episodic memory. Rats learn two paired associates (flavours of food and their spatial locations) and then remember one of them when cued with the other item.

In summary, it has been reviewed various relevant studies of episodic memory in animals defined by its content (i.e. spatio-temporal; context). A more recent version of the definition of episodic-like memory includes structure and flexibility (de Kort et al, 2005). This work shows that jays are able to remember the what-where-when elements of the caching episode (content) but also that these components are integrated (structure) and updated and generalized over situations (flexibility).

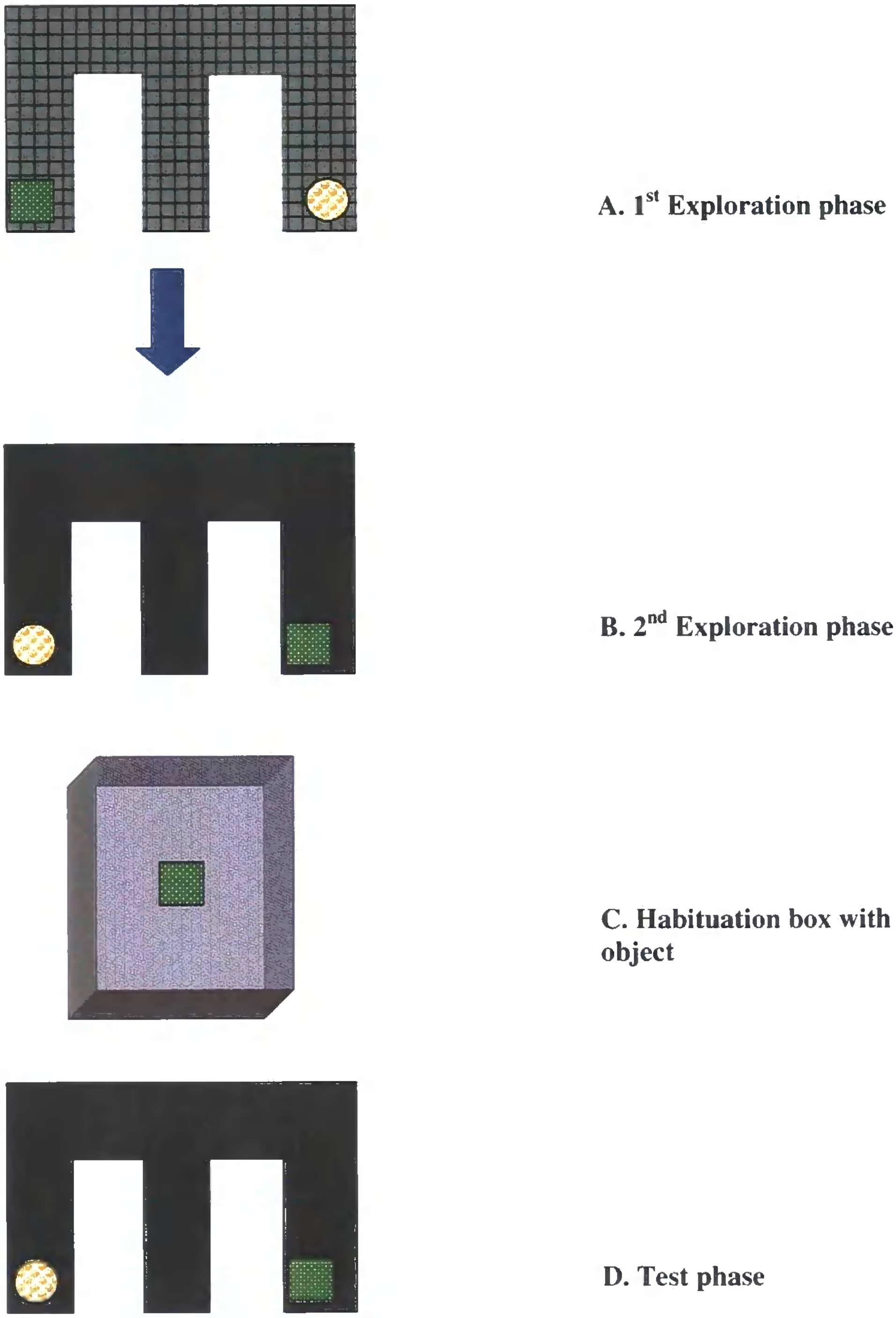
1.3: Development of the E maze to study episodic memory in the rat

The task described above (Eacott and Norman 2004) relies on familiarity of the objects since the objects are visible, but human memory involves both, familiarity and recollection. Therefore, Eacott et al (2005) designed an E shaped maze which permits to study specifically recollection due to the use of hidden objects, and so cannot be solved solely on the basis of familiarity as other recognition tasks. The task also relies on the natural tendency rats have to explore novelty aspects of the environment (Ennaceur and Delacour, 1988).

The E shaped maze has three arms whereby the central arm constitutes the start arm and the two outer arms where two different objects are located. The E-maze includes two contexts differentiated by visual and tactile cues and the objects swap location between contexts (figure 1.2). The rat encodes what-where-which information during the training trials and is then habituated to one of the objects presented before to generate novelty preference. Therefore, in the final trial, the rat is expected to turn preferentially to the object less recently explored or the non habituated one. As commented previously, the configuration of the E maze allows the study specifically of recollect aspects of the experience when the objects are positioned out of sight in the arms of the maze. As an overview, Figure 1.2 represents a schema of the events held in a session of the what-where-which task with objects hidden. The figure shows how the rat has to recall the position of the object within that context and that the paradigm cannot be solved on the basis of familiarity since the objects are not visible. The task gives opportunity to study recall attributes of episodic memory and more specifically remembrance of what-where-which elements of past episodes. Also, the task uses the natural exploratory behaviour which is also discussed as an important component of episodic memory (Easton and Eacott, in press).

Briefly, this study (Eacott et al, 2005) showed that both groups including those performing with visible and hidden objects turn towards the novel (non habituated) object above the level expected by chance. The animal's "percent correct first turn" over 8 days on the what-where-which task with objects visible showed an average of 64.8% significantly above the level expected by chance ($p < 0.001$, one-tailed). When objects were hidden from the start arm, the animals showed recollection of the location of the objects within a context with an average of 65.2% over 16 trials significantly above the chance level ($p < 0.0001$, one-tailed).

Figure 1.2: Outline of the order of events in the what-where-which task, objects hidden. Here is represented an example training session whereby one type of context (seen grey with a mesh) is present in the first sample phase followed by another context (seen black) in the second sample phase, habituation to object and the final test phase, in this case, within black context. The two different objects swap opposite locations for each of the contexts.



A. 1st Exploration phase

B. 2nd Exploration phase

C. Habituation box with object

D. Test phase

It was initially studied the recall performance on the what-where task with objects hidden (Chapter 2; Zinkivskay, 2006). This task shows a unique context per session and it is different between days. Two probe trials were carried out to control for any odour cues in the E maze. The probe trials were the same as a normal day or training session except for that there were no objects at test in the E maze. The animals performance showed that they were able to remember the position of the objects within the E maze showing recall of what-where and that there was no evidence that the animals would guide the behaviour on the basis of odour cues when no object were present at test (probe trials). This task confirmed that the exploration of relatively novel objects was a sufficient reinforcement for the rats to seek hidden objects and that the habituation phase was a reliable method to generate object preference of novelty. Also, the first turn was a good indicator of memory for the location of objects within a context in an E-shaped maze and could be used as an indicator of rat memory for unique events. Therefore, it was ensured that this behavioural methodology could be used to test other types of memory such as what-where-which.

Following those results in the what-where task with objects hidden, it was carried out the study of what-where-which task with object visible and hidden from the start arm as it was also included two probe trials one at the end of each of the two tasks (Chapter 3; Zinkivskay, 2006). The results obtained in the what-where-which task with objects visible showed that the animals turned toward the non habituated objects significantly above chance both in the objects visible and objects hidden tasks. There was no difference in the recall performance between the training group and the probe trials. There was no correlation between the individual rat performance on the objects visible and objects hidden tasks and they did not differed significantly.

Therefore, these results provided evidence for episodic-like recall in the rat. In order to study the memory retention in the rat, the following episodic-like memory paradigm was designed (Chapter 4; Zinkivskay, 2006). It compared the recall accuracy in the what-where-which task with objects hidden between three conditions differing in the habituation phase but all the other procedures remaining the same. The first experiment explored the performance when habituating the animal 5 minutes to one object followed by a 15 min delay in the home cage before being returned to the E maze for the final test phase. A second design was characterized by the use of the delay of 15 min before the habituation to the object and a third and last experiment increased the delay to 1 hour before the habituation. In summary, the delay of 15 min after the habituation was found to be sufficient to the objects become equally relatively novel and the increase on the delay previous habituation was found to affect the recall of the past events by decreasing it. The rats were able to recollect past experiences at delay of 15 min which supports the results obtained on the recognition task described by Eacott and Norman (2004). However, animals were not able to remember past events after a delay of 1 hour differing with those results obtained in the recognition task (Eacott and Norman, 2004).

The role of the fornix on the what-where-which episodic memory task in the E maze has recently been studied (Zinkivskay, 2006; Eacott and Easton, 2007). It was shown that the animals with fornix damage were impaired for the recollect aspects of memory, whilst they showed similar exploration patterns of the objects and therefore familiarity intact. Along with these results, the role of the fornix was examined by studying the performance on the open field what-where-which, episodic memory task (Eacott and Norman, 2004). A severe significant impairment on the task was observed. Since in this task objects were visible when the animals made a choice, it could be solved purely by familiarity mechanisms. The impairment caused by either fornix or hippocampal damage could be due somehow to a dysfunction in spatial memory. Therefore, it is of interest to study these aspects in a control task for the what-where-which memory task that does not include to role of the context to solve the task.

Therefore, the initial aim of the thesis is to study more profoundly the involvement of the fornix in episodic memory by taking the same group of animals described above (Zinkivskay, 2006; Eacott and Easton, 2007). Animals with damage in the hippocampal system have spatial and navigational impairments. For that reason, it is of interest to test whether the impairment observed in recollection may be caused by a dysfunction in spatial memory or in the retrieval of a complete what-where-which triad.

Chapter 2: Role of the fornix and what-where memory

2.1: Introduction

It has been suggested that there are two anatomical systems underlying the processes of recollection and familiarity (Aggleton and Brown, 1999). One pathway entails the encoding of episodic information and the recollection aspects of recognition and generally it is critical for normal episodic memory. This pathway comprises the link from the hippocampus to the mamillary bodies, anterior thalamic nuclei, via the fornix. The second pathway corresponds to the other process arising from recognition that is, stimulus familiarity ("knowing"). This pathway requires the perirhinal cortex in the temporal lobes, which projects both to the medial dorsal thalamus directly or indirectly through the entorhinal cortex and to the prefrontal cortex. Any damage of this hippocampal-anterior thalamic axis causes anterograde amnesia (Delay and Brion, 1969; Aggleton and Saunders, 1997). Specifically, clinical studies in patients with fornix damage (Gaffan and Gaffan, 1991; Aggleton et al, 2000) and selective fornix lesion with non-human primates (Gaffan, 1994) shows that the fornix damage gives rise to an anterograde amnesia and reveals its essential role in episodic memory. The role of the fornix was examined by studying the performance on the open field what-where-which, episodic memory task (Eacott and Norman, 2004). A relevant impairment on the task was observed. Since in this task objects were visible when the animals made a choice, it could be solved purely by familiarity mechanisms. Recently, it has been reported that the fornix lesioned animals were impaired in recall on the what-where-which memory task (Eacott and Easton, 2007). Rats with fornix damage were impaired for the recollect aspects of memory, whereas they showed similar exploration patterns of the objects and therefore familiarity intact.

Animals with damage in the hippocampal system have spatial and navigational impairments (Olton et al, 1978; Olton et al, 1979; Smith and Milner, 1981; Wilson and McNaughton, 1993; Hanesson and Skelton, 1998). Therefore, in the light of the finding of deficit in recall of what-where-which memory, it is essential to test the performance of the same group of animals on a spatial control task. The spatial task is the same as the what-where-which paradigm except that there is no role of context. Essentially, the control task explores whether there may be effects of the fornix lesions on what-where memory. In other words, it studies whether the deficits found in recall of what-where-which are caused by deficits in recall of what-where memory.

This chapter experiments then take the same group of fornix lesion and sham lesion animals described before (Eacott & Easton, 2007) and test them on the what-where control task (experiments 1 and 2). Results from previous work in the what-where objects hidden version of the task in unoperated rats (Chapter 2; Zinkivskay, 2006) showed that the animals recall what-where information significantly above chance (61%) when objects are out of sight. On the light of those results, it is expected that the sham group would perform at a similar level and that the performance of the fornix group would vary depending on the degree of involvement of the fornix structure to the execution of the what-where spatial memory task compared to the what-where-which task. Results observed before (Chapter 3; Zinkivskay, 2006) showed that there was no correlation between individual rat performance on the objects visible and objects hidden versions and they did not differed significantly. Therefore, it is worth doing the objects hidden version of the what-where task (experiment 2) in addition to the objects visible version (experiment 1). Because the performance on this task appears to be weak, it is also explored some possible reasons for this poor performance.

2.2: Experiment 1 (What-where; Objects visible) and Experiment 2 (What-where; Objects hidden)

2.2.1: Methods and Materials

2.2.1.1: Subjects

Fourteen male Dark Agouti rats (Bantin and Kingman, Hull, UK) were used. Testing began when the animals were 13-14 months. They were housed in groups of two or four rats in opaque plastic cages (960 cm^2 x 20 cm height and 1575 cm^2 x 20 cm height, respectively) in diurnal conditions (12 hr light/dark cycle). All testing took place during the light phase. Throughout the study, all animals had *ad libitum* access to food and water. There were two groups of 7 animals on the basis of the surgery method applied, one group having an electrolytic lesion of the fornix (Group Fx) and the other one with sham surgery (Group Sham). These two groups were exactly the same animals as from Zinkivskay's data (unpublished MSc thesis, 2006; Eacott and Easton, 2007). Therefore, these animals were not tested for 6 months between Zinkivskay's testing and the present experiment. The same group of animals performed all the experiments described in this chapter.

2.2.1.2: Apparatus

Testing was conducted in a wooden E-maze (60 cm long x 37 cm wide x 21 cm high, with three arms, each 15 cm wide x 21 cm long). The apparatus was the same maze as used by Zinkivskay in previous work. The E maze has three arms with a start arm in the middle to hold the rat and two outer arms. The apparatus consists of two different contexts and a set of objects. The inner surface of the maze is either smooth, matt black (Context X) or grey on walls and black floor all covered with a wire mesh (Context Y), to provide two different visual and tactile contexts (figure 2.1). A low light level camera was positioned above the maze and all the testing sessions were recorded onto DVD. As part of the procedures, there was a holding cage or habituation box into which the rat was placed between presentation of contexts and at habituation. This holding cage was a home cage that would hold four rats (1575 cm^2 x 20 cm height), and the floor of the holding cage was covered in sawdust. Two random objects (A and B) were placed either visible to the animal in the backbone of the maze or out of sight in the arms at either end of the maze. The objects used were obtained from a variety of sources with the following collection criteria: a) there would be four copies for use respectively in the exploration, habituation and test phases to avoid any scent marking; b) they should be stable enough for the rats to explore them; c) they should be of a broadly similar size maintaining a minimum height in order to be distinctive enough; d) both objects should be equally balanced regarding the ornamentation and e) they should be made of a material that cannot be gnawed easily by the rats. Examples of objects included duplo blocks, bottles, toys, jars, ornamentation objects. The object might be a combination of objects to form a complete copy of an object. Figure 2.2 gives a representation of some example objects used in the training procedures. Animals performed one session per day and the objects were session unique so there was no object presented for more than one day in an animal's experience. The same apparatus was used for all the experiments described in this chapter.

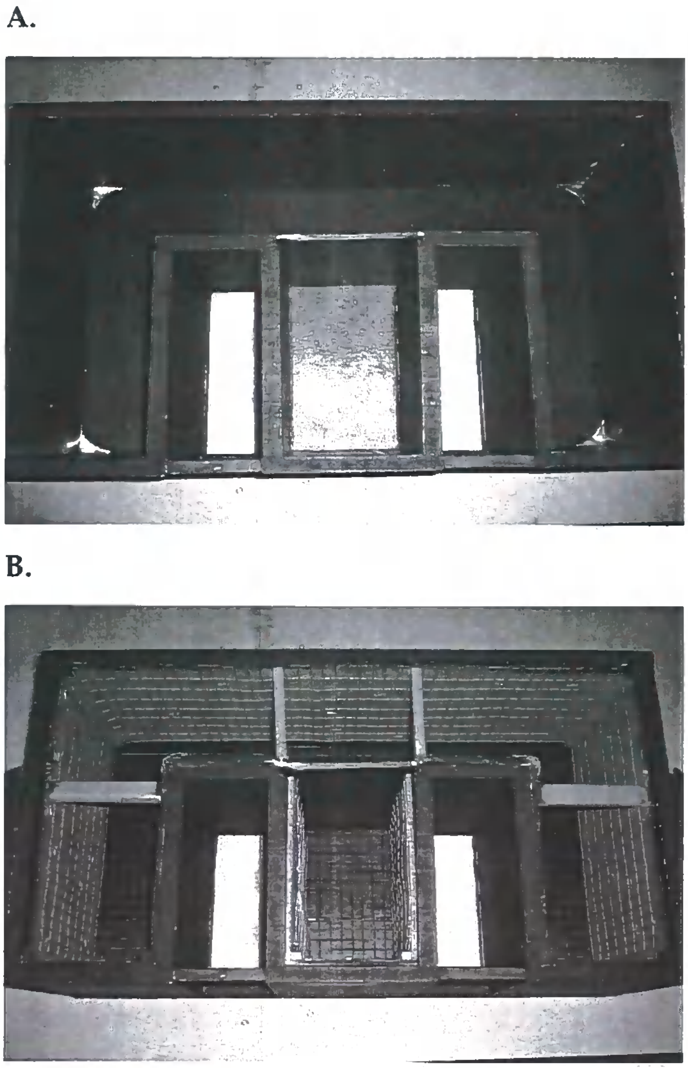


Figure 2.1: Photographic illustration of the two contexts in the E maze. Figure 2.1.A represents the E maze in context X and figure 2.1.B corresponds to context Y. The two outer arms can be observed at the two sides of the photograph as the start arm is located between them in the middle of the E maze.

Figure 2.2: Example objects used in the training procedures. These are random objects A and B used in two different sessions or days. The objects are duplo blocks, bottles, toys, jars, ornamentation objects or a combination of objects to form a complete copy of an object.

2.2.1.3: Behavioural Methods

2.2.1.3a: Experiment 1: What-where; Objects visible

Habituation

Because the rats had previously been tested in this apparatus on a similar procedure only a brief period of re-habituation was necessary. The rats were habituated during two 30 minute sessions (on two different days) in each context to the E maze in phase 1 of pre training. In phase 2, the rats were habituated to the procedures used in the what-where task and two different objects were positioned so that they were visible from the end of the start arm.

Each session consisted of a first exploration phase of five minutes within one context followed by eight minutes in the holding cage without any object present. Then, the rat was returned to the E maze for five minutes for a final test phase in the same context as before and with the objects in the same positions. There were two sessions to habituate the animal to each of the two contexts. By this procedure, the rats were familiarized with the schedule of events and the presence of two visible objects at either side of the maze and it taught the animals that the location of objects was stable within a given context.

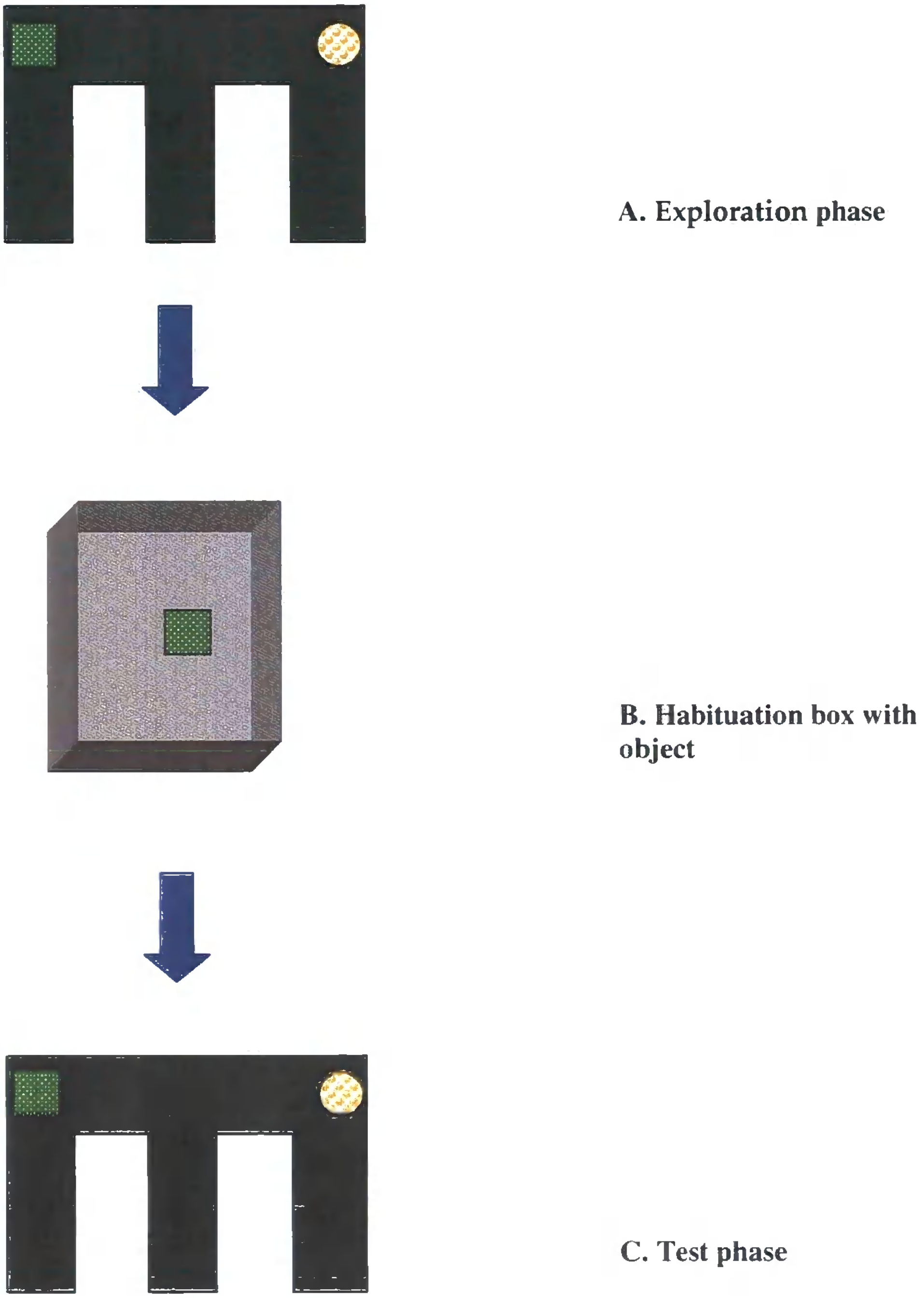
Training

The training phase consisted of eight sessions of two 2 minutes each separated by a habituation phase of 8 minutes. Animals performed one session per day. For each session and between each 2 minute phases in the maze, the rat was transferred to the holding cage for 8 minutes where it was allowed to explore and habituate to one of the objects (figure 2.3). The presentation of contexts X and Y was alternated and counterbalanced between days. Therefore, 4 sessions were conducted in context X and 4 in context Y. The position of the objects within a context was counterbalanced in such a way that the number of right and left turns at test towards the non habituated or habituated objects remained the same. The selection of the object to explore at the habituation phase was counterbalanced so that the number of A and B objects were the same per session.

2.2.1.3b: Experiment 2: What-where; Objects hidden

A total of sixteen sessions were performed at the rate of one session per day. The same schedule of events and procedures as was explained for experiment 1 above was followed except for the location of the objects in the E maze, which were out of sight in the end arms of the maze (figure 2.4).

Figure 2.3: Outline of the order of events in experiment 1; What-where, objects visible. This is an example session of training whereby context X and two objects A and B randomly selected and visible from the start arm are present in the E maze.

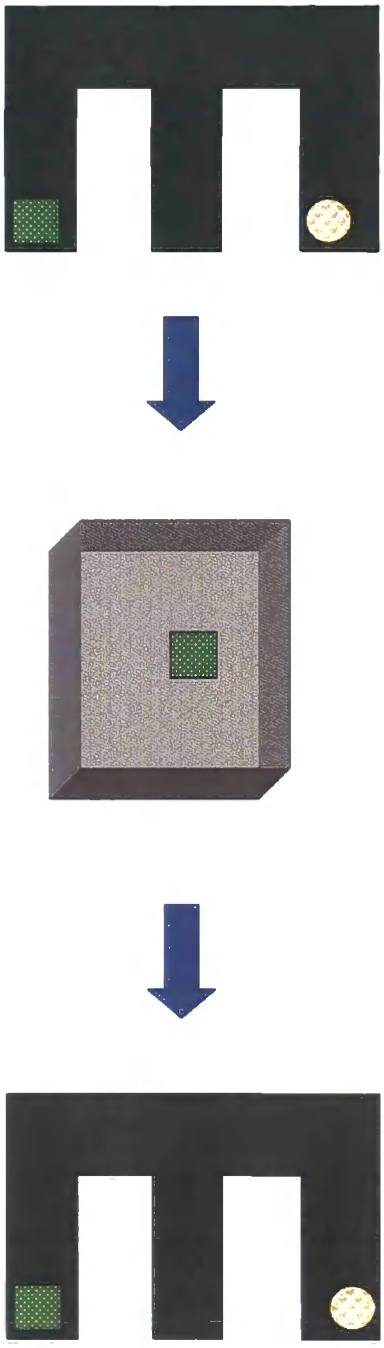


A. Exploration phase

B. Habituation box with object

C. Test phase

Figure 2.4: Outline of the order of events in experiment 2; What-where, objects hidden. This is an example of one training session whereby context X and two objects A and B randomly selected and out of sight from the start arm are present in the E maze.



A. Exploration phase

B. Habituation box with object

C. Test phase

2.2.1.4: Data analysis

The experiments were based on the natural tendency the rats have to explore the novel aspects of their environment (Ennaceur and Delacour, 1988). Animals would typically explore the object to which they were not habituated in the holding cage (Experiments 1, 2 and 3) or that one that was suited in a novel configuration of what-where (Experiment 4), according to their innate preference for novelty. The difference between the time spent exploring non habituated and habituated objects or novel and familiar objects in place was calculated as a proportion of the total time spent exploring both objects (Ennaceur and Delacour, 1988). This ratio is named the D2 score. On this measure, a value of zero reveals no difference in exploration of the two objects and therefore no preference for either object. Values higher than zero reveal greater exploration of novelty. Moreover, a value of 1 means a complete preference to novelty whereas a value of -1 means a complete preference to the non novel object.

2.2.2: Results

2.2.2.1: Experiment 1: What-where; Objects visible

The sham group showed a mean percent of turns towards the non habituated object of 59.82% (SE = 6.07) that is not significantly above chance (t (6) = 1.62, p = 0.16, 2-tailed). The fornix lesion group is performing at chance level, turning towards the non habituated object the 51.79% (SE = 5.05) of the time. The fornix data is not significantly different from chance (t (6) = 0.35, p = 0.74, 2-tailed). The Independent Sample t Test comparing the results from the two groups shows that there is not a significant difference between them (t (12) =1.02, p = 0.33, 2-tailed). When considering D2 scores for both groups, the animals do not have a significant preference for either object either within the sham group (M_{D2}= 0.05, SE = 0.08, t (6) = 0.68, p = 0.52, 2-tailed) or in the fornix group (M_{D2}=-0.09, SE = 0.07, t (6) = -1.21, p = 0.27, 2-tailed). Accordingly, there is not a significant difference in the D2 scores recorded for the sham and fornix lesion animals (t (12) =1.33, p = 0.21, 2-tailed).

2.2.2.2: Experiment 2: What-where; Objects hidden

For the present task, neither sham nor fornix animals performed significantly different from the level expected by chance (Sham: M = 50.00%, SE = 3.05; Fornix: M = 52.68%, SE = 3.57). The One Sample t Test shows that the sham group does not differ from chance (t (6) =0.00, p = 1, 2-tailed) and neither do the fornix lesioned animals (t (6) = 0.75, p = 0.48, 2-tailed). When comparing the "percent correct first turn" between both groups, there is not a significant difference between them (t (12) = -0.57, p = 0.58, 2-tailed). There is a highly significant difference in the D2 scores recorded for the sham and fornix lesion animals (t (12) = -3.84, p = 0.002, 2-tailed). The difference is based on the significant preference for the relatively novel object in the fornix group (M_{D2} = 0.14, SE = 0.03, t (6) = 4.11, p = 0.006, 2-tailed). However, the sham animals do not show a preference for exploring novelty (M_{D2} = -0.06, SE = 0.04, t (6) = -1.59, p = 0.16, 2-tailed).

2.2.3: Discussion

The results obtained in experiment 1 show that the what-where visible objects task does not work in either group of animals. These data demonstrate that there is no preference for either object. Previous results (Chapter 3; Zinkivskay, 2006) showed that there was no correlation between individual rat performance on the objects visible and objects hidden versions and they did not differed significantly. Therefore, since the results with objects visible do not predict in all cases the performance in the what-where task with objects hidden, experiment 2 assesses the animals' performance in this version of the task. Findings from previous work in the what-where, objects hidden version of the task in unoperated rats (Chapter 2; Zinkivskay, 2006) showed that the animals recall what-where information significantly above chance (61%). However, results from current experiment 2 show no evidence for recall in either group of animals. However, since there is limited evidence for an object preference for the relatively novel one as expressed by their D2 scores (fornix animals in experiment 2), these data do not address the question of the effects of fornix lesions on recall. The two aspects of episodic memory in regard to know and remember are linked to distinct neural systems (Aggleton and Brown, 1999). They differ in the patterns of performance when statistically analyzing the receiver operating characteristics (ROC) curves (Yonelinas et al, 1998). It has also been supported recently by behavioural evidence of this dissociation in rats (Eacott and Easton, 2007). Therefore, these behavioural studies with lesion animals demonstrate the dissociation of recall from familiarity based recognition memory. In summary, different results might be observed in regard to the recollection and familiarity measures referring to first turn and D2 ratios scored.

Since the what-where task does not work in either group of animals, we returned to the what-where-which task with objects visible, a task that these same animals had previously performed well.

2.3: Experiment 3 (What-where-which; Objects visible) and Experiment 4 (What-where; Objects visible)

2.3.1: Behavioural Methods

2.3.1.1: Experiment 3: What-where-which; Objects visible

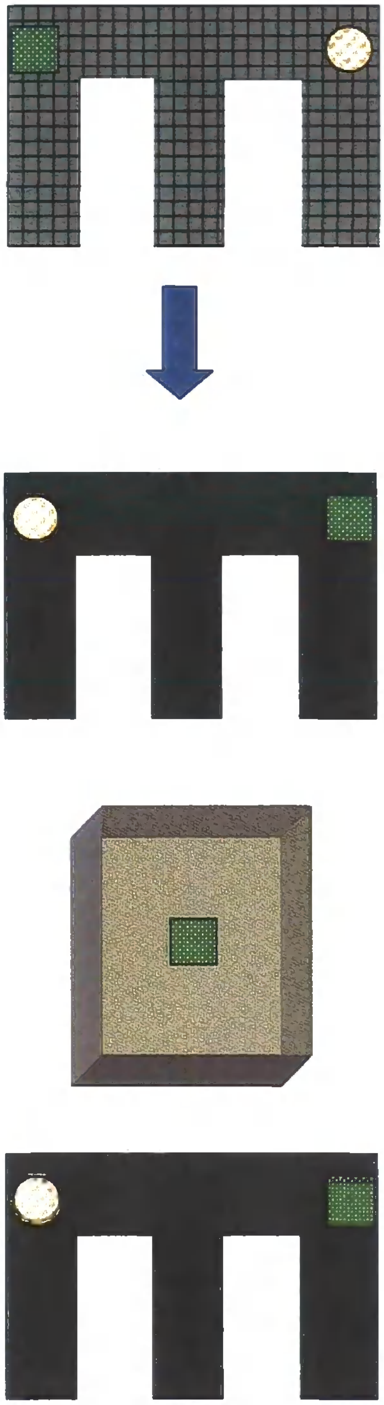
Habituation

In phase 3 of pre-training, the rats were habituated to the next training procedure and as main criterion the objects were visible from the start arm. Briefly, the procedure is based on stage 3 of pre-training from Eacott et al (2005). The exploration phase consisted of two 2 minutes phases in the maze separated by a delay of 1 minute in which the rat was transferred to the holding cage. The first two phases showed the two contexts and were counterbalanced for order of presentation. The position of the objects was specific to each context showing opposite location between contexts. Then, the rat was transferred to the holding cage without an object present for 8 minutes after which it was returned to the E-maze for a 2 minute test phase in the presence of one of the earlier contexts. By using this procedure, the rats were familiarized with the schedule of events and it shows the animals that the location of the objects remained stable within a given context, even with multiple events.

Training

The rats performed the training during eight sessions at the rate of one session per day. The procedure used is the same as for the habituation phase except for the habituation to one object during the 8 minutes interval (figure 2.5). The methodological procedure was based on phase 4 of pre-training from Eacott et al (2005), except for the duration of the phases that is 2 minutes instead of 3 minutes.

Figure 2.5: Outline of the order of events in experiment 3; What-where-which, objects visible. Here is represented an example training session whereby context Y is present in the first exploration phase followed by context X in the second phase, habituation to object and the final test phase, in this case, within context X. The two objects A and B swap opposite locations for each of the contexts.



A. 1st Exploration phase

B. 2nd Exploration phase

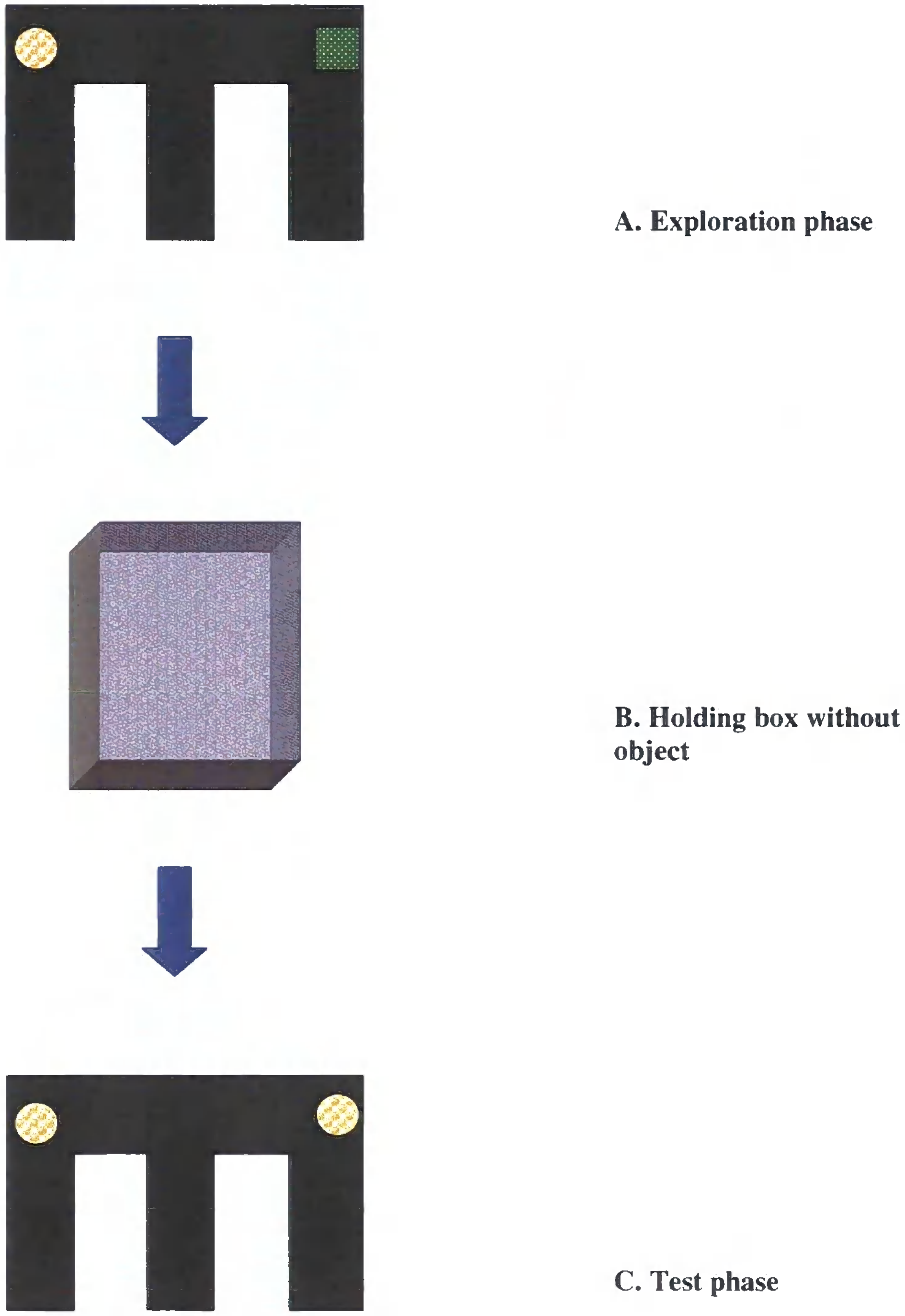
C. Habituation box with object

D. Test phase

2.3.1.2: Experiment 4: What-where; Objects visible

The experiment procedure indicated here was based on Experiment 3 described by Eacott and Norman (2004) and studied the recognition memory for what-where since the objects were visible. The present experiment was carried out over four sessions on four different days. Each day the rat performed a 2 minutes phase within a context after which it was transferred to the holding cage without an object present for 8 minutes. Finally, it returned to the E-maze for the last 2 minute test phase in the same context but with two copies of the same object at either end of the maze. This experiment was performed as a control to study the preference to novelty or novel object in place in this group of animals. Figure 2.6 gives an outline of the procedure.

Figure 2.6: Outline of the order of events in the E maze in experiment 4. As described, the events shown are the same as for experiment 1 except that there is no habituation to the object and there are two copies of the same object in the E maze at test phase.



2.3.2: Results

2.3.2.1: Experiment 3: What-where-which; Objects visible

The "percent correct first turn" shows that animals do not turn preferentially towards the non-habituated object, either in the sham group (M = 57.14%, SE = 6.16, t (6) = 1.16, p = 0.29, 2-tailed) or the fornix group (M = 51.79%, SE = 5.23, t (6) = 0.34, p = 0.74, 2-tailed). Accordingly, there is not a significant difference between the two groups (t (12) = 0.66, p = 0.52, 2-tailed). There is a significant difference in the D2 scores recorded for the sham and fornix lesion animals (t (12) = 2.36, p = 0.04, 2-tailed). This observation is explained by the fact that the sham animals show a preference to the relatively novel object (M_{D2} = 0.22, SE = 0.09, t (6) = 2.40, p = 0.05, 2-tailed) whereas the fornix rats do not explore significantly different from no preference level (M_{D2} = -0.08, SE = 0.09, t (6) = -0.91, p = 0.4, 2-tailed). The "percent correct first turn" between the three experiments that are, what-where with objects visible, what-where with objects hidden and what-where-which with objects visible, was compared in relation to the two surgery groups. A mixed ANOVA was performed and it was found that there was no significant difference between the three experiments (F (2, 24) = 0.34, p = 0.72). There was no interaction effect between experiment and group of lesion animal (F (2, 24) = 0.50, p = 0.62, 2-tailed). Therefore, the progression throughout the three experiments of the "percent correct first turn" in both groups occurred in a similar manner. The test for the differences between the two groups alone found no significant difference between them (F (1, 12) = 1.55, p = 0.24, 2-tailed). Figure 2.7 represents the "percent correct first turn" of the animals during all three experiments briefly explained before. Figure 2.8 shows the D2 scores for the two groups of animals and the three experiments 1, 2 and 3.

2.3.2.2: Experiment 4: What-where; Objects visible

There is no significant difference in the "percent correct first turn" between sham and fornix lesioned animals (t (12) = 0.55, p = 0.59, 2-tailed). The mean percentage of turns to the relatively novel object in the sham group (M = 50.00%, SE = 7.71) is similar to the fornix group (M = 42.86%, SE = 10.51). Both groups do not perform differently from the level expected by chance (Sham: t (6) = 0.00, p = 1, 2-tailed; Fornix: t (6) = -0.68, p = 0.52, 2-tailed). For the D2 ratio, there is no significant difference between the sham and fornix lesioned animals (t (12) = -0.16, p = 0.88, 2-tailed). Also, there is no significant preference for either object in either the sham (M_{D2} = 0.01, SE= 0.18, t (6) = 0.05, p =0.96, 2-tailed) or the fornix group (M_{D2} = 0.04, SE = 0.14, t (6) = 0.32, p = 0.76, 2-tailed).

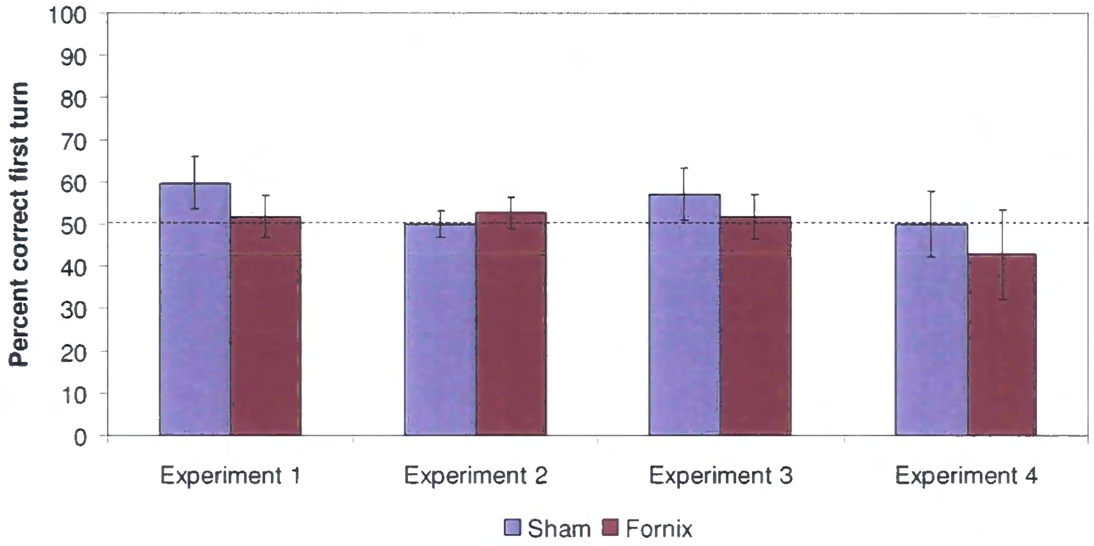


Figure 2.7: First turn performance of the two groups of animals in experiments 1, 2, 3 and 4. The bars represent means and standard errors.

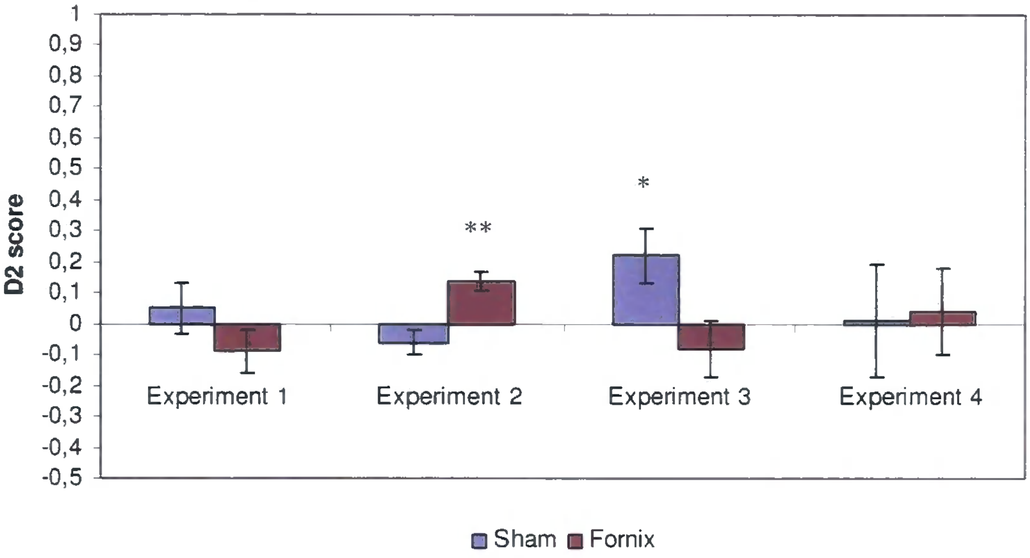


Figure 2.8: Exploration ratios of the two groups of animals in experiments 1, 2, 3 and 4. The bars represent means and standard errors. Asterisks (**) represent statistical significance of the "D2 score" mean within the fornix group compared to no preference, zero ($p < 0.01$); Asterisk (*) represents statistical significance of the "D2 score" mean within the sham group compared to no preference, zero ($p \leq 0.05$).

2.3.2.3: Performance in the what-where-which task with objects visible

In order to compare the results obtained in the present study with those of previous work performed with the same group of animals (Zinkivskay, 2006), a Paired t Test was performed. Experiment 3 from this chapter includes eight days of training on what-where-which objects visible as it was used in previous work. To outline here, the previous work had the following results regarding the sham and fornix animals. The sham group had the mean average of turns towards novelty equal to 67.86% (SE = 7.65). This group performed significantly above chance (t (6) = 2.33, p = 0.03, 1 tailed). The fornix group turned towards novelty the 64.29% (SE = 7.43) of the time which was significantly above chance level (t (6) = 1.92, p = 0.05, 1 tailed). There was no significant difference between the two groups of sham and fornix animals (t (12) = 0.33, p = 0.74, 2-tailed).

A paired t test was carried out to compare these data to the "percent correct first turn" of these same animals in experiment 3 of the current chapter. A marginally significant difference was found within the sham group in the final mean average of correct turns towards the novelty (t (6) = 2.17, p = 0.07, 2-tailed) (figure 2.9). The comparison within the fornix group of the two experiments indicates that there is not significant difference in the "percent correct first turn" (t (6) = 1.76, p = 0.13, 2-tailed) (figure 2.9).

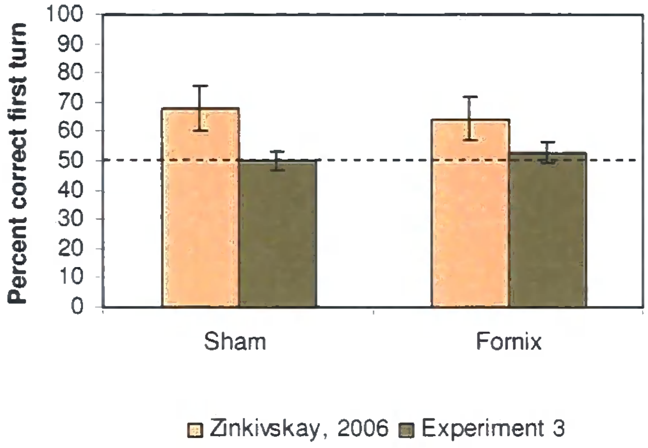


Figure 2.9: First turn performance in the what-where-which task with objects visible comparing experiment 3 to previous work (Zinkivskay, 2006) and in relation to the sham and fornix animals. The bars represent means and standard errors.

2.3.3: Discussion

Results from previous work in the what-where-which task, objects visible task (Zinkivskay, 2006) showed that the animals turned toward the non habituated objects significantly above chance for either group of sham and fornix animals. Here, limited evidence for a preference for the non habituated object is found (sham animals in experiment 3) and no evidence for a preference for the novel object in the sham group, which might be as they are now older and experienced or that they may not habituate in the same way to objects. Experiment 4 investigates these two possibilities since it does not require habituation to the object. No evidence for a preference for the non habituated object is found in experiment 4.

Experiments from this chapter show that animals still perform the task as they show exploration of the objects. However, there is limited evidence of a preference for the non habituated object (fornix group in experiment 2 and sham group in experiment 3) and no evidence for a preference to the novel object in experiments 1 and 4 in either group of animals. Therefore, the data does not address the question of the effects of fornix lesions on recall and familiarity.

Animals appear unable to show a preference for one or other object based on novelty or relatively familiarity, or to show recall based on these preferences. Therefore, the possible conclusions to be made on the basis of the results explained above are the following. These animals were middle-aged (13-14 months) when the testing started. Aging has been shown to have an effect in cognitive functions within the memory domain (Small et al, 1999). Specifically the age-related decline was reflected in specific aspect of memory that were, the acquisition and early retrieval of new information although not memory retention. This aspect has also been studied in the rat by showing age-related decreases in the performance on learning and memory tasks (Wyss et al, 2000). Therefore, it may be possible that these animals show a poor performance due to a more advance age than when they performed the tasks on previous experimental work with good performance. Previous published demonstrations of good performance in this task used animals that were naïve, while these animals had been previously tested by Zinkivskay. Therefore, these animals may not be able to perform well any of these tasks because they are too experienced. The relatively novel object constitutes a rewarding element for the animals when exploring at test in the E maze. The experience the animals received with objects on previous training might lead to a non motivational state. In addition to the postulated explanations, it is also possible that these animals may not be able to perform well because there are different procedures between these experiments and those referred to previous work and that these differences are crucial to the performance. This aspect will be then discussed more fully later. Therefore, following the present results chapter 3 will take a naïve group of animals and explore the different aspects of the procedure to investigate which might be influential in modifying the performance on the task.

Chapter 3: Factors in the E maze

3.1: Introduction

In chapter 2 experienced animals with sham lesions or lesions of the fornix were poor at a task of what-where and a task of what-where-which. As discussed, this might have been a result of the age of the rats, the experience of the rats or small differences in the experimental procedure compared to earlier work. To test these hypotheses, a naïve group of rats was used in the studies in this chapter, and therefore the age or the animals' experience should not be factors influencing performance. The learning abilities of rats decrease with age (Rapp et al, 1987) showing acquisition deficits in aged rats although the performance was found to be at similar level compared to the young group. Prolonged periods of training may lead to animals that are highly experienced and consequently have no interest or motivation to perform the task. When using a completely naïve group of rats, these animals have not been trained before in the task. Therefore, it is expected they would show exploration and motivation to perform the task. It is worth noting that this normal group of rats had no surgery method applied which may affect the performance on the task. This situation allows studying other aspects that may influence the results of the task and not the contribution of an anatomical structure in the cognitive attribution of the task. Therefore, since the age or the animal's experience could not have an effect on the data, it was possible to test explicitly whether there was any procedural detail differing from previous work that may affect the performance. In summary, this study clearly answers the expounded question whether the performance in chapter 2 reflected something about the task methodologies or something about those animals in regard to the age or the experience.

3.2: Experiment 1: What-where; Objects visible

3.2.1: Introduction

The what-where task with objects visible in chapter 2 did not work. The animal's performance might have been poor because the age of the rats, the experience of the rats or some variation in the procedure compared to previous work. Therefore, we need to test the performance in this task with a complete naïve and young group of rats in experiment 1.

3.2.2: Methods and Materials

3.2.2.1: Subjects

A naïve group of sixteen Dark Agouti rats (Bantin and Kingman, Hull, UK) were used in the behavioural study. The animals were housed in groups of four rats in diurnal conditions (12 hr light/12 hr dark cycle) in opaque plastic cages (1575 cm^2 x 20 cm height) and all testing was carried out during the light phase. Throughout the study, all animals had *ad libitum* access to food and water. The same group of animals performed all the experiments described in this chapter. Testing began when the animals were approximately 10 weeks old.

3.2.2.2: Apparatus

The apparatus was as described in chapter 2.

3.2.2.3: Behavioural Methods

3.2.2.3a: Habituation

The procedures followed for the what-where task with objects visible from chapter 2 (experiment 1) were used here, except that exploration phases (the sample phase and test phase) were two minutes long rather than the five minutes in chapter 2. This change was to bring the current experiment in line with the procedure from previously published work (Eacott et al, 2005).

3.2.2.3b: Training

Eight training sessions completed the present study following the same methodological procedures as for chapter 2, experiment 1.

3.2.3: Results

The mean percent of first turns toward the novel object was 48.8% (SE = 3.0) and was not significantly different from chance (t (15) = -0.39, p = 0.70, 2-tailed) (figure 3.1). The average D2 score for the group was not significantly different from the no preference level (M = -0.01, SE = 0.04, t (15) = -0.20, p = 0.85, 2-tailed) (figure 3.2).

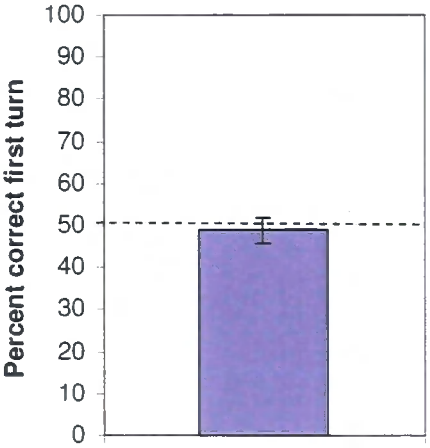


Figure 3.1: First turn performance in experiment 1; What-where, objects visible. The bars represent means and standard errors.

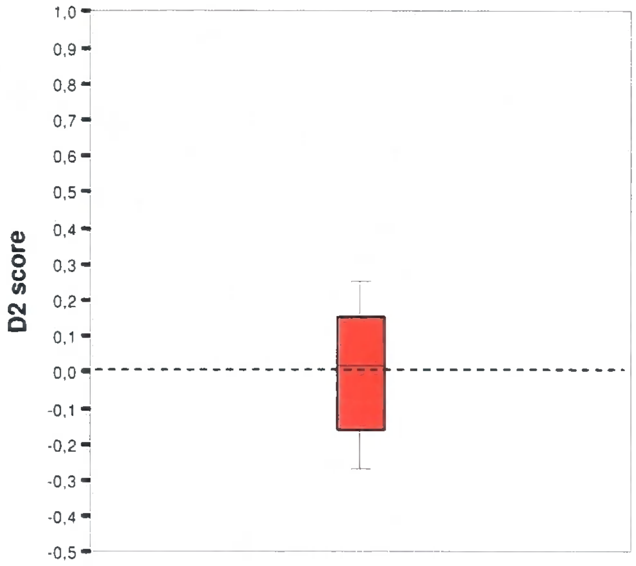


Figure 3.2: Exploration ratios in experiment 1; What-where, objects visible.

We studied whether the exploration time of the objects would have an effect on the performance of the task and whether there was a correlation between the time spent exploring the objects and the recall measures and/or D2 scores. We hypothesized that the time 5 seconds would be a minimum baseline of exploration when performing the task. On this basis, there were only three cases whereby the exploration was lower than the baseline. The data was split in two groups; group 1 (N = 13) by showing a mean exploration time higher than 5 seconds and group 2 (N = 3) when the mean exploration was less or equal than 5 sec (figure 3.3).

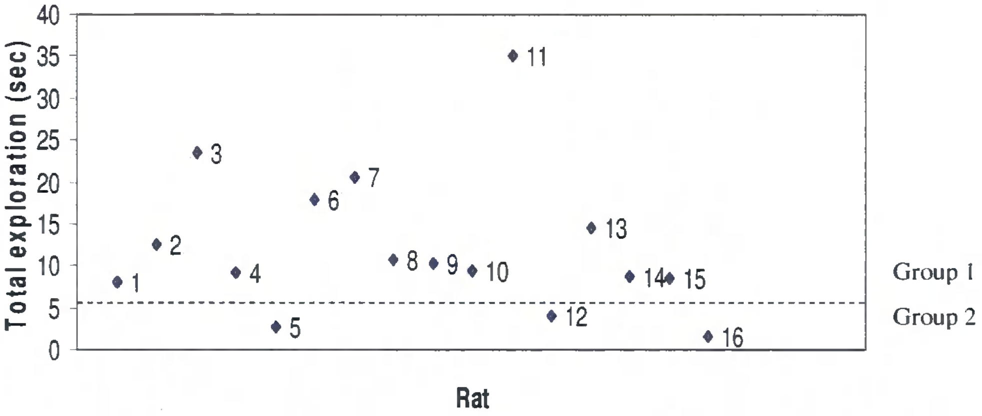


Figure 3.3: Individual distribution of the total time exploring both objects at test in experiment 1; What-where, objects visible.

The mean of the exploration time of the objects at test for the whole group was 12 seconds (SE = 2) which was significantly above the 5 second level (t (15) = 3.43, p = 0.004, 2-tailed). The mean percent of correct turns in group 1 exploring more than 5 seconds was 48.6% (SE = 3.4) which was not different from chance level performance (t (12) = -0.42, p = 0.68, 2-tailed). The "percent correct first turn" of group 2 exploring 5 seconds or less than that was 50% (SE = 7.2) hardly different to that from group 1 and again not different from chance (t (2) = 0.00, p = 1.00, 2-tailed). An independent samples t test compared these two values and it was found no significant difference between them (t (14) = -0.18, p =0.86, 2-tailed). Figure 3.4 compares the "percent correct first turn" of these two groups. The mean D2 score for group 1 was -0.01 (SE = 0.05) which was not different from the no preference level (t (13) = -0.26, p = 0.80, 2-tailed) and for group 2 was -0.01 (SE = 0.05) which also resulted to be not significantly different from the no preference level (t (2) = -0.26, p = 0.82, 2-tailed). The independent samples t test was carried out and it was found no significant difference between the two values (t (7.13) = -0.36, p =0.73, 2-tailed) by assuming not equal variances.

Figure 3.5 represents the exploration ratios in relation to the two groups defined.

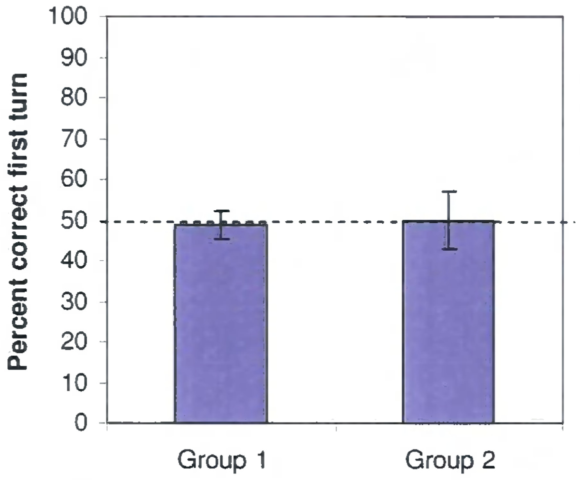


Figure 3.4: First turn performance of the two groups split on the basis of showing a mean exploration time > 5 sec (group 1) and ≤ 5sec (group 2). The bars represent means and standard errors.

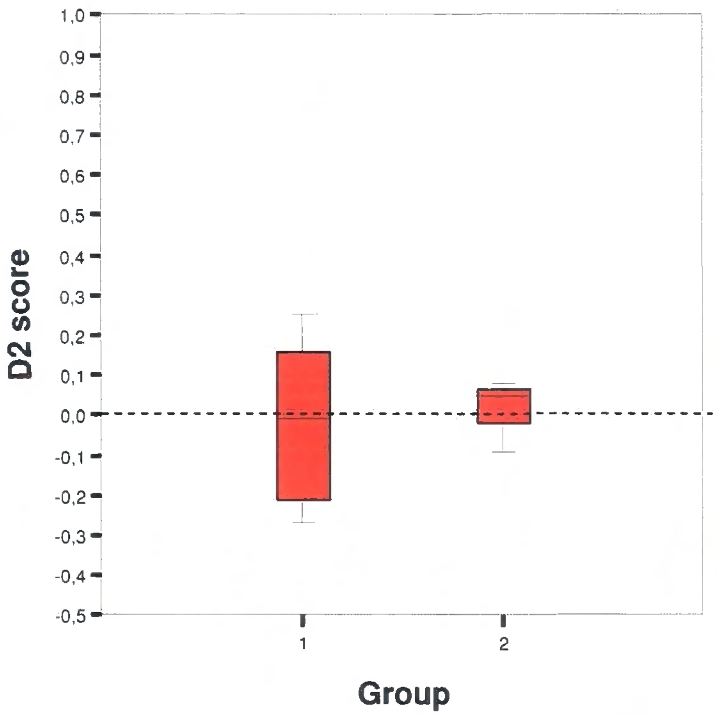


Figure 3.5: Exploration ratios in relation to the two groups split on the basis of showing a mean exploration time > 5 sec (group 1) and ≤ 5 sec (group 2).

A correlation bivariate analysis of the whole group was performed for the "time spent exploring the objects" as the independent variable and the "mean percent of correct turns as the dependent variable" and it was found that there was not a significant correlation between them (r =0.34, p = 0.19, 2-tailed) (figure 3.6).

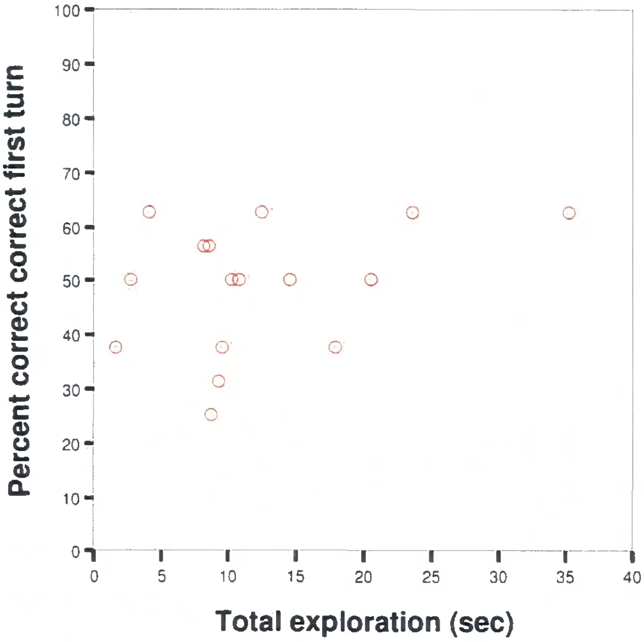


Figure 3.6: Representation of the first turn performance versus the total exploration (seconds).

Also, this analysis was carried out for the two variables time spent exploring the objects and the D2 scores as the dependent variable and it was found that there was no correlation between them (r = -0.22, p = 0.40, 2-tailed) (figure 3.7). A curve estimation by regression for the best fit to the data was performed for the two variables "percent of correct turns" and "D2 scores" in relation to an independent variable or "total exploration time". It was found that there was no significant equation modelling the distribution for each of the variables.

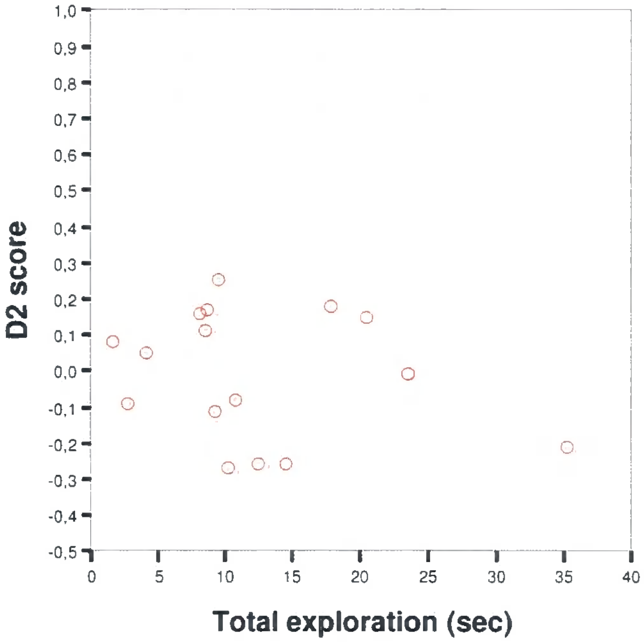


Figure 3.7: Representation of the exploration ratios versus the total exploration (seconds).

3.2.4: Discussion

The what-where task with objects visible did not work in this normal, young and naïve group of animals. This group of animals had not performed the task before and therefore they were not too experienced or too old which were possible explanations for poor performance in chapter 2. Another possible reason would be that the animals were not exploring the objects and therefore had no motivation to make a behavioural response. The task relies on the objects being interesting and therefore if the animals are not interested enough to explore the objects, then they are unlikely to be motivated to work in the task. This was not the case since the animals showed evidence of object exploration. In addition, there was no evidence of a correlation between the time spent exploring the objects and the recall measures or familiarity judgements. This was observed by comparing groups of low and higher exploration rates, by performing a correlation analysis and curve estimation by regression. Although a what-where recognition task in the open field has been previously demonstrated (Eacott and Norman, 2004), the E-maze based task might be different enough to produce different results. Apart from Eacott and Norman using an open field maze, the tasks differ in that there is only a habituation to an object in the E-maze task. This suggests that the results might be due to some aspect of the E-maze procedure at, for example, habituation. In previous experiments (Eacott et al, 2005; Eacott and Easton, 2007) performance on an objects visible version of the what-where-which task has not always predicted performance when objects are hidden from view. Therefore, experiment 2 will test the performance of these same young naïve animals on an objects hidden version of the what-where task.

3.3: Experiment 2: What-where; Objects hidden

3.3.1: Introduction

Experiment 2 examined performance on the what-where task from experiment 1, but now with objects hidden. In previous work (Section 3.3.6; chapter 3; Zinkivskay, 2006), there was no correlation between the objects visible and objects hidden task for individual rat performances. It was suggested that the choice to explore objects in the objects visible task may be influenced by the immediate object appearance which would not bias the first turn performance in the objects hidden task since the objects were not immediately visible form the start arm. Therefore, there was found no correspondence between individual performances in both tasks so it is worth doing the what-where task with objects hidden.

3.3.2: Behavioural Methods

The procedures were the same as described in chapter 2 for experiment 2 except for the animals were run for eight trials instead of sixteen. Timings were 2 minutes at exploration phases (the sample phase and test phase), as for experiment 1.

3.3.3: Results

The animals made their first turn towards the novel object 52.0% (SE = 4.1) of the time which was not significantly different from chance (t (15) = 0.48, p = 0.64, 2-tailed). A paired t test comparing the "percent correct first turn" in experiments 1 and 2 revealed that there was no significant difference (t (15) = -0.57, p = 0.58, 2-tailed) (figure 3.8). There was a marginally significant preference to the novel object based on the obtained D2 ratios (M = 0.11, SE = 0.06, t (15) = 1.94, p = 0.07, 2-tailed). Also, when comparing the D2 in both experiments, there was no significant difference between them (t (15) = -1.64, p = 0.12, 2-tailed) (figure 3.9).

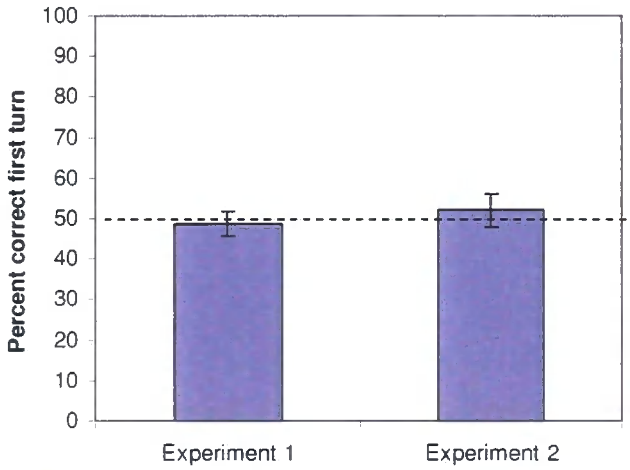


Figure 3.8: Recall performance in relation to experiments 1 and 2 compared. The bars represent means and standard errors.

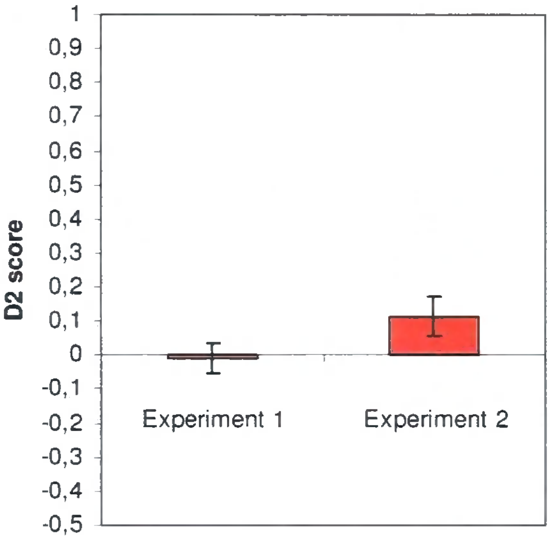


Figure 3.9: Exploration ratios in relation to the experiments 1 and 2 compared. The bars represent means and standard errors.

The mean time spent exploring the two objects at test was 11 seconds (SE = 2). A paired t test was carried out to compare these data with that obtained in previous experiment 1 (12 sec). It was found that there was no significant difference between them (t (15) = 0.79, p = 0.44, 2-tailed). The average number of turns made to the right at test was 40.6% (SE = 5.17), to the left was 50.8% (SE = 5.98) and there were 8.5% (SE = 4.38) of trials in which a choice was not made. A paired t test was carried out to compare the left-right turns made at test in the E maze and it was found that there was no significant difference (t (15) = -0.98, p = 0.34, 2-tailed) (figure 3.10).

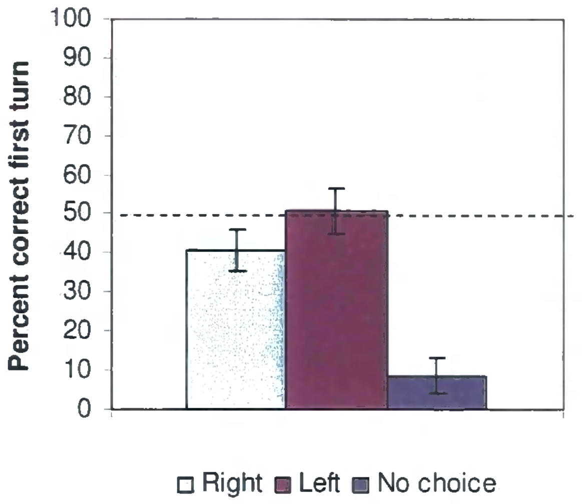


Figure 3.10: Choice made at test expressed by turns to the right, left and no choice, experiment 2. The bars represent means and standard errors.

A paired t test showed that there was no significant difference between "percent correct first turn" when tested in context X (56.3%) or context Y (47.7%) (t (15) = 1.65, p = 0.12, 2-tailed) (figure 3.11). The average D2 scores within context X was 0.12 (SE = 0.09) and within context Y was 0.10 (SE = 0.09). A paired t test found no significant difference between these two values (t (15) = 0.13, p = 0.90, 2-tailed) (figure 3.12).

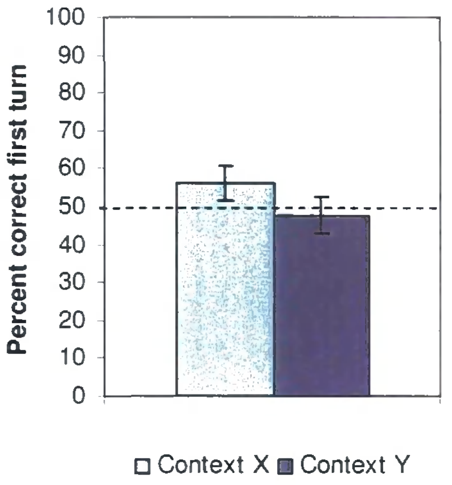


Figure 3.11: Recall performance in relation to test context X and context Y, experiment 2. The bars represent means and standard errors.

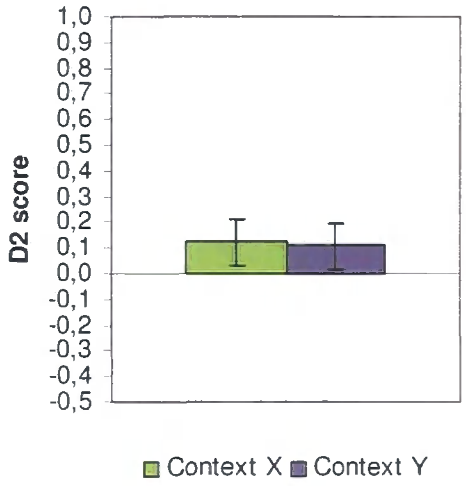


Figure 3.12: Compared mean D2 score in relation to test context X and context Y, experiment 2. The bars represent means and standard errors.

3.3.4: Discussion

These results showed that the what-where task did not work with objects visible (experiment 1) nor with objects hidden (current experiment) and the animals did not show an object preference. There was a significant exploration of the objects that was not different from previous experiment 1. Therefore, it could not be the case that the objects were not interesting enough to perform these tasks as the animals showed motivation. There was no side bias expressed by the mean percent of turns to each right-left direction in the E maze. There was no bias of the results based on an effect of the type of context on the first turns to the novel object in the exploration ratios of the objects.

There are two possible explanations for the poor performance in this task, and by the animals in chapter 2. Firstly, the poor performance may be because these animals may find the what-where task especially difficult to perform since there is no contextual information associated with the position of the objects as in the what-where-which task. Secondly, the poor performance may be because there might be small differences in the procedure compared to published work (Eacott et al, 2005) that were influencing the performance. To test these two hypotheses experiment 3 will test these animals on the what-where-which task with objects hidden (Eacott et al, 2005) which has been seen to work in other experiments.

3.4: Experiment 3: What-where-which; Objects hidden

3.4.1: Introduction

The what-where-which task with objects hidden has been previously shown to work in a normal group of rats (Eacott et al, 2005). Experiment 3 explores the performance on this task of the animals that cannot perform the what-where task in experiments 1 and 2. If these animals can perform well on the what-where-which task then this would suggest that the what-where task is too difficult for the animals. If the animals cannot perform well on the what-where-which task either, then this will suggest that there could be differences in procedure between the experiment here and those previously published which might affect the performance.

3.4.2: Methods and Materials

3.4.2.1: Subjects

The same group of animals that performed experiments 1 and 2 were used.

3.4.2.2: Apparatus

The apparatus was as described in chapter 2 the same as was used in experiments 1 and 2.

3.4.2.3: Behavioural Methods

3.4.2.3a: Habituation

We reviewed the whole procedure and the following issues were addressed. We looked at any possible aspect that could affect the animals stress. Firstly, the transport of the animals upstairs to the behavioural testing room which was not an issue in previous published work (Eacott et al, 2005), was considered. From this point on animals were carried upstairs for testing in their home cages covered with a white cloth. This was in place of the rat's carrier carton boxes used in experiments 1 and 2. Secondly, the room in which animals were held prior to testing, and the testing room were lit with minimum intensity. The animals were habituated for two days to these new aspects of the procedure by transporting them to the behavioural testing room but without any training session being held. The methodological procedure was the same as explained in chapter 2, experiment 3 except for the objects were out of sight. Figure 3.13 outlines the schedule of events in the what-where-which task with objects out of sight. The holding cage used between the two contexts exploration and at the habituation phase was the same type as in chapter 2 that is, a four rat cage (1575 cm^2 x 20 cm height) and with sawdust on the floor.

3.4.2.3b: Training

The animals performed the what-where-which task with objects hidden for eight days, following the procedure outlined in Chapter 2, experiment 3 (figure 3.13).

Figure 3.13: Illustration of the events of what-where-which task with objects hidden.

3.4.3: Results

It was observed that the rats' "percent correct first turn" was marginally significantly below the level expected by chance (M = 43.4%, SE = 3.6, t (15) = -1.83, p = 0.09, 2-tailed) (figure 3.14). Therefore, a further analysis looked at "percent correct first turn" in two days blocks and found a clear pattern showing a progressive decrease in the mean percent of correct turns over the experiment. It is worth noting that incorrect turns to the habituated object were recorded as zero, correct choices in the direction of the non habituated object as one and when no choice was made at test 0.5 was recorded. For the first block, the mean percent was 54.7% (SE = 8.0) which was found to be not significantly different from chance (t (15) = 6.0, p = 0.60, 2-tailed). The second block showed a mean percent of 51.6% (SE = 8.7) which was not significantly different from chance (t (15) = 0.18, p = 0.86, 2-tailed). The third block showed a mean percent 40.6% (SE = 7.9) not significantly different from chance (t (15) = -1.19, p = 0.25, 2-tailed). Nevertheless, the last block of two days showed an average percent of 26.6% (SE = 8.4) which was found to be significantly below chance (t (15) = -2.80, p = 0.01, 2-tailed) (figure 3.15). A repeated measures ANOVA was performed to test whether any of the blocks differed from each other. The test of within subjects effect showed that there was not a significant main effect of block (F (3, 45) = 2.22, p = 0.10).

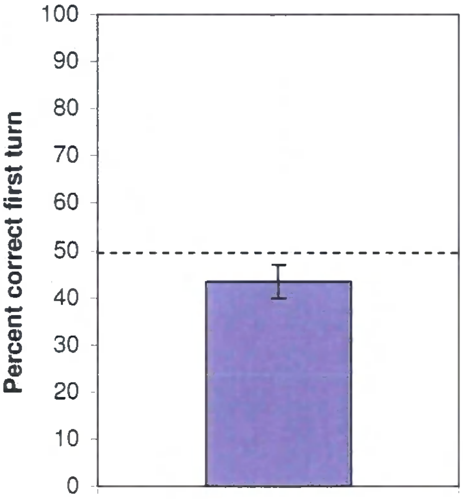


Figure 3.14: Recall performance in experiment 3. The bars represent means and standard errors.

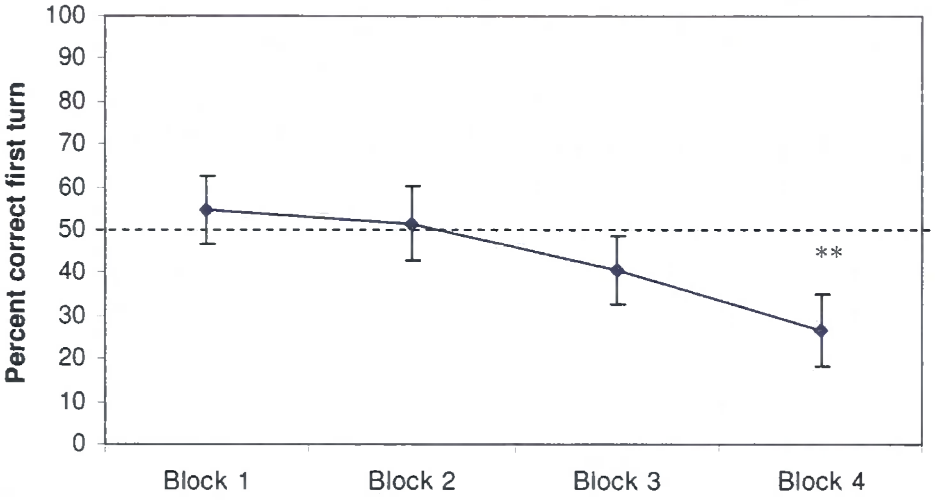


Figure 3.15: Behavioural pattern on the percent of correct turns throughout experiment 3. The line graph represents means and standard errors. Asterisks (**) represent statistical significance of the "percent correct first turn" mean in block 4 compared to chance, 50% ($p \leq 0.01$).

The D2 score indicates a marginal preference for the novel object (M = 0.10, SE = 0.05, t (15) = 2.04, p = 0.06, 2-tailed) (figure 3.16). By looking at two day blocks of testing, the following results were obtained. The repeated measures ANOVA found that there was that there was not a significant main effect of block (F (1.6, 24.2) = 2.71, p = 0.10) (figure 3.17).

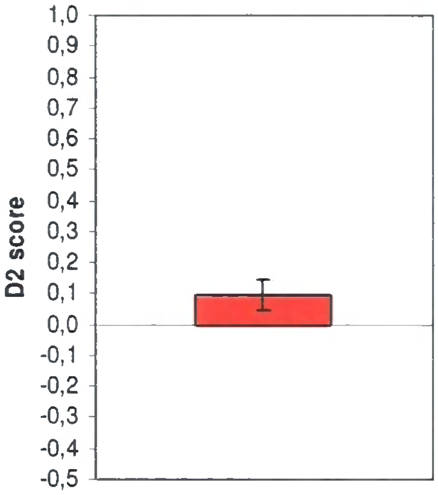


Figure 3.16: Exploration ratios in experiment 3. The bars represent means and standard errors.

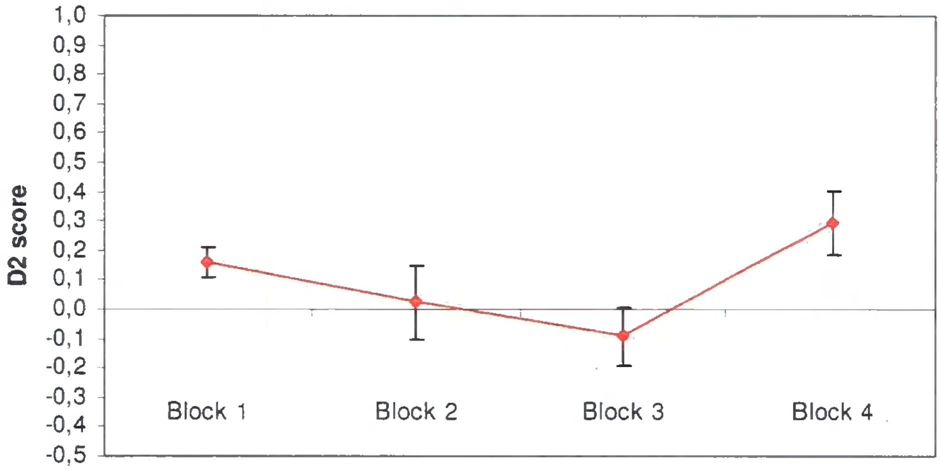


Figure 3.17: Behavioural pattern on the D2 scores throughout experiment 3. The line graph represents means and standard errors.

The average of the total time for exploration of the two objects at test was 12 seconds (SE = 1). A paired samples t test was carried out to compare the present data with experiment 2, objects hidden. It was found that there was no significant difference between them (t (15) = -0.36, p = 0.72, 2-tailed). The mean percent of correct turns to the right at test was 51.6% (SE = 5.1) and to the left 43.8% (SE = 4.8) (7.8%, no choice). A paired t test was performed to compare the mean percent of turns to the right and left and it was found that there was no significant difference (t (15) = 0.83, p = 0.42, 2-tailed) (figure 3.18).

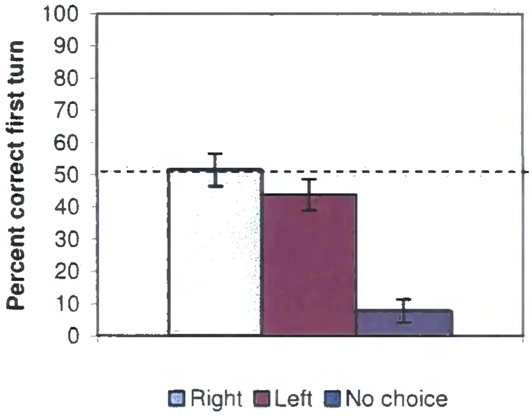


Figure 3.18: Choice made at test expressed by turns to the right, left and no choice, experiment 3. The bars represent means and standard errors.

The mean percent of correct turns to the novel object, when the context at test was context 2 (most recent context, second context), was 37.0% (SE = 6.5) which was approaching significance below chance (t (15) = -1.99, p = 0.07, 2-tailed). When the context at test was context 1 (earliest context, context 1), this value was 48.2% (SE = 6.3) that resulted to be at chance level and not significantly different from it (t (15) = -0.29, p = 0.78, 2-tailed). Although the "percent correct first turn" in the second context appeared weaker, a paired t test showed no significant difference between these two values (t (15) = -1.01, p = 0.33, 2-tailed) (figure 3.19). To study further why the "percent correct first turn" in context 2 was almost below chance, it was analyzed by blocking the data for two days. There was no evidence of a difference for the first block (t (9) = -1.00, p = 0.34, 2-

tailed) neither did the second block (t (8) = -0.43, p = 0.68, 2-tailed) nor the third block (t (8) = -1.10, p = 0.30, 2-tailed). However, the last block indicated a significant effect of the order of the contexts (t (9) = -2.33, p = 0.05, 2-tailed). When the test context was context 2, the mean D2 score was 0.04 (SE = 0.08) which was not significantly different from no preference (t (15) = 0.51, p = 0.62, 2-tailed) and when it was context 1 was 0.15 (SE = 0.07) significantly above no preference (t (15) = 2.16, p = 0.05, 2-tailed). A paired t test was performed a paired t test and there was no significant difference between the two values (t (15) = -1.02, p = 0.32, 2-tailed) (figure 3.20). It is worth pointing out that whereas recall was below chance in context 2 (37%), D2 showed a positive value.

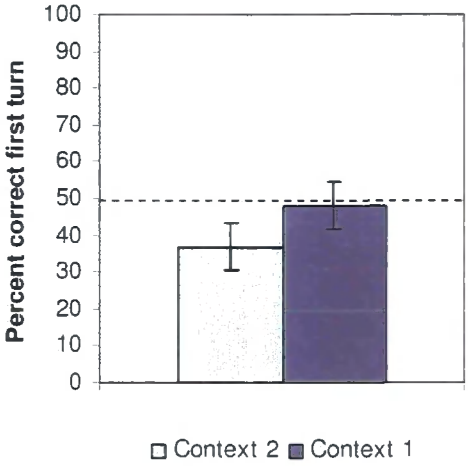


Figure 3.19: Recall performance in relation to the test context being the same or different as the most recent context 2, experiment 3. The bars represent means and standard errors.

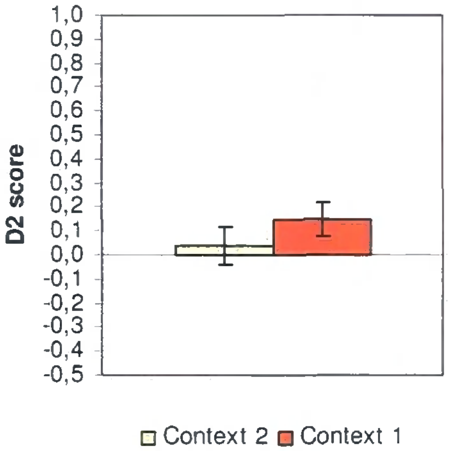


Figure 3.20: Compared D2 score in relation to the test context being the same or different as the most recent context 2, experiment 3. The bars represent means and standard errors.

The mean percent of turns to the novel object within context X was 37.2% (SE = 7.0) marginally significant below chance (t (15) = -1.84, p = 0.09, 2-tailed) and in context Y 51.0% (SE = 5.9) that was not significantly different from chance (t (15) = 0.18, p = 0.86, 2-tailed). Although "percent correct first turn" in context X (the wooden context) appeared poorer, a paired t test found no significant difference between these two values (t (15) = -1.38, p = 0.19, 2-tailed) (figure 3.21). Since the "percent correct first turn" in context X was almost below chance we wanted to study this issue more profoundly. With this purpose, an analysis of blocks of two days was carried out to test any possible effect of the type of context on the turns made. The first block showed a significant effect of the type of context on the recall data (t (8) = -4.91, p = 0.001, 2-tailed) although there was no evidence of an effect for the others comparisons (2nd block: t (9) = 0.58, p = 0.58; 3rd block: t (9) = 1.00, p = 0.34, 2-tailed) and 4th block: t (8) = 1.00, p = 0.35, 2-tailed). D2 scores in context X or Y at test were also compared, and the mean D2 in context X was 0.19 (SE = 0.08) and in context Y was 0.01 (SE = 0.06). A paired t test showed a marginally significant difference (t (15) = 1.85, p = 0.08, 2-tailed) between the D2 scores in these two contexts (figure 3.22). Following the finding of a possible effect on the D2 scores by the type of context at test, we analyzed also by blocking the data per two days. There were not significant differences between the D2 scores obtained for each context in none of the blocks (First block: t (8) = -0.70, p = 0.50, 2-tailed; second block: t (9) = -0.45, p = 0.67; third block: t (8) = 1.09, p = 0.31, 2-tailed; fourth block: t (9) = 1.51, p = 0.17, 2-tailed). Table 3.1 summarizes the conditions underlying the procedure and table 3.2 outlines the outcome results from this experiment (mean percent, mean D2 and their significance levels).

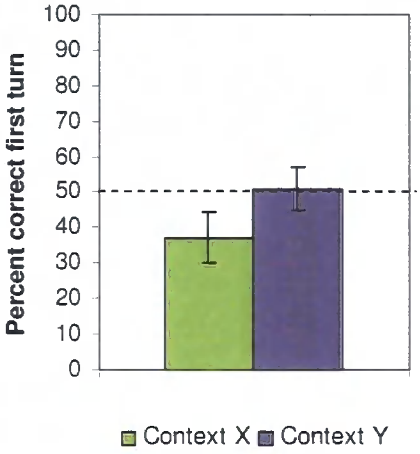


Figure 3.21: Recall performance in relation to the test context, experiment 3. The bars represent means and standard errors.

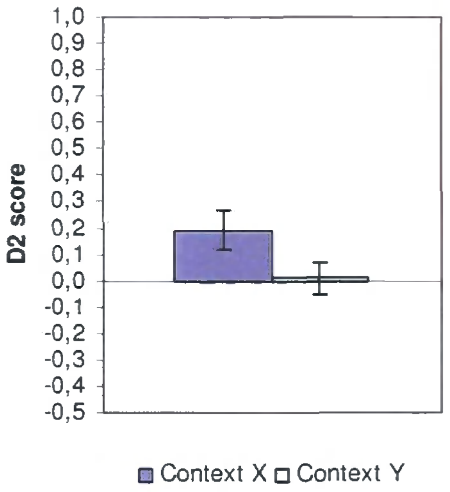


Figure 3.22: Compared mean D2 score in relation to the test context, experiment 3. The bars represent means and standard errors.

Experiment	Conditions
3	- Holding box was a big cage for four rats (1575 cm^2 x 20 cm height) and sawdust on the floor - The position of the habituation object was about in the centre in the habituation cage - Exploration phases (the sample phases and test phase) times were 2 minutes as in experiments 1, 2 - Second rat from the parallel training was positioned in the holding box prior test

Table 3.1: Summary of the conditions in experiment 3.

Experiment	3
N° of trials	8
Percent correct first turn	43.4%
One Sample t Test	t=-1.83 p=0.09
D2 score	0.10
One Sample t Test	t=2.04 p=0.06

Table 3.2: Outline of the results in experiment 3.

3.4.4: Discussion

A possible preference for the novel object was seen in the D2 scores of the animals and although it was very poor, almost significantly above the no preference level. The pattern was variable and showed that there was a preference for the non-habituated object which decreased throughout the study showing no preference and finally it recovered to be significantly above no preference. The recollection data (turnings towards the novel object) appeared to have a tendency to be below chance, i.e. there was a marginally significant preference to turn towards the habituated object. In addition to this, a progressive tendency to turn towards the habituated object was observed throughout the eight days of the study, being significantly below chance at the last two days. Therefore, this suggests that there might be some effect at habituation generating a preference for the habituated object. This would explain the progressive increase of turns towards habituated object over the novel one even significantly towards it in the last part of the study and also the decrease on the initial preference for the novel object expressed by the D2 score.

Poor performance on the task could not be explained by lack of object interest, as there is a significant amount of exploration of the objects at test. There was no side preference in animals' turning behaviour, which could bias the results. Although recall performance when tested in the second context appeared to be poorer than recall performance when tested in the first context, there was no significant difference in recall performance in either condition. It is worth noting that whereas recall was almost below chance when tested in context 2, the D2 score showed a positive value. There was some evidence of a possible effect by the order of the context on the recall performance at test when contexts were the same or different as the most recently explored one, i.e. there was a significant difference in the last block of two days. There was no significant effect of which context (X or Y) was used at test (although recall performance in context X appeared weaker). It did appear, however, that there was more exploration of the novel object within context X at test (though only marginally significant). When blocking the data per two days of training, there was a possible effect on the first block since a significant difference was found. This is interesting because although there was an apparent preference for the novel object in context X, recall performance appeared to be below chance, though not significantly so.

In contrast to previously published data (Eacott et al, 2005; Eacott and Easton, 2007), these animals presumably recalled both objects, but they had a poor preference for the non habituated object which leads them to turn towards the object present in the habituation box. Therefore, these aspects will be explored in the following experiment 4. It may be possible that some procedure affected this low performance and that there might be some factor at habituation that guides the performance into the wrong direction. All testing to this point (but not in the previously published work: Eacott et al, 2005; Eacott and Easton, 2007) has had sawdust in the habituation box with the object. The sawdust can retain the scent marks from con-specifics in the form of urine or feces that give information about the individual identities (Beynon and Hurst, 2004). The presence of odour scents from the con-specifics in the habituation box might create a preference to habituated object since it would be associated with social interaction and would increase interest in that object. In experiment 4 we will test if the sawdust in the habituation cage is an influential factor driving the performance into the wrong direction.

The what-where-which task with objects hidden did not work as there was not a clear preference for the non habituated object (expressed on the D2 scores) nor a tendency to turn towards the habituated one when they had to make a choice at test. Therefore, we need to review the procedure to investigate whether there was any difference from previous work (Eacott et al, 2005) at habituation, i.e. sawdust in the habituation cage, which may affect this poor performance.

3.5: Experiment 4: What-where-which; Objects hidden

3.5.1: Introduction

Sawdust in the habituation box has been used up to now and it has been observed that it was directing the performance in the task in the wrong way by showing a tendency to turn towards the habituated object and a not a clear preference for the novel object expressed by the D2 scores. Therefore, we will test whether there is any difference or improvement in the performance by removing it from the habituation box.

3.5.2: Behavioural Methods

3.5.2.1: Habituation

A number of alterations were made to the previous procedures to ensure the procedures matched those of the published data as closely as possible. Firstly, the habituation box which was a large home cage with sawdust on the floor was replaced by a small cage (960 cm^2 x 20 cm height) without sawdust on the floor. The sawdust in the habituation cage might accumulate the odour scent from the con-specifics and therefore it may generate a preference to the habituated object since it was associated with social interaction. Therefore, by removing sawdust in the habituation cage, it may be improved the preference to the novel object. Secondly, the position of the object in the habituation box was changed to alternate between the four quadrants of the cage between days. The alternation of the object in the habituation box might have an effect on motivation and therefore it may improve the accuracy of results. The rats were habituated to the new holding cage during one 8 minutes phase. Finally, timings for exploration phases were prolonged to 3 minutes instead of 2 minutes used before. The reason that the times were increased was based on that as much time the animals had to explore the objects, it was expected that the performance would be more accurate. Otherwise, all procedures were identical to those of experiment 3.

As part of the general procedure of the task, animal pairs were run in parallel, with one animal having its sample phase while another habituates. Therefore, for all the testing from previous chapter experiments and the experiments 1 to 4 described in this chapter (but not in previously published data), the rats have had slightly different experiences. The first of the two rats run in the pair had gone straight into the E-maze apparatus, whilst the second animal of the pair tested in parallel had gone into the habituation cage (without any objects present) first. This was a difference from the previously published procedure and a difference in the procedure of rats within the same experiment. Therefore, the two groups performance was studied here to investigate whether this aspect affected the performance somehow.

3.5.2.2: Training

A total of eight training sessions completed this study.

3.5.3: Results

The average performance of correct choices was improved (M = 55.9%, SE = 4.3). Nevertheless, there was not a significant difference from the level expected by chance (t (15) = 1.38, p = 0.19, 2-tailed). A paired t test was carried out to compare the mean percent of correct turns between experiments 3 and 4 and showed a significant difference between "percent correct first turn" on the two tasks (t (15) = -2.27, p = 0.04, 2-tailed) (figure 3.23). A repeated measures ANOVA was carried out to compare the "percent correct first turn" between blocks of two days data. It was found that there was no significant difference on the "percent correct first turn" between the four blocks (F (3, 45) = 1.77, p = 0.17) (figure 3.24).

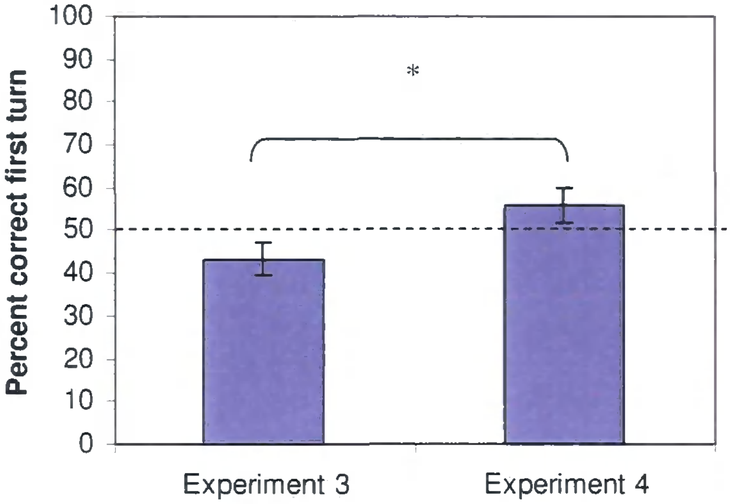


Figure 3.23: Compared recall performance in experiments 3 and 4. The bars represent means and standard errors. Asterisk (*) represents statistical significance of the "percent correct first turn" means between experiment 3 and experiment 4 ($p < 0.05$).

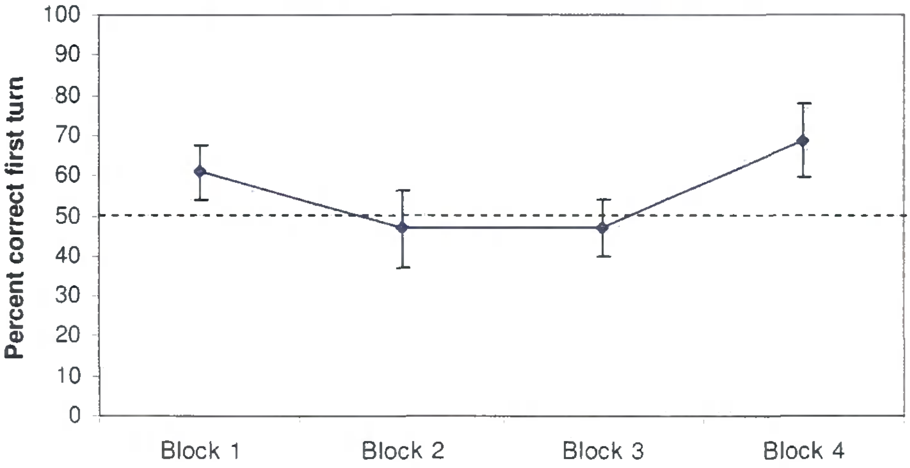


Figure 3.24: Behavioural pattern on the percent of correct turns throughout experiment 4. The line graph represents means and standard errors.

When analyzing the D2 score, there was a significant difference above the level of no preference (M = 0.15, SE = 0.05, t (15) = 3.12, p = 0.007, 2-tailed). When comparing the preference to the novel object based on the D2 scores, there was not a significant difference between both experiments (t (15) = -0.79, p = 0.44, 2-tailed) (figure 3.25). A repeated measures ANOVA was performed to compare the D2 values obtained in the four blocks and it was found there was no significant difference between them (F (3, 45) = 0.41, p = 0.75)(figure 3.26).

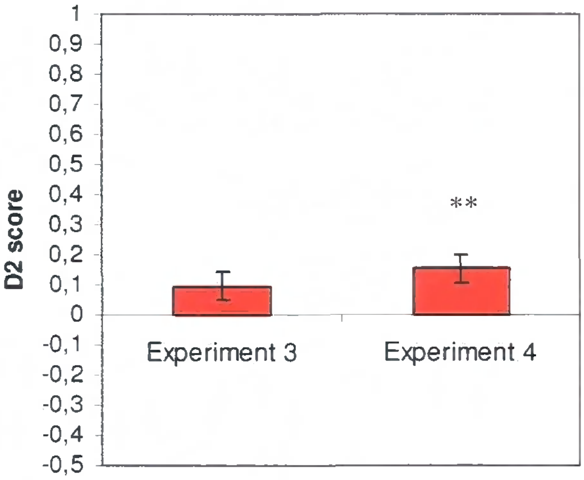


Figure 3.25: Compared exploration ratios in experiments 3 and 4. The bars represent means and standard errors. Asterisks (**) represent statistical significance of the "D2 score" mean compared to no preference, zero ($p < 0.01$).

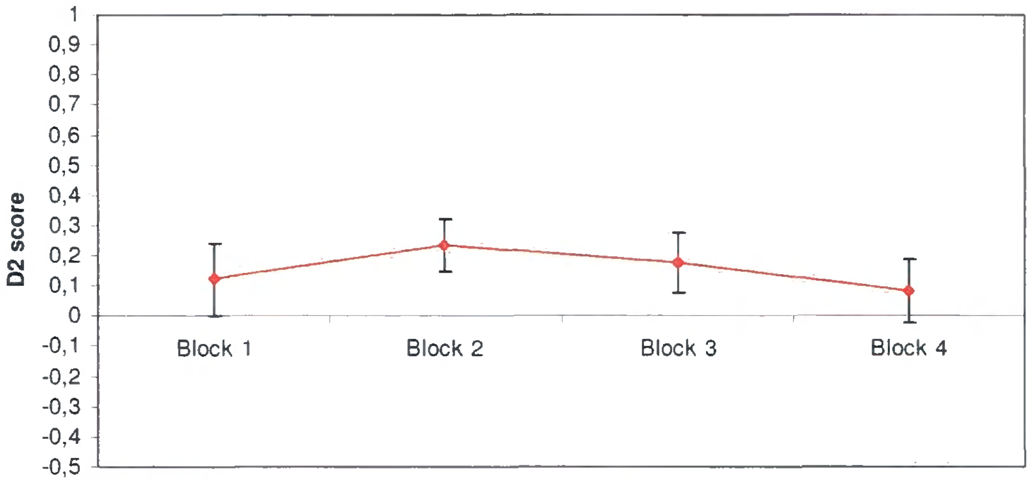


Figure 3.26: Behavioural pattern on the D2 scores throughout experiment 4. The line graph represents means and standard errors.

The mean of total time spent exploring both objects at test was 17 seconds (SE = 1). A paired t test was performed to compare this total exploration time between experiments 3 and 4 and no significant difference was found (t (15) = 0.34, p = 0.74, 2-tailed). The percent mean turnings to the right at test was 42.2% (SE = 6.0) and to the left 57% (SE = 6.0), leaving 6.3% (SE = 3.02) for which there was no choice. There was no significant difference between the turns made to the right and left (t (15) = -1.26, p = 0.23, 2-tailed). The percent mean of correct turns to the novel object when the context from the second sample phase was the same as in the test phase was 57.8% (SE = 7.0). When the second sample context was different from the test context this value was 46.6% (SE = 8.6). A paired t test was performed which showed a non significant difference between these two values (t (15) = 0.88, p = 0.39, 2-tailed). The number of correct turns to the novel object at test when context X was present was 56.2% (SE = 5.1). When the context Y is at test, the number of correct turns was 55.5% (SE = 6.3). A paired t test was performed to compare these results finding that there was not a significant difference between these two values (t (15) = 0.08, p = 0.93, 2-

tailed). The average for the D2 scores obtained within context X was 0.18 (SE = 0.07) and within context Y was 0.22 (SE = 0.07). The paired t test revealed that there was not a significant difference between the D2 ratios obtained in context X and Y (t (15) = -0.34, p = 0.74, 2-tailed).

The analysis of the performance of the two groups of animals, differing from being transferred directly or indirectly (through a holding cage) to the E maze, was carried out. An independent samples t test was performed and it was found that there was no significant difference between the groups expressed by the mean percent of correct turns (t (15) = 0.45, p = 0.66, 2-tailed) (figure 3.27). Also, when comparing the D2 scores for the two groups, it was found that there was no significant difference between them (t (15) = 0.07, p = 0.94) (figure 3.28). Table 3.3 summarizes the conditions underlying the procedure. Table 3.4 summarizes the data obtained in experiments 3 for the mean percent of correct turns, the mean D2 score and the related significance p-values.

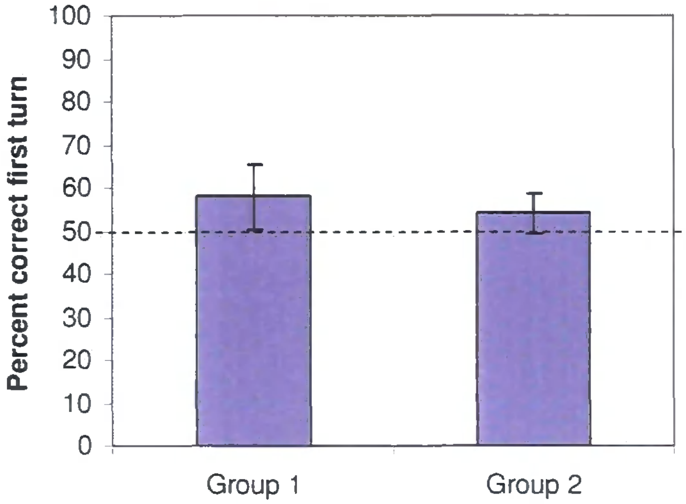


Figure 3.27: Recall performance in relation to the two groups 1 and 2, experiment 4. The bars represent means and standard errors.

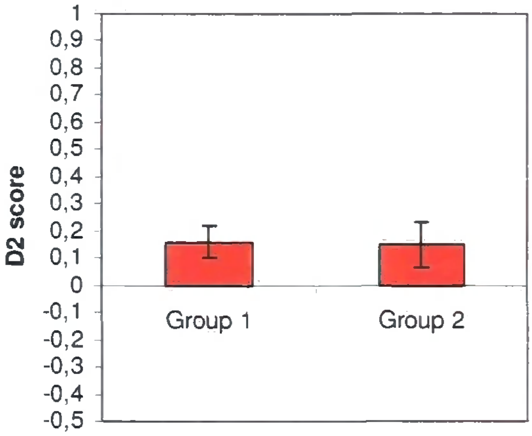


Figure 3.28: Exploration ratios in relation to the two groups 1 and 2, experiment 4. The bars represent means and standard errors.

Experiment	Conditions
3	- Holding box was a big cage for four rats (1575 cm^2 x 20 cm height) and sawdust on the floor - The position of the habituation object was about in the centre in the habituation cage - Exploration phases times were 2 minutes - Second rat from the parallel training was positioned in the holding box prior test
4	- Exploration phases times were prolonged to 3 minutes - Holding box was a small cage for two rats (960 cm^2 x 20 cm height) and no sawdust on the floor - The position of the habituation object was alternated to the four quadrants of the cage between days - Second rat from the parallel training was positioned in the holding box prior test

Table 3.3: Summary of the conditions in experiments 3 and 4.

Experiment	3	4
N° of trials	8	8
Percent correct first turn	43.4%	55.9%
One Sample t Test	t=-1.83 p=0.09	t=1.38 p=0.19
D2 score	0.10	0.15
One Sample t Test	t=2.04 p=0.06	t=3.12 p=0.007**

$p < 0.01$**

Table 3.4: Outline of the results in experiments 3 and 4.

3.5.4: Discussion

There was a clear improvement in the performance comparing the two experiments 3 and 4 based on the recollection measures (turns to the novel object). However, overall recall performance did not differ from chance level and there was no significant main effect when looking at blocked performance. There was evidence of a significant preference to the novel object on the basis of the D2 score. The factors at habituation that were changed were the following: the sawdust on the floor of the habituation box was eliminated, timing for exploration phases was prolonged to 3 minutes and the position of the habituation object alternated between one of four positions.

Sawdust in the habituation cage might accumulate the odour scent from the con-specifics which may create a preference to the habituated object since it is associated with social interaction. Therefore, the tendency to turn towards the habituated object and the low preference to the novel object observed in experiment 3 may be explained by the presence of sawdust in the habituation cage. The alternation of the object in the habituation box might have had an effect on the motivational state to perform the task and it is suggested that it may improve the accuracy of results. Timings for exploration phases were also increased to 3 minutes which could have improved learning processes and therefore recall for specific object-place-context configurations. There was no effect on the performance by the order of the rats or by being transferred directly or indirectly to the E maze previous training session. The performance in the task is going into the good direction but still is not significant above chance. Therefore, we will continue in experiment 5 with the same study.

3.6: Experiment 5: What-where-which; Objects hidden

3.6.1: Introduction

The performance of the task is still poor but is improving over the course of the eight days of experiment 4, and therefore we need to continue the training to reach the level of significance above chance. The procedure was reviewed and any possible factor affecting the results is target of modification. However, a modification was introduced here that did not match what was done in the published experiments (Eacott et al, 2005). This modification referred to the transfer of the animals to the home cages between the two sample phases exploration, instead of the empty habituation cage as had been the procedure to this point.

3.6.2: Behavioural Methods

The rats performed the training for eight days. The procedure was reviewed to eradicate possible factors that might bias the results. There was not significant difference in the performance between the two groups of animals in previous experiment, those differing from being transferred directly or indirectly to the E maze to start training. However, in order to match the procedures used in previous published work and to avoid any possible biasing factor, all animals received the same experience when being tested. Therefore, from now on the second rat in the pair was not placed in the habituation box prior to training.

Exclusively, in this experiment a modification was made so that the animals were transferred to the home cages between the presentations of the two sample contexts during the 1 minute interval, rather than into the empty habituation cage as had been the procedure to this point.

3.6.3: Results

The mean percent of choices to the novel object was not significantly different from chance (M = 44.5%, SE = 4.6, t (15) = -1.20, p = 0.25, 2-tailed). The D2 score showed no significant preference to the novel or habituated object (M = 0.01, SE = 0.1t (15) = 0.25, p = 0.81, 2-tailed). A repeated measures ANOVA was performed to compare the "percent correct first turn" in this experiment to that of experiments 3 and 4 (figure 3.29). It was found that there was a marginally significant difference (F (2, 30) = 2.83, p = 0.08). There was a marginally significant difference on the recollection measures of this experiment comparing to the previous experiment 4 (t (15) = 1.98, p = 0.07, 2-tailed) and not significantly different from experiment 3 (t (15) = -0.19, p = 0.85, 2-tailed). As reviewed in previous experiment 4 section, there was a significant difference on the "percent correct first turn" between experiments 3 and 4 (t (15) = -2.27, p = 0.04, 2-tailed) (figure 3.29).

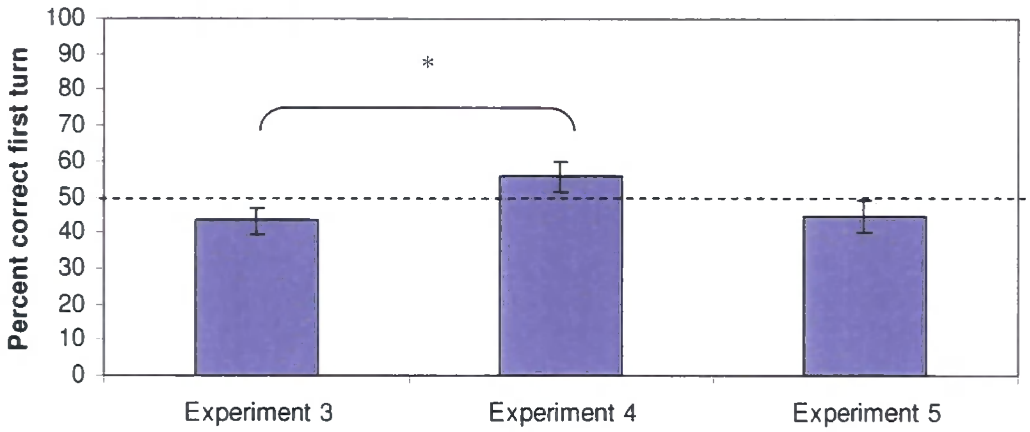


Figure 3.29: Recall performance in relation to the experiments 3, 4 and 5. The bars represent means and standard errors. Asterisk (*) represents statistical significance of the "percent correct first turn" means between experiment 3 and experiment 4 ($p < 0.05$).

A repeated measures ANOVA was carried out to compare the mean D2 scores between the experiments 3, 4 and 5. It was found that there was no significant difference between them (F (2, 30) = 1.79, p = 0.18). A paired t test was performed to compare the performance between the experiments 4 and 5 and it was found that there was a marginal difference between them (t (15) = 1.99, p = 0.06). Also, there was not a significant difference between the experiments 3 and 5 (t (15) = 1.07, p = 0.30). To summarize here, there was not a significant difference between experiments 3 and 4 (t (15) = -0.79, p = 0.44, 2-tailed) (figure 3.30).

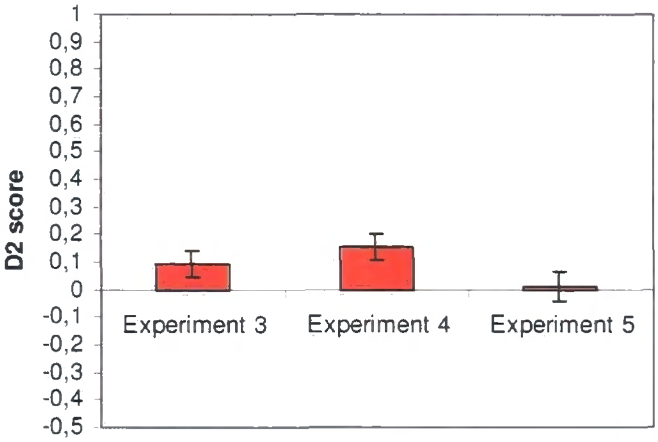


Figure 3.30: Exploration ratios in relation to the experiments 3, 4 and 5. The bars represent means and standard errors.

The average total time spent exploring both objects at test was 16 seconds (SE = 3) which was significantly different from 5 sec exploration (t (15) = 3.88, p = 0.001, 2-tailed). A repeated measures ANOVA was carried out to compare this result with those from previous experiments 3 and 4. It was found that there was no significant difference between them (F (2, 30) = 0.25, p = 0.78). An independent-samples t test was carried out to compare the performance of the two different groups of animals on the basis of where they were placed previous to starting training in previous experiments and there was a non significant difference in the performance expressed by the correct turns to the novel object (t (15) = -0.17, p = 0.87, 2-tailed). The mean percent of correct turns for the group of animals that were transferred directly from the home cages to the maze to start the training (group 1) was 43.8% (SE = 8.8) and for the second half of the total group of animals that were transferred from the holding cage to the maze (group 2) was 45.3% (SE = 3.3) (figure 3.31). The mean for the D2 score in the group 1 was 0.04 and for the group 2 was -0.01. The paired t test confirmed that there was no significant difference on the D2 scores between these two groups (t (15) = 0.5, p = 0.62, 2-tailed) (figure 3.32). Table 3.5 summarizes the conditions underlying the procedure. Table 3.6 summarizes the data obtained in experiments 3 for the mean percent of correct turns, the mean D2 score and the related significance p-values.

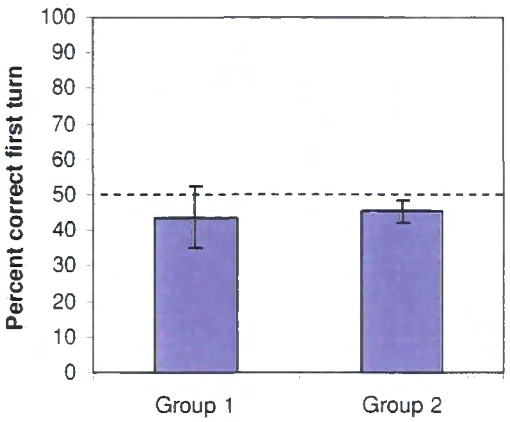


Figure 3.31: Recall performance in relation to groups 1 and 2, experiment 5. The bars represent means and standard errors.

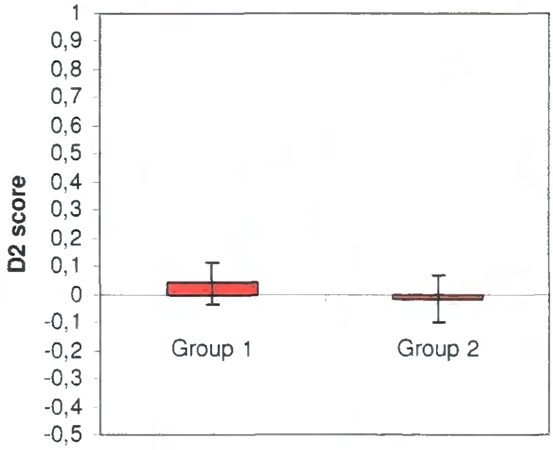


Figure 3.32: Exploration ratios in relation to groups 1 and 2, experiment 5. The bars represent means and standard errors.

Experiment	Conditions
3	- Holding box was a big cage for four rats (1575 cm^2 x 20 cm height) and sawdust on the floor - The position of the habituation object was about in the centre in the habituation cage - Exploration phases times were 2 minutes - Second rat from the parallel training was positioned in the holding box prior test
4	- Exploration phases times were prolonged to 3 minutes - Holding box was a small cage for two rats (960 cm^2 x 20 cm height) and no sawdust on the floor - The position of the habituation object was alternated to the four quadrants of the cage between days - Second rat from the parallel training was positioned in the holding box prior test
5	- The position of the habituation object was alternated to the four quadrants of the cage between days - Exploration phases times were 3 minutes - Second rat from the parallel training was not positioned in the holding box prior test - The animals were transferred to the home cages between the presentation to the two contexts

Table 3.5: Summary of the conditions in experiments 3 to 5.

Experiment	3	4	5
N° of trials	8	8	8
Percent correct first turn	43.4%	55.9%	44.5%
One Sample t Test	t=-1.83 p=0.09	t=1.38 p=0.19	t=-1.20 p=0.25
D2 score	0.10	0.15	0.01
One Sample t Test	t=2.04 p=0.06	t=3.12 p=0.007**	t=0.25 p=0.81

p < 0.01**

Table 3.6: Outline of the results in experiments 3 to 5. Asterisks (**) represent statistical significance of the "D2 score" mean compared to no preference, zero (p < 0.01).

3.6.4: Discussion

The performance of the task appeared to be poorer compared to previous experiment 4 (there was a marginally significant difference) and the first turn performance was not significantly above chance. Also, the animals did not show a preference to explore one of the objects above the other. Whether the animals were transferred directly or indirectly from the home cages to the maze was demonstrated to have no effect on the performance and the two groups performed about the same. The cage used between the two sample phases exploration did not significantly affect first turn or object preference. We need to continue the task under the favourable conditions which will include the allocation of the animals in the holding cage between contexts exploration.

3.7: Experiment 6: What-where-which; Objects hidden

3.7.1: Introduction

Experiment 6 was designed to mimic as closely as possible the previous published procedures (Eacott et al, 2005) in which all rats follow the following sequence: 1. Home cage; 2. E maze sample phase 1 (3 minutes); 3. Small habituation cage, no sawdust, no objects (1 minute); 4. E maze sample phase 2 (3 minutes); 5. Habituation cage, no sawdust, with object (8 minutes); 6. E maze test (3 minutes).

3.7.2: Behavioural Methods

The training was formed by a total of twenty four trials. The methods were those used in experiment 5 but animals were not transferred to the home cages between the two sample phases exploration in order to match the procedures used in previous published work. The temperature of the testing room was increased to carry out the behavioural study (due to some cold weather affecting the temperature of the rooms). For blocks 1 and 2, some new objects purchased on shop were added to the general stock used which had not been cleaned or previously explored by rats. In block 3 or last block of training, the majority of the objects used for training purposes were objects newly bought from shop were and had not been cleaned or previously explored by rats. Otherwise, the conditions for the three blocks of this experiment were the same. Summarizing, these conditions were: a) Habituation cage without sawdust and small cage for two rats used at habituation phase and between the two contexts presentation as a holding box, b) the exploration phases times were 3 minutes instead of 2 minutes, c) the position of the habituation object was moved to the four quadrants of the cage alternatively, d) all rats started training moving from the home cages directly to the E maze, e) during the 1 minute interval between the two contexts exploration, the rat was held in the habituation box. The procedures now matched those of previously published data (Eacott et al, 2005).

3.7.3: Results

The study of the results was performed by analyzing each block of eight days. A repeated measures ANOVA was performed to compare the "percent correct first turn" between the three blocks of experiment 6. There was no difference between the three blocks (F (2, 30) = 1.75, p = 0.19) (figure 3.33). A repeated measures ANOVA was performed to compare the D2 score between the three blocks of experiment 6 (F (2, 30) = 0.00, p = 1.00), by showing very steady D2 values (figure 3.34). A repeated measures ANOVA was performed to study whether there was a difference in the total exploration time of the objects comparing blocks 1, 2 and 3 from experiment 6 (figure 3.35). There was no significant difference between them (F (2, 30) = 2.08, p = 0.14).

For the first block of eight trials (block 1), the animals were performing at the level expected by chance expressed by the "percent correct first turn" (M = 48.4%, SE = 5.1, t (15) = -0.31, p = 0.76, 2-tailed). Regarding the exploration times, the D2 score indicated that there was not a significant preference for one of the objects (M = 0.13, t (15) = 1.77, p = 0.10, 2-tailed). The average of the total time exploring both copies of the objects at test was 20 sec (SE = 3) which was significantly above 5 sec exploration (t (15) = 5.90, p = 0.00, 2-tailed).

In relation to the second block of eight trials (block 2), it was observed that the animals were now performing significantly above chance level expressed by the "percent correct first turn" (M = 60.2%, SE = 3.1, t (15) = 3.26, p = 0.004, 2-tailed). The D2 ratio was significantly above the zero value and therefore it showed a preference to the novel object (M = 0.13, t = 2.76, p = 0.01, 2-tailed). There was a significant exploration of the objects expressed by the total amount of time exploring both copies at test being 17 sec (SE = 3) which was significantly above 5 sec exploration (t (15) = 4.68, p = 0.00, 2-tailed).

In the last block of eight days or block 3, the "percent correct first turn" was poor and not different from chance (M = 51.6%, SE = 5.2, t (15) = 0.30, p = 0.77, 2-tailed). The preference to the novel object indicated a D2 score marginally significant above the no preference status (M = 0.13, t (15) = 1.96, p = 0.07, 2-tailed). The average of the total time spent exploring both objects at test was 20 sec (SE = 2) which was significantly above 5 sec exploration (t (15) = 6.96, p = 0.00, 2-tailed).

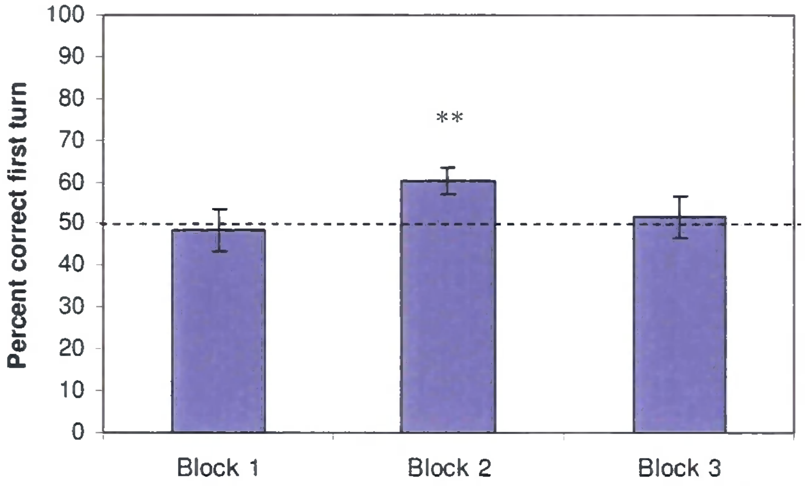


Figure 3.33: Recall performance in experiment 6 throughout the three blocks. First block (block 1), second block (block 2) and third block (block 3). The bars represent means and standard errors. Asterisks (**) represent statistical significance of the "percent correct first turn" mean compared to chance, 50% ($p < 0.01$).

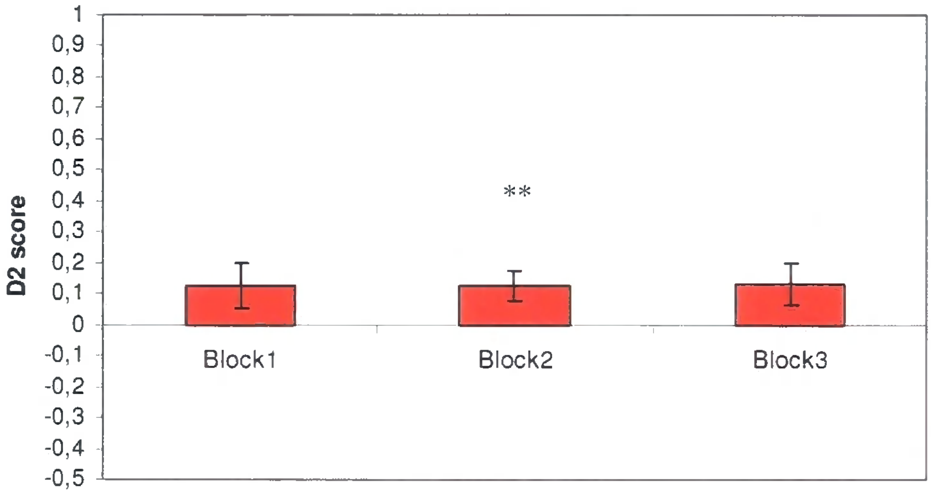


Figure 3.34: Exploration ratios in experiment 6 throughout the three blocks: First block (block 1), second block (block 2) and third block (block 3). The bars represent means and standard errors. Asterisks (**) represent statistical significance of the "D2 score" mean compared to no preference, zero ($p \leq 0.01$).

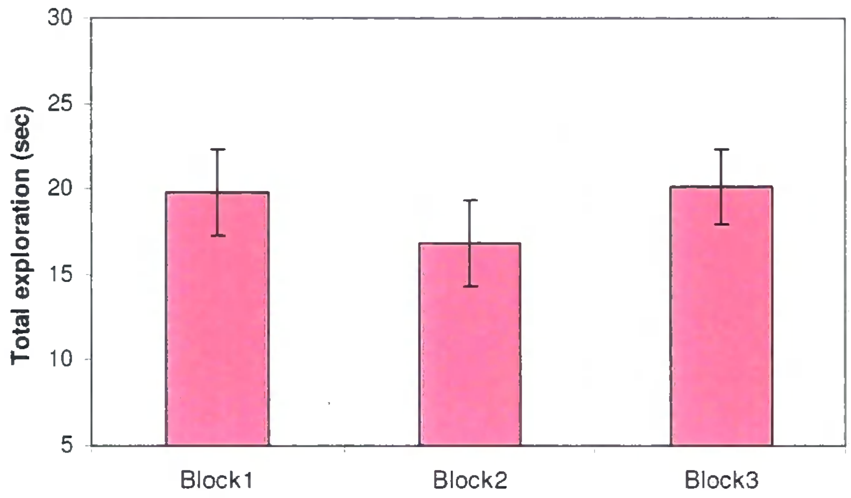


Figure 3.35: Total exploration of the objects at test in experiment 6 throughout the three blocks: First block (block 1), second block (block 2) and third block (block 3). The bars represent means and standard errors.

The correlation between the total exploration and the mean percent of correct turns was studied. There was no evidence of the correlation in the first block ($r = 0.33$, $p = 0.22$, 2-tailed) nor in the second block ($r = 0.10$, $p = 0.73$, 2-tailed). There was evidence in the last block 3 that the correlation was a significant one ($r = -0.60$, $p = 0.01$, 2-tailed). A curve estimation of the model was then studied. The best predictors of the model were the compound, growth, exponential and logistic equations (figure 3.36). The estimation was $R^2 = 0.42$ and the independent variable total exploration was very significant in predicting the values of the mean percent of correct turns ($p = 0.006$, 2-tailed). It is worth noting that at test, incorrect turns to the habituated object were recorded as zero, correct choices in the direction of the non habituated object were recorded as one and a third score (0.5) was recorded when no choice was made at test.

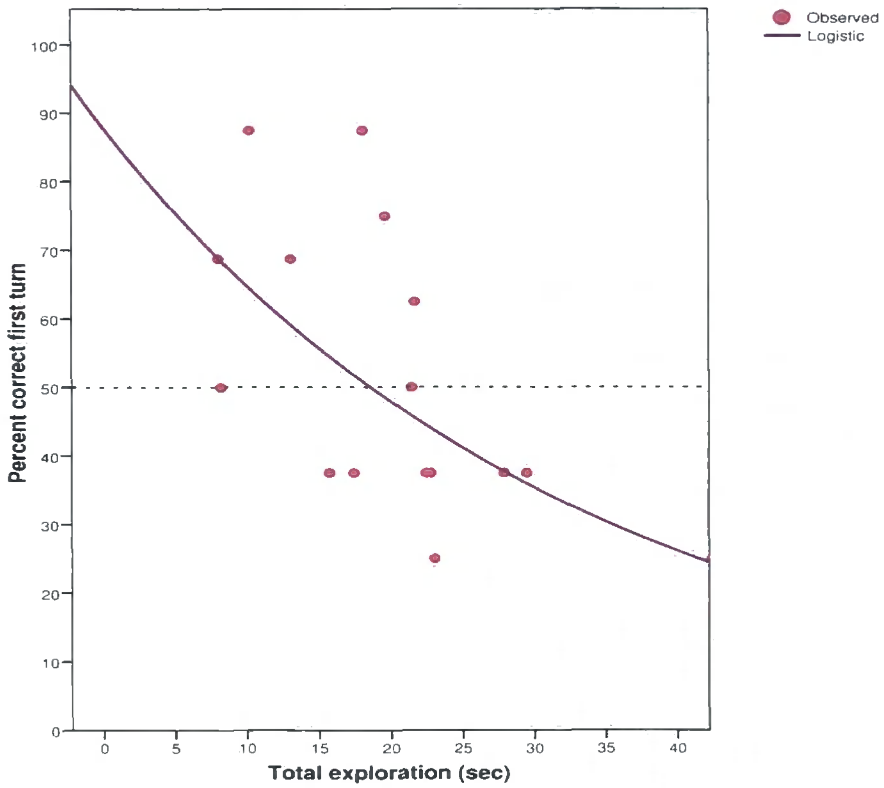


Figure 3.36: Curve estimation of the two variables total exploration as the independent variable and the mean percent of correct turns as the dependent variable.

It was investigated whether there was a correlation between the total exploration and the D2 scores. There was not correlation in none of the three blocks of this experiment (block1: r = -0.29, p = 0.29, 2-tailed, block 2: r = 0.40, p = 0.13, 2-tailed, block 3: r = -0.21, p = 0.45, 2-tailed).

Table 3.7 outlines the conditions of each of the experiments from this chapter.

Table 3.8 summarizes the data obtained in the experiments described above indicating the mean percent of correct turns, D2 values and the corresponding significance levels.

Experiment	Conditions
3	- Holding box was a big cage for four rats (1575 cm^2 x 20 cm height) and sawdust on the floor - The position of the habituation object was about in the centre in the habituation cage - Exploration phases times were 2 minutes - Second rat from the parallel training was positioned in the holding box prior test
4	- Exploration phases times were prolonged to 3 minutes - Holding box was a small cage for two rats (960 cm^2 x 20 cm height) and no sawdust on the floor - The position of the habituation object was alternated to the four quadrants of the cage between days - Second rat from the parallel training was positioned in the holding box prior test
5	- The position of the habituation object was alternated to the four quadrants of the cage between days - Exploration phases times were 3 minutes - Second rat from the parallel training was not positioned in the holding box prior test - The animals were transferred to the home cages between the presentation to the two contexts
6	- New objects were incorporated to the training - The position of the habituation object was alternated to the four quadrants of the cage between days - Exploration phases times were 3 minutes - Second rat from the parallel training was not positioned in the holding box prior test - The temperature in the testing room was conditioned by increasing it

Table 3.7: Conditions along the experimental approach.

Experiment	3	4	5	6, block 1	6, block 2	6, block 3
N° of trials	8	8	8	8	8	8
Percent correct first turn	43.4%	55.9%	44.5%	48.4%	60.2%	51.6%
One Sample t Test	t= -1.83 p=0.09	t=1.38 p=0.19	t= -1.20 p=0.25	t=-0.31 p=0.76	t=3.26 p=0.002**	t=0.30 p=0.77
D2 score	0.10	0.15	0.01	0.13	0.13	0.13
One Sample t Test	t=2.04 p=0.06	t=3.12 p=0.007**	t=0.25 p=0.81	t =1.77 p =0.10	t=2.76 p=0.01**	t=1.96 p=0.07

$p \leq 0.01$**

Table 3.8: Recollection and familiarity processes data; Mean percent of correct turns to the novel object, mean D2 scores and the correspondent significance values.

3.7.4: Discussion

For the first part of eight days, there was no preferential turn to one object and although there was a positive D2 score it was not different from non preference. Poor performance on the task could not be explained by lack of object interest, as there was a significant exploration of the objects at test. However, in the second part of eight days the task worked properly by showing a clear preference to the novel object expressed by the D2 score and significant turnings made to the relatively novel object. In the last part of eight days, the first turn performance of the task had been seriously affected expressed in the recollection data which is not different from chance although the D2 scores remain very steady throughout all three blocks from the experiment by differing in the significance values and the performance did not differ significantly between the three blocks. There was some evidence of a negative correlation between the total exploration of the objects and recall performance in block 3. However, there was no significant correlation in blocks 1 and 2 of training.

3.8: Discussion

To summarize here, procedural differences may have contributed to different results in chapter 2 and previously published work. This possibility was investigated in Chapter 3. There was some evidence that the removal of sawdust improved the performance. The first turns to the non habituated object increased significantly in experiment 4 (without sawdust) compared to experiment 3 (with sawdust) whereby a significant preference to turn in the direction of the habituated object was found in the last block of experiment 3. However, recall measures were not different from chance in both experiments 3 and 4. Also, the D2 score was not significantly different between experiments 3 and 4, although it was significantly above no preference level only in experiment 4. In regard to the timings for exploration phases that were increased to 3 minutes, as they would improve learning opportunities, it was suggested that they could result in better memory for specific object-place-context configurations, even if the animals were not actively exploring the objects for more time. The alternation of the object in the habituation cage, as it may improve the motivation when performing the task, was suggested to improve the results on the performance of the task. Whether the animals were transferred directly or indirectly to the E maze to start the sample phase 1 did not significantly affect the performance of the task. The allocation of the animals in the home cages during the training session and between the two contexts exploration was concluded not to affect significantly first turn or object preference. When the conditions were as published work (Eacott et al, 2005) in experiment 6, there was some evidence of recall and significant preference to the non habituated object (block 2) although performance between blocks did not differ significantly.

Chapter 4: The effect of certain modifications on the what-where-which task and the role of the olfactory cues

4.1: Introduction

Chapter 3 showed that the task worked well by the absence of sawdust in the habituation box although the "percent correct first turn" may be relatively low at about 60%. Therefore, this chapter aims to improve performance on the E maze task such that recall of what-where-which is more robust. Therefore, this chapter describes various experimental approaches which are a variation of the what-where-which episodic memory task with objects hidden. Experiment 1 aimed to improve animal's interest in the non habituated, relatively novel object. By removing the habituated object at test from the E maze, it was expected that the preference for the relatively novel object, the object that remains, would improve. Therefore, the habituated object was removed from the maze at test showing an "empty arm" and a "full arm" with the relatively novel object. Subsequently, experiment 2 studied the presence on this empty arm of a novel third copy of object C. Therefore, the animal's choice at test was between an object that has seen before but not been habituated to and a completely novel object never seen before. It is worth noting that the complete novel object C was made to be as much interesting as it may be to improve the preference for this object. Based on the preference the animals have for novelty, it was expected that animals would seek the complete novel object rather than the relatively novel one. This experiment was a cued recall task whereby the object present in the holding box between sample and test phases acted as a cue for the rat to know where to turn in the E maze to seek novelty or complete novel object C. In this sense, the habituation phase as described before was now a cueing phase. Experiment 3 studied the presence of the object C by presenting one of the two objects for shorter periods of time in the holding box after a delay. Therefore, based on the preference for complete novelty and with the aim of developing the cued recall task, different conditions were also explored that differed in the use of increasing delays between sample and test phases and also by decreasing the time spent with the cue object. Finally, experiment 4 aimed to test the hypothesis whether animals may have used unintended cues (i.e. olfactory cues) in the maze that would guide the behavioural response at test rather than memory. This probe was characterized by modifying the conditions at test whilst all the other procedures remained the same as in the cued recall task. Specifically, these different conditions at test were the absence of the two objects, the use of two identical copies of the complete novel object C and the use of two identical copies of the relatively novel object in the arms of the maze. It was expected that the response at test would vary depending on the animals using memory to find novel objects or olfactory cues.

4.2: Experiment 1: What-where-which; Non habituated object at test

4.2.1: Introduction

This experiment investigated the effect on the performance of the what-where-which task with objects hidden (Eacott et al, 2005) by the absence of the habituated object in the E maze at test. Therefore, it showed at test a "full arm" with the non habituated, relatively novel object and an "empty arm" without the habituated object. This study was interesting to carry out because we were trying to improve the animal's interest in the non habituated object. By assuming that removing the habituated object may be one way to increase interest in the object that remains that was, the relatively novel object, it was expected that the performance would improve. Therefore, the animal should be motivated to find the object that it has not been habituated to and which is the only object left in the E maze at test. The same group of animals performing chapter 3 experiments was taken here. Therefore, it was possible to study this effect in experienced animals which had previous training in the task.

4.2.2: Methods and Materials

4.2.2.1: Subjects

The same group of animals that performed chapter 3 experiments was used in these chapter experiments. Testing began when the animals were about 7 months old.

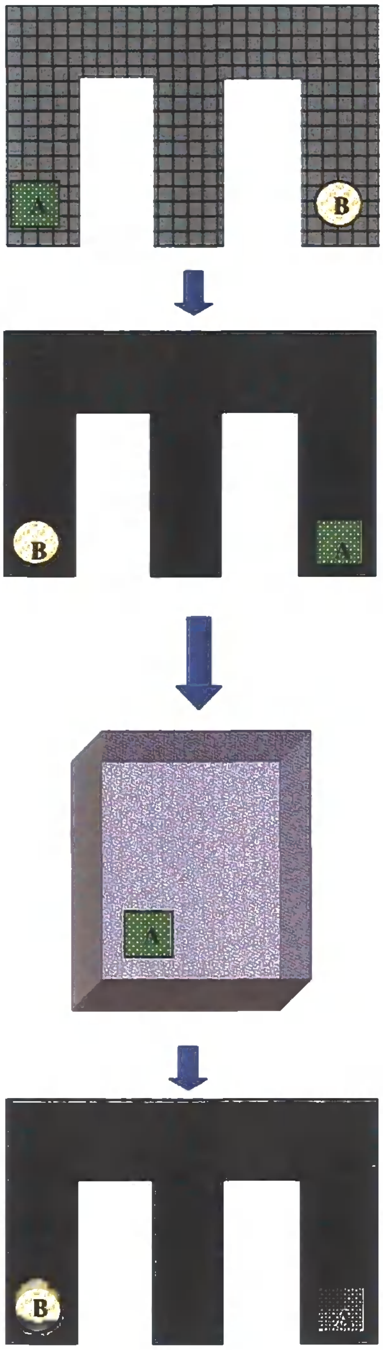
4.2.2.2: Apparatus

The apparatus was the same E-maze as described in chapter 2 and 3.

4.2.2.3: Behavioural Methods

The procedure used was the same as in experiment 6 from previous chapter 3 regarding the schedule of events for the what-where-which task with objects hidden and the conditions summarized in table 3.7. These conditions referred to the use of alternated position of the object in the habituation box, timing, animals being transferred directly from the home cages to the maze to start training. It is worth noting that from now onwards the same main stock of objects compiled at that experiment was used, these included the objects newly purchased from shop. There were no new objects added to this main stock in this chapter experiments. Nevertheless there were the following differences in the procedures: a) at test there was just one object, the one to which the animals had not been habituated; b) cleaning of the object used for the habituation with azo-wipes (disinfectant wipes) between animals; c) clean habituation boxes were used for the second half of the animal group. Conditions "b" and "c" remained for the next experiments as they consisted of general lab regulations. These two modifications were incorporated in the light of the experiments from chapter 3 and from other work in the lab that were showing odour to be a potential issue affecting the performance. To summarize here, experiment 3 showed preference to the habituated object (i.e. there was a significant percent of turns to that object in the last block of two days). These results showed that the preference to novelty was not well-formed which led to the poor recall data. Confirming that the sawdust in the habituation box may have affected the performance in the wrong way, experiment 4 showed that by taking the sawdust out from the habituation box the performance was significantly higher at the recall measures. Those observations showed that the sawdust, as it accumulated the odour scents from the con-specifics, may have affected the task. Experiment 6 showed that new objects from shop may have affected also the performance in the wrong way since there was a negative correlation between total time spent exploring objects and the recall measure in block 3. Since the animals may be more interested in smell at the habituation box, when the sawdust was around, the animals may be interested in the objects. These animals may be interested in smells which could explained the interest in the objects in experiment 6 but not a special motivation to explore novel objects over the habituated one. All together, those observations brought us to the need of reviewing the procedures used in the task to avoid presence of odour either from other con specifics or from other sources. Therefore, we included the cleaning of all the objects for habituation with alcohol impregnated wipes and the change to clean habituation boxes for the second half of the training group. Therefore, "b" and "c" conditions constituted general methodologies to carry out the training of the task and option "a" specifically conferred a variation to the what-where-which task. Figure 4.1 gives an outline of the events followed in experiment 1. There was a total of twenty four sessions performed.

Figure 4.1: Illustration of the events in the E maze in experiment 1; What-where-which task with objects hidden, no habituation object at test. It is being represented an example training session whereby context Y is present in the first sample phase followed by context X in the second sample phase, habituation to object and the final test phase, in this case, within context X. The two objects A and B swap opposite locations for each of the contexts. What is the relevant feature at the present experiment is the absence of the habituated object in the E maze at test.



A. 1st sample phase; context Y

B. 2nd sample phase; context X

C. Habituation box with object

D. Test phase; context X, no habituation object

4.2.3: Results

Experiment 1 was a total of 24 days that were analyzed in blocks of eight days in order to increase the reliability of the measure as day-by-day performance relies on only one trial per animal. The exploration ratio data or D2 score, was calculated as the ratio of the difference between the times spent by an animal within the arm of the E-maze in which the non-habituated object would normally appear (but now didn't) and the arm containing the non-habituated object to the total time spent exploring both arms of the E-maze at test. There was a clear preference and longer exploration of the empty arm expressed by the mean D2 value of -0.18 (SE = 0.03) which was significantly below the no preference level (t (15) = -5.30, p = 0.00, 2-tailed). The analysis of the three blocks of eight days showed very steady D2 values (figure 4.2). A repeated measures ANOVA was performed to study whether there was any difference between the 3 blocks. There was not a main effect (F (2, 30) = 0.11, p = 0.90) and the blocks did not differed from each other (p = 1.00, 2-tailed).

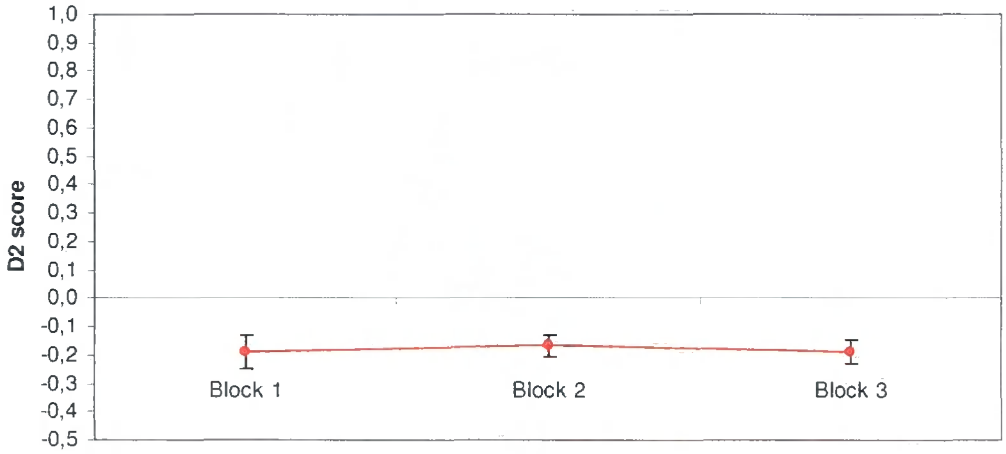


Figure 4.2: Exploration ratios of the three blocks of eight days throughout experiment 1. The line graph represents means and standard errors.

In contrast to the D2 scores which were significantly below the non-preference level, the mean recall (first turn) "percent correct first turn" over the three blocks of eight days was not significantly different from chance (M = 47.4%, SE = 2.6, t (15) = -1.00, p = 0.33, 2-tailed). The analysis by blocks of eight days was performed also for the mean percent of correct turns to the relatively novel object (figure 4.3). A repeated measures ANOVA was run to test any main effect by the block of training and it was found that there was no effect (F (2, 30) = 1.70, p = 0.2). The means did not differed from each other (blocks pair 1-2: p = 0.19, 2-tailed; blocks pair 1-3: 0.59, 2-tailed; blocks pair 2-3: p = 1.00, 2-tailed).

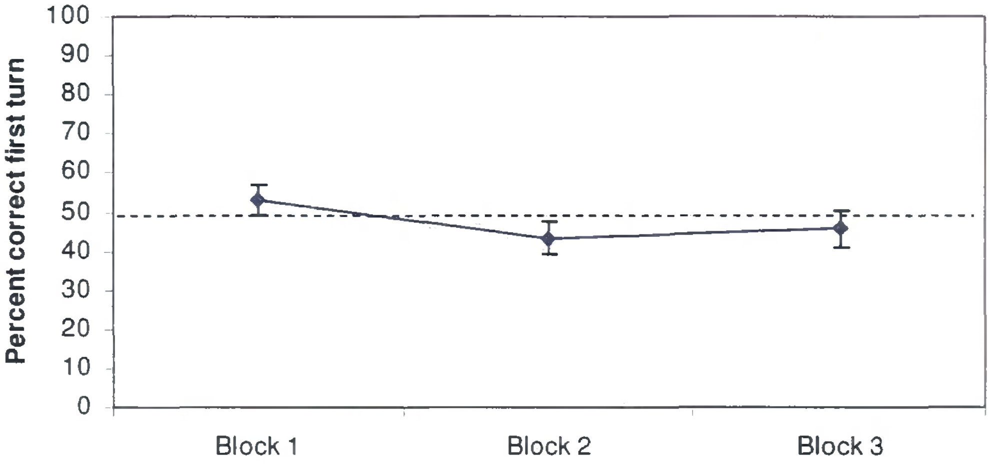


Figure 4.3: Recall performance of the three blocks of eight days throughout experiment 1. The line graph represents means and standard errors.

We investigated whether there was a relationship between animals having negative D2 scores and their recall performance. There was no significant correlation between the variable D2 and the variable mean percent of correct turns (r = -0.28, p = 0.30, 2-tailed). The linear regression was very poor (R^2=0.08) and the variable D2 was not significant (t = -1.08, p = 0.30, 2-tailed). Figure 4.4 shows the data points obtained by representing the D2 score in the X axis and the percent of correct turns to the novel object in the Y axis. However, the sample size was relatively small and lower than 30 (N = 16). It would be appropriate to collect more pairs of data points to perform more accurate and precise analysis, and then a significant result might be confirmed or even rejected. The correlation between these two variables by blocks of eight days was also studied. The absence of correlation was found more robust as the significance values increased throughout the three blocks (block 1: r = 0.12, p = 0.66, 2-tailed; block 2: r = 0.20, p = 0.50, 2-tailed; r = -0.00, p = 0.99, 2-tailed). Again, the sample size was small and by acquiring more pairs of data points, one might confirm or reject the correlation.

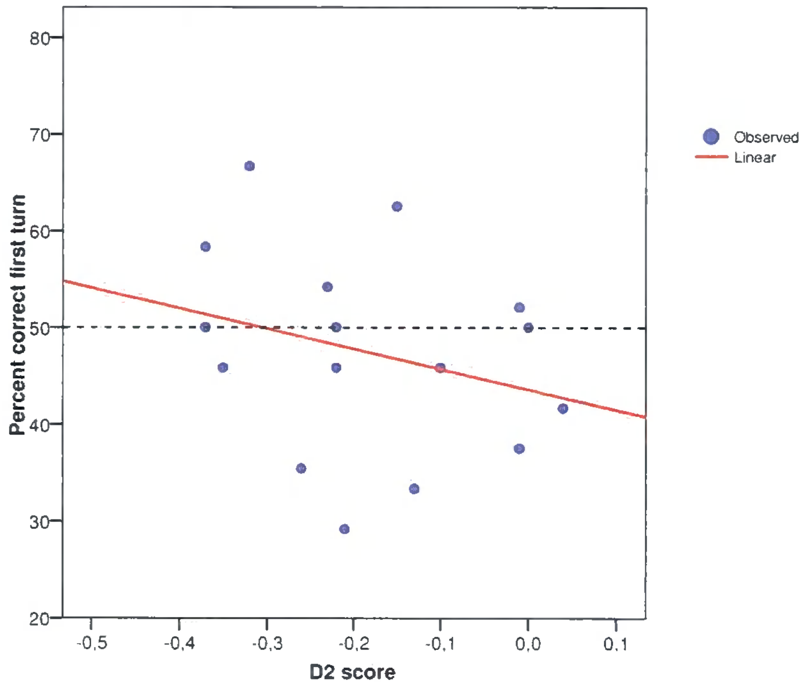


Figure 4.4: Representation of the data points of D2 scores (X axis) vs. percent of correct turns to the relatively novel object (Y axis) and the estimation obtained by linear regression.

4.2.4: Discussion

The animals showed a marked preference for the empty arm based on D2. It was found that the values for the D2 ratios were very stable from the beginning throughout all the study, when blocking the experiment in three blocks of eight days. This preference to the empty arm was clearly different to the results obtained when both objects are present at test (chapter 3, experiment 7). When both objects were present at test, the animals explored both copies but with greater exploration of the novel object. Therefore, it is suggested that the empty arm, being novelty at test in the animals experience, is rewarding, and therefore the animals seek it out. The recall measures showed a more variable pattern which was affected by the preference to the empty arm. There was no correlation between the D2 scores and the mean percent of correct turns for individual animals. There was also a marked difference from showing both objects at test. In summary, our expectation that the empty arm would be boring, it actually appears to be interesting to the animals as they turn towards it and spent time in it. On the other hand, the preference for the empty arm is observed by very stable familiarity judgements but the recall measures show a learning curve according to previous experience in experiment 1.

4.3: Experiment 2: What-where-which; Complete novel copy of object C in the empty arm

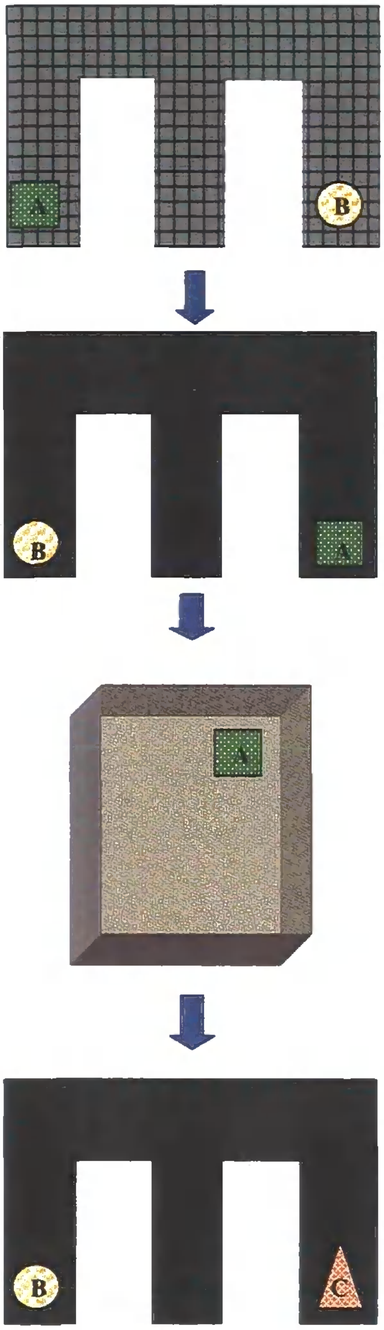
4.3.1: Introduction

Because experiment 1 demonstrated the animals prefer the empty arm (which is a novel experience), experiment 2 develops this preference animals have for absolute novelty by modifying the previous what-where-which task to include absolutely novel (rather than relatively novel) objects. This experiment was performed in the same way as experiment 1 except that at test, where the habituated object should be (and where the empty arm was in experiment 1), we now presented completely novel object C. By replacing the habituated object, the animal has a choice between a relatively novel object (which it has seen before but not been habituated to) and a completely novel object which it has never seen before. Placing the novel object at the site of the habituated object should reinforce the animals' preference for this arm from experiment 1 where it was empty and they found this to be rewarding.

4.3.2: Behavioural Methods

There were a total of twenty four trials performed. There were two modifications at this experiment. First, the design included a copy of a complete new object C in the empty arm. Object C was made on the basis of being as much interesting it can be (i.e. higher size and/or height, irregular surfaces with holes, nooks or channels, more ornamentation, playground items, less seen items, etc). The same items stock was used to form the copy of object C. Therefore, examples of objects included duplo blocks, bottles, toys, jars, ornamentation objects and the object might be a combination of objects to form a complete copy of an object. For the last 16 trials all the objects were cleaned between each 4 rats-cage. In addition, as was done previously, the habituation objects were cleaned between each animal. The cleaning of all the objects was performed for the same reason as the cleaning of the habituation object explained in experiment 1. To be based on the results from chapter 3 experiments and other work in the lab, it was found odour as an important aspect since scent marking from the con specifics in the habituation box drive the performance in the wrong direction and the animals may have smelt a shop odour when new objects were used. Therefore, it is worth doing this method to avoid this issue. Figure 4.5 shows the schedule of events in the experiment 2.

Figure 4.5: Illustration of the events in the E maze in experiment 2. As an example, it is represented a first sample phase within context Y followed by the second sample phase within context X with the objects in opposite locations. The final test phase takes place in context X and what is relevant at the present task is the presence of a third copy of a novel object C whereby habituated object would be.



A. 1st sample phase; context Y

B. 2nd sample phase; context X

C. Habituation box with object

D. Test phase; context X, habituation object is replaced by object C

4.3.3: Results

The mean D2 score for the whole group was 0.61, (SE = 0.02) and therefore it revealed a highly significant preference for the completely novel object C (t (15) = 36.31, p = 0.00, 2-tailed). As for experiment 1, the 24 days of testing were blocked into three blocks each of eight days of training. A repeated measures ANOVA was performed and a main effect was found (F (2, 30) = 8.55, p = 0.001). There was a significant difference between first and third blocks (p = 0.003, 2-tailed) but not between first and second blocks (p = 0.20, 2-tailed) nor between second and third (p = 0.14, 2-tailed) (figure 4.6).

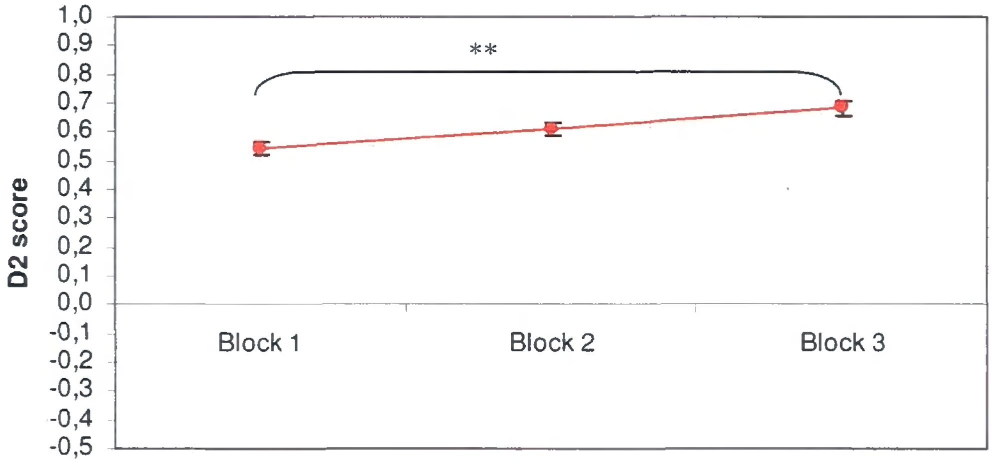


Figure 4.6: Exploration ratios in the three blocks of eight days throughout experiment 2. The line graph represents means and standard errors. Asterisks (**) represent statistical significance of the "D2 score" means between block 1 and block 3 ($p < 0.01$).

It is worth remembering that a correct turn is now counted as towards the novel object which has replaced the habituated object. This turn was noted as incorrect turn before the experiment with object C. The mean percent of turns towards the complete novel object C was 59.9% (SE = 2.6) which was significantly above chance (t (15) =3.88, p=0.001, 2-tailed). Since the average was about 60%, this means that 40% of correct turns in experiment 1 would be the same in this experiment. A repeated measures ANOVA was performed to study whether there was any effect by blocks of eight days and a significant effect was found (F (2,30) = 5.78, p = 0.01). There were significant differences between first and second blocks (p = 0.04, 2-tailed), first and third blocks (p = 0.02, 2-tailed) but not between second and third blocks (p = 1.00, 2-tailed). Figure 4.7 represents the first turn performance throughout the 3 blocks in experiment 2.

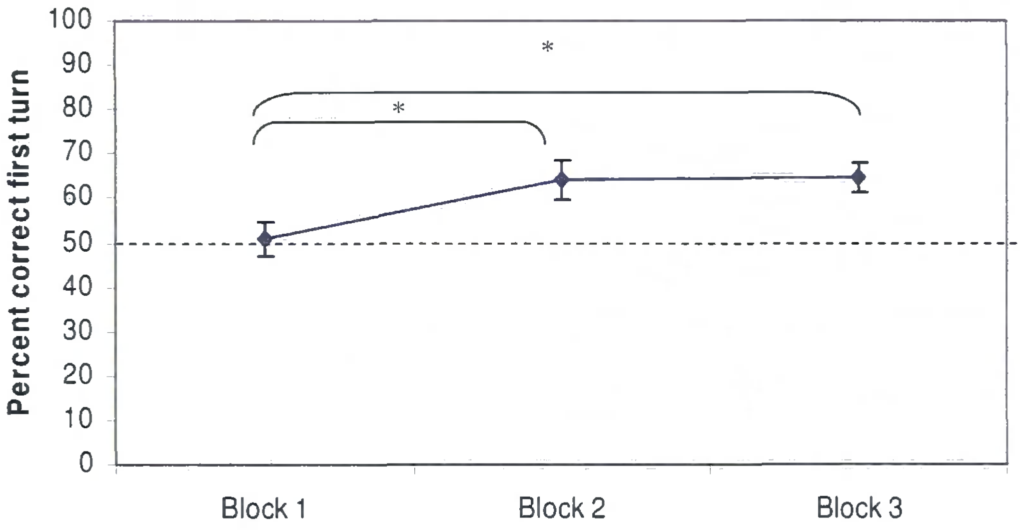


Figure 4.7: Recall performance in the three blocks of eight days throughout experiment 2. The line graph represents means and standard errors. Asterisks (*) represent statistical significance of the "percent correct first turn" means between block1, block 2 and between block 1, block 3 ($p < 0.05$).

4.3.4: Discussion

The animals showed a marked preference to the completely novel object C expressed by the mean D2 scores. A progressive improvement in the exploration ratios was observed, and this increased constantly throughout the experiment. The animal's preference to the complete novel object over the relatively novel one was due to the relatively novel object having seen twice before, whereas the complete novel object have never seen before. In addition to this, the novel object C was made to be more interesting which may have had an effect on D2 performance. These should affect the object preference and motivation, but not the ability for cued recall per se. The object presented in the holding box previous the test phase was a cue for the rat since it was associated with the arm whereby the complete novel object C was located. Therefore, the task has changed from being a habituation-based task to a cued recall task. The preference for object C was also reflected in a learning curve of the recall data which changed from being chance to be above it in second and third blocks showing more accurate data for the last block. In contrast to the D2 progression, the curve for the recall measures increased until second block after which it seemed to stabilize. The significant main effects between trial blocks for both measures (recall and D2 data) suggested that animals were learning the rules of the cued recall task, rather than it being based on spontaneous behaviour as the habituation based tasks may have been. In summary, the task of complete novelty showed good results and its potential to study cued recall aspects which will be developed in the following experiment.

4.4: Experiment 3: What-where-which, cued recall

4.4.1: Introduction

In experiment 2, the animals learned to turn in the direction of a completely novel object on the basis of a habituation phase with a different object (associated with that location). Therefore, performance on experiment 2 was not a result of habituation, but rather the habituation phase was cueing the animals' later recall of where to turn in that context. There are several types of Cued Recall Tests performed in humans to assess the level of impairment in disorders such as Alzheimer's disease (such as the Category Cued Recall test (Vogel et al, 2007). By using the cued recall test in humans, it can be valuated the severity of the Alzheimer's disease impairment since the test is highly sensitive (Ivanoiu et al, 2005; Dierckx et al, 2007). Therefore, the development of a cued recall task in the rat is important. Although there have been previous cued recall tasks in rats (Day et al, 2003), uniquely experiment 3 requires the animal to use what, where and which information in its recollection. It has to remember where the cuing object used to be in that context in order to know where to go to find the completely novel object. Experiment 2 showed that the task with complete novel objects can be developed as a task of cued recall. Therefore, in order to develop experiment 2 to be more similar to human cued recall tasks, experiment 3 reduced the time spent with the cuing object. Therefore, the cue stage was characterized by presenting one of the objects in the holding box (previously the habituation box) after a delay. Also, experiment 3 aimed to test the capacity of rats for remembering after prolonged periods of time. With the aim of developing the cued recall task and improve recall performance, experiment 3 will then explore the performance on different conditions by increasing the total delay between the sample and test phases and by decreasing the time spent with the cueing object.

4.4.2: Behavioural Methods

The training was run under the same procedure as depicted in experiment two. The main modification was at what was previously described as the habituation phase and now as the cueing phase. There was now a delay without any object in the cueing box followed by the presence of one of the objects as a cue object. To bring the procedures in line with previous work (Zinkivskay, 2006), the time for exploration phases (the sample phases and test phase) was 2 minutes long rather than 3 minutes. The time of 3 minutes was incorporated to the procedures of the task previously (experiment 4, chapter 3). At that point, to be based on the poor results obtained in experiment 3, we reviewed the procedures to improve the performance and the time for exploration phases, among other, was modified and increased to 3 minutes to have more level of accuracy in the final results although the outcomes from the results would have not changed. Five different conditions were performed consecutively. The progression on these conditions relied on the observation of a good performance, by showing recall. First condition (cued recall 4:4) used the same delay between sample and test phases as in experiment 2 but now some of that will be empty delay (4 minutes) and some will involve the animal seeing the cueing object (4 minutes). Cued recall 4:4 trained the animals for four days. Second condition that was carried out (cued recall 5:5) explored the performance in the cued recall task by increasing the total time frame between the sample and the test phases (10 minutes). The cueing was similar to previous cued recall 4:4 but increased to five minutes the presentation to the object and after a five minutes delay without object. Cued recall 5:5 trained the animals during sixteen trials. Third condition (cued recall 7:3) explored the recall performance in the cued recall task by decreasing exposure to the cue object while holding total delay constant (10 minutes). Here, the cue object was presented for only three minutes after a delay of seven minutes. Cued recall 7:3 trained the animals for four consecutive trials. Following cued recall 7:3, a fourth condition was performed (cued recall 9:1) which studied whether the animals could do the task across 10 minute delay when only cued for one minute. Cued recall 9:1 was performed during eight trials. Finally, the fifth condition (cued recall 19:1) trained the rats for four trials by increasing the total delay between sample and test phases to 20 minutes. At the cueing phase, the animal was transferred to the holding cage or habituation box for 19 minutes without any object followed by the presentation of the cue object the next 1 minute. Table 4.1 shows the summary for the schedules of time used for each of the tests for cued recall.

4.4.3: Results

For cued recall 4:4, the following results were observed. The mean percent of correct turns to the completely novel object C was 70.3% (SE = 4.7) which was found to be significantly above chance (t (15) = 4.33, p = 0.001, 2-tailed). Regarding the D2 score, there was a high preference to explore object C (M = 0.57, SE = 0.05) which was significantly above the no preference level (t (15) = 11.65, p = 0.00, 2-tailed). The following results were observed in the cued recall 5:5. There were two blocks of eight days of training completing a total of sixteen days studied. The first block showed a mean percent not different from the chance level (M = 52.7%, SE = 4.3; t (15) = 0.635, p = 0.53, 2-tailed). The second block raised it up to 63.7% (SE = 4.7) which was significant above chance (t (15) = 2.9, p = 0.01, 2-tailed). There was not significant difference between the two blocks compared by the paired t test (t (15) = -1.75, p = 0.10, 2-tailed). The overall mean for the two blocks was 58.2% (SE = 3.3) which was significant above chance (t (15) = 2.52, p = 0.02, 2-tailed). Regarding the D2 score data, the first block showed a mean of 0.45 (SE = 0.04) completely significant above no preference (t (15) = 10.55, p = 0.00, 2-tailed). The second block gave a mean D2 score of 0.52 (SE = 0.04) completely significant above no preference (t (15) = 12.15, p = 0.00, 2-tailed). The average of the two blocks was 0.48 (SE = 0.03) being significantly above the no preference level (t (15) = 17.27, p = 0.00, 2-tailed). Because the recall performance was very poor in block 1 and not different from chance, we aimed to explain why the first turn performance was so poor in the first block. We then checked whether there was any side bias or other factors such as order of the context or type of context at test biasing the results obtained in the first block and compared to the results from the second block to investigate whether there was any difference. For the first block, the mean percent of correct turns to the right at test was 45.5% (SE = 6.3) and to the left 54.1% (SE = 5.9) (1%, no choice). A paired t test was performed to compare the mean percent of turns to the right and left and it was found that there was no significant difference (t (15) = -0.71, p = 0.49, 2-tailed) (figure 4.8). For the second block, the mean percent of correct turns to the right at test was 49.5% (SE = 4.7) and to the left 48.7% (SE = 5.3) (2%, no choice). A paired t test was performed to compare the mean percent of turns to the right and left and it was found that there was no significant difference (t (15) = 0.08, p = 0.94, 2-tailed) (figure 4.8). To investigate if there was any difference on those results between first and second blocks, a paired t test was performed for each of the turns to the right or left. It was found that there was no significant difference in the turns made to the right (t (15) = -0.63, p = 0.54, 2-tailed) neither did the turn to the left (t (15) = 0.87, 0.40, 2-tailed) (figure 4.8).

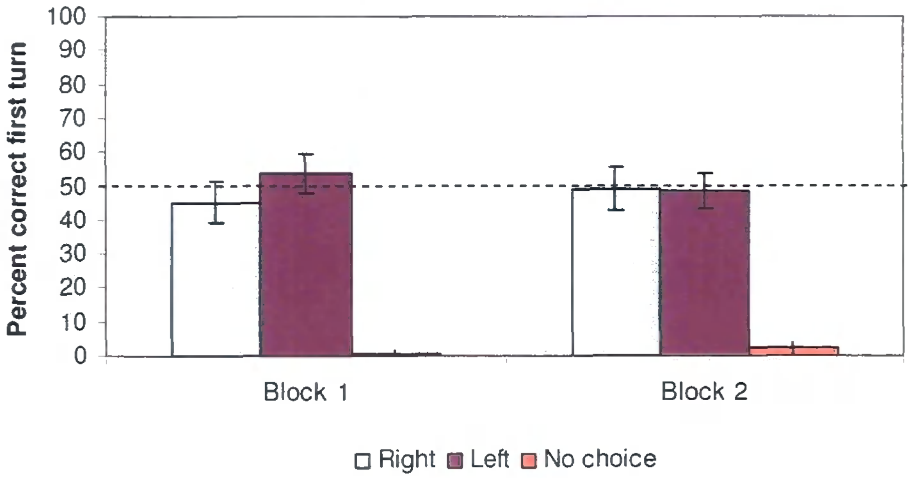


Figure 4.8: Choice made at test expressed by turns to the right, left and no choice in relation to the two blocks of training (eight days), cued recall 5:5 from experiment 3. The bars represent means and standard errors.

It was then studied whether the order of the contexts present in the sample phases affected the results since the possibilities were that the context at test would be the most recent context explored (context 2, second sample phase) or the earliest context explored (context 1, first sample phase). For the first block, the mean percent of correct turns to the novel object when the context at test was context 2 was 50.9% (SE = 5.8). When the context at test was context 1, this value was 54.1% (SE = 6.4). A paired t test was performed and it was found that there was no significant difference between these two values, (t (15) = -0.40, p = 0.70, 2-tailed) (figure 4.9). When the test context was the context 2, the mean D2 score was 0.50 (SE = 0.05) and when it was context 1 it was 0.37 (SE = 0.08) which was found not to be significantly different (t (15) = 1.31, p = 0.21, 2-tailed) (figure 4.10). For the second block, the mean percent of correct turns to the novel object when the context at test was context 2 most recently explored was 56.4% (SE = 6.3). When the context at test was context 1, this value was 69.0% (SE = 6.5). A paired t test was performed and it was found that there was not a significant difference between these two values (t (15) = -1.55, p = 0.14, 2-tailed) (figure 4.9). When the test context was the context 2, the mean D2 score was 0.58 (SE = 0.06) and when it was context 1 it was 0.46 (SE = 0.05) which was found not to be significantly different (t (15) = 1.59, p = 0.13, 2-tailed) (figure 4.10).

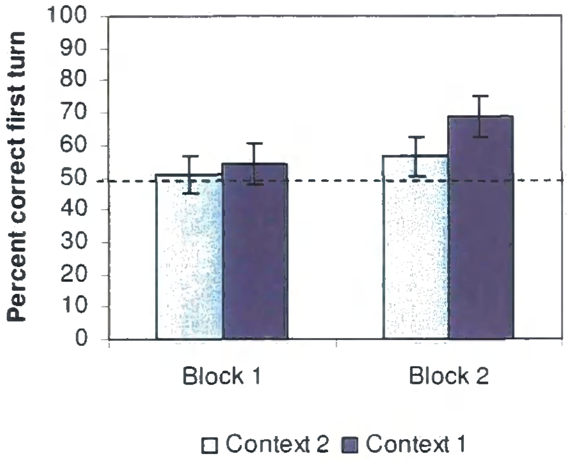


Figure 4.9: Recall performance in relation to the test context being context 2 most recently explored or context 1 the earliest context explored. Comparison of the two blocks of training (eight days), experiment 4. The bars represent means and standard errors.

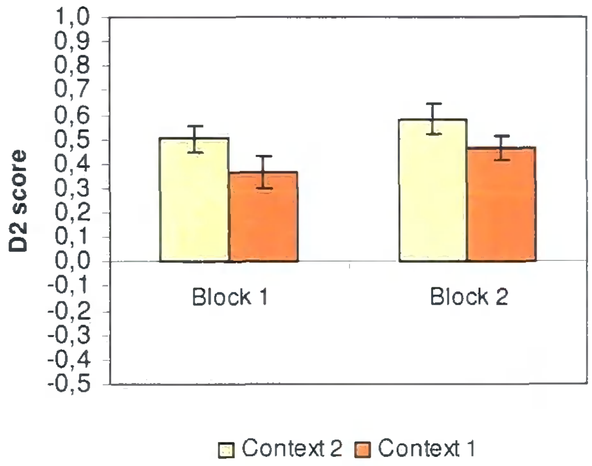


Figure 4.10: Exploration ratios in relation to the test context being context 2 most recently explored or context 1 the earliest context explored. Comparison of the two blocks of training (eight days), experiment 4. The bars represent means and standard errors.

Then, it was tested if the type of context might have affected the results in block 1 and compared the results with those of the second block. For the first block, the mean percent of turns to the novel object within context X was 50.1% (SE = 7.2) and in context Y was 52.7% (SE = 5.8). A paired t test found no significant difference between these two values (t (15) = -0.29, p = 0.78, 2-tailed) (figure 4.11). D2 scores in context X or Y at test were also compared, and the mean D2 in context X was 0.37 (SE = 0.08) and in context Y was 0.51 (SE = 0.05). A paired t test showed no significant difference (t (15) = -1.55, p = 0.14, 2-tailed) between the D2 scores in these two contexts (figure 4.12). For the second block, the mean percent of turns to the novel object within context X was 69.5% (SE = 5.7) and in context Y was 57.4% (SE = 6.5). A paired t test found no significant difference between these two values (t (15) = 1.59, p = 0.13, 2-tailed) (figure 4.11). D2 scores in context X or Y at test were also compared, and the mean D2 in context X was 0.47 (SE = 0.06) and in context Y was 0.56 (SE = 0.06). A paired t test showed not significant difference (t (15) = -0.97, p = 0.35, 2-tailed) between the D2 scores in these two contexts (figure 4.12).

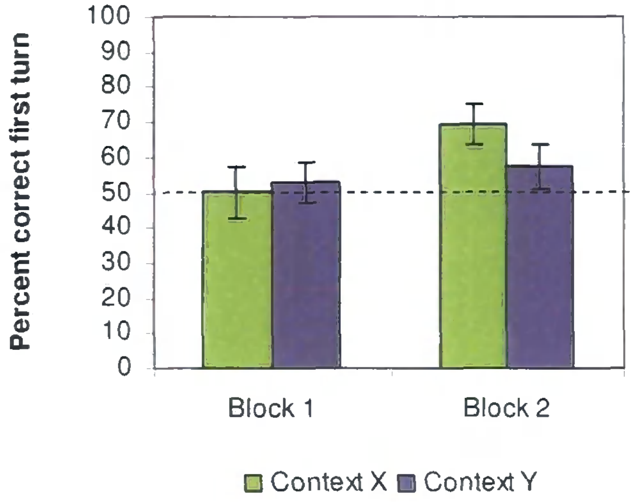


Figure 4.11: Compared recall performance within context X and context Y in relation to the two blocks of training (eight days), experiment 4. The bars represent means and standard errors.

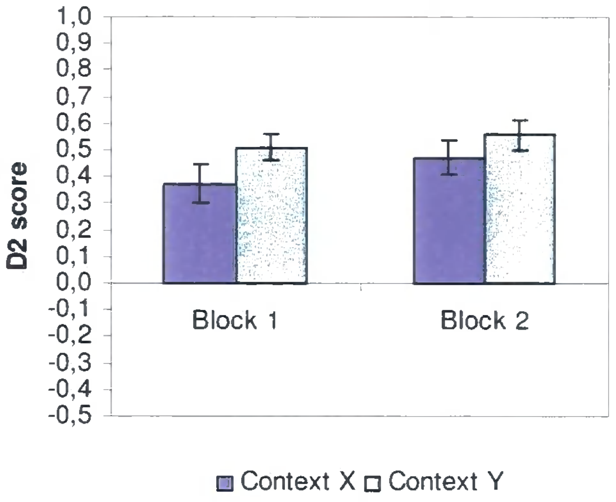


Figure 4.12: Compared mean D2 score within context X and context Y in relation to the two blocks of training (eight days), experiment 4. The bars represent means and standard errors.

Therefore, with the aim of developing the cued recall task, this experiment explored the performance in the cued recall task with objects hidden by decreasing exposure to the cue object while holding total delay constant (10 minutes). For cued recall 7:3 these results were observed. The animals showed a high level of correct turns towards the novel object 75.8% (SE = 4.6). These results were significantly above the level of 50% or chance (t (15) = 5.57, p = 0.00, 2-tailed). This data showed a highly significant preference for the completely novel object C (M = 0.62, SE = 0.06, t (15) = 10.62, p = 0.00). In the light of the results obtained for cued recall 5:5 and 7:3, it was demonstrated that the animals showed recall after 10 minute delay and that the performance rose up when shorter cueing times (i.e. 3 minute long rather than 5 minutes was found to increase recall data). Therefore, it was interesting to study this performance by using shortest cueing time (1 minute) which was expected to improve even more the performance. For cued recall 9:1, the mean percent of correct turns to the novel object was 58.6% (SE = 2.5) significantly above chance (t (15) = 3.38, p =

0.004, 2-tailed). The mean D2 score was 0.47 (SE = 0.04) significantly above no preference level (t (15) = 11.95, p = 0.00, 2-tailed). These results supported those obtained in previous work (Zinkivskay, 2006) that showed that the animals remembered after delay of 15 minutes when performing the what-where-which memory task although the performance was lower compared to delays of 8 minutes. On those experiments, the remembrance capacity after 1 hour delays was studied and an impairment in recall in the same task was observed. Therefore, it was interesting to carry out this study by using 20 minute delay that was in the interval between the 15 minute and 1 hour delays. For cued recall 19:1, the data showed that the mean percent of turnings towards the completely novel object was 66.4% (SE = 6.7) which was significantly above the chance level (t (15) = 2.44, p = 0.03, 2-tailed). In relation to the D2 score, there was a high preference to the novel object by showing a mean D2 of 0.61 (SE = 0.5) that was significantly above the no preference level (t (15) = 11.30, p = 0.00, 2-tailed). Table 4.1 summarizes the recollection measures and D2 scores along with their statistical analysis from the experiments carried out in this chapter.

A repeated measures ANOVA showed that there was a significant difference between the experiment 2 and different conditions within experiment 3 in the mean percent correct first turn (F (5, 75) = 2.99, p = 0.02) (figure 4.13). It is worth noting that the Mauchly's test of sphericity was approaching significance although still not significant (p = 0.06, 2-tailed), for samples with non equal sizes or number of trials. The paired t test found a significant difference between experiments cued recall 4:4 and cued recall 5:5 (t (15) = 2.14, p = 0.05, 2-tailed) The performance was better in previous cued recall 4:4 (70%) than 5:5 (58%). The paired t test found also significant differences between cued recall 5:5 and cued recall 7:3 (t (15) = -4.81, p = 0.00, 2-tailed) and between experiment 2 and cued recall 7:3 (t (15) = -3.15, p = 0.007, 2-tailed). The performance was much better in cued recall 7:3 (76%) compared to previous cued recall 5:5 (58%)

and better than experiment 2 for the task of complete novelty (60%). The paired t test found a significant difference between cued recall 7:3 and cued recall 9:1 (t (15) = 3.75, p = 0.002, 2-tailed) and between cued recall 4:4 and cued recall 9:1 (t (15) = 2.11, p = 0.05, 2-tailed). The performance got worse at cued recall 9:1 compared to previous cued recall 7:3 (76%) and cued recall 4:4 (75%).

A repeated measures ANOVA was performed to compare the D2 scores between experiment 2 and the different conditions in experiment 3 and a significant main effect was found (F (5, 75) = 2.81, p = 0.02) (figure 4.14). The paired t test found a significant difference between experiment 2 and cued recall 5:5 (t (15) = 3.65, p = 0.002, 2-tailed) The performance was better in previous experiment 2 (0.61) than 5:5 (0.48). The paired t test found also significant differences between experiment 2 and cued recall 9:1 (t (15) = 3.91, p = 0.001, 2-tailed) and between cued recall 9:1 and cued recall 19:1 (t (15) = -2.22, p = 0.04, 2-tailed). The performance was much better in experiment 2 (0.61) and cued recall 19:1 (0.62) compared to cued recall 9:1 (0.47). The paired t test found a significant difference between cued recall 5:5 and cued recall 19:1 (t (15) = -2.27, p = 0.04, 2-tailed).

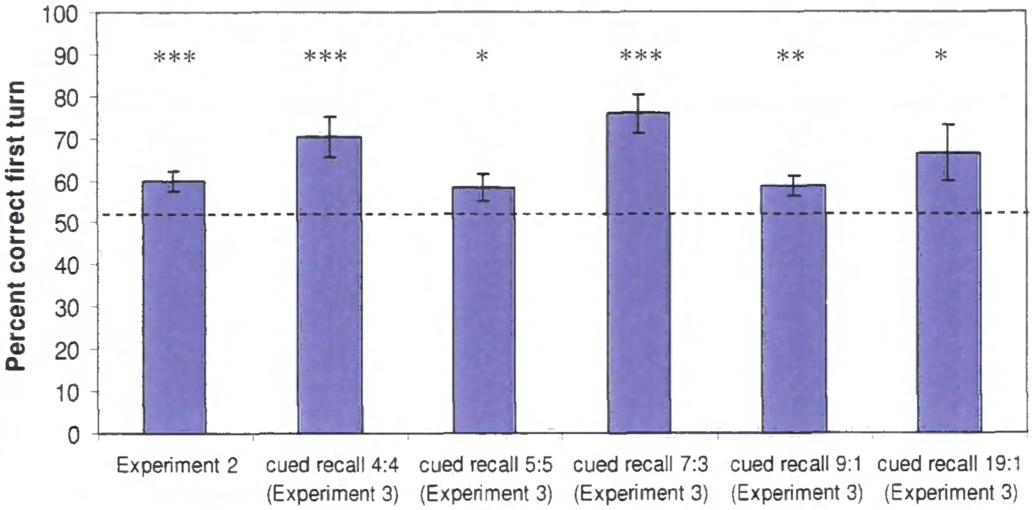


Figure 4.13: Recall performance compared throughout experiment 2 and the various conditions within experiment 3 (cued recall 4:4, 5:5, 7:3, 9:1 and 19:1). The bars represent means and standard errors. Asterisks (***) represent statistical significance of the "percent correct first turn" mean compared to chance, 50% ($p \leq 0.001$). Asterisks (**) represent statistical significance of the "percent correct first turn" mean compared to chance, 50% ($p < 0.01$). Asterisks (*) represent statistical significance of the "percent correct first turn" mean compared to chance, 50% ($p < 0.05$).

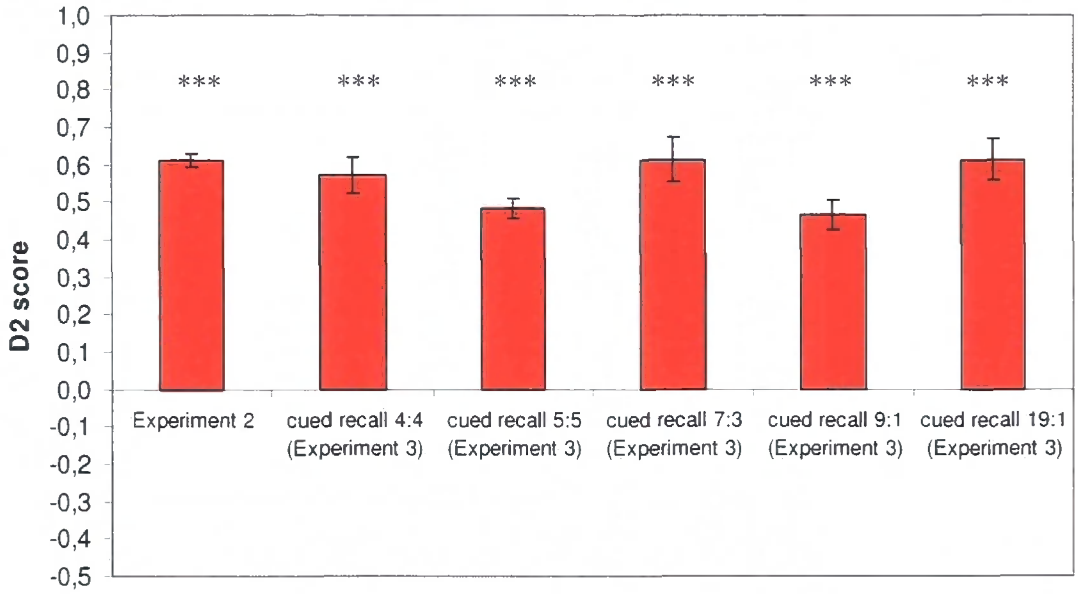


Figure 4.14: Exploration ratios compared throughout experiment 2 and the various conditions within experiment 3 (cued recall 4:4, 5:5, 7:3, 9:1 and 19:1). The bars represent means and standard errors. Asterisks (***) represent statistical significance of the "percent correct first turn" mean compared to chance, 50% ($p < 0.001$).

Experiment	1	2	3 (4:4)	3 (5:5)	3 (7:3)	3 (9:1)	3 (19:1)
Time schedule	8	8	4:4	5:5	7:3	9:1	19:1
Objects	No hab. object at test	Complete novel object C at test	Complete novel object C at test	Complete novel object C at test	Complete novel object C at test	Complete novel object C at test	Complete novel object C at test
N º trials	24	24	4	16	4	8	4
Percent correct first turn	47.4%	59.9%	70.3%	58.2%	75.8%	58.6%	66.4%
One sample t test	t=-1.00 p=0.33	t=3.88 p=0.001	t=4.33 p=0.001	t=2.52 p=0.02	t=5.57 p=0.00	t=3.38 p=0.004	t=2.44 p=0.03
D2 ratio	-0.18	0.61	0.57	0.48	0.62	0.47	0.61
One sample t test	t=-5.30 p=0.00	t=36.31 p=0.00	t=11.65 p=0.00	t=17.27 p=0.00	t=10.62 p=0.00	t=11.95 p=0.00	t=11.30 p=0.00

Table 4.1: Recollection and familiarity processes data.

4.4.4: Discussion

As with experiment 2, there was a clear preference for the completely novel object C as expressed by both the D2 and recall measures across the different conditions, and significant first turns towards the novel object, suggesting cued recall. Therefore, the addition of an "empty delay" without object present did not impair the performance of the task. These results demonstrate that the animals remembered where to go in the E maze to seek novelty at test, when the cueing to the object lasted only 4 minutes (and the delay between sample and test phases remained 8 minutes in total). This provides more evidence that the task with completely novel objects can be developed as a task of cued recall. The recall performance of the animals when the total delay between sample and test phases was increased to 10 minutes (cued recall 5:5) was poorer compared to the previous condition. Especially, the recall performance of the first block of eight days was very poor and not different from chance. However, there was no effect on the performance by the possible biasing factors studied that were, side of the turnings made, order of the contexts and the type of context at test neither in the first block nor in the second block of training. In summary, the animals showed the capacity for remembering the encoded information from the sample phases after prolonged periods of time of up to 10 minutes although the recall data showed to be poorer compared to 4:4, delays of eight minutes and cueing for 4 minutes. Therefore, following condition (cued recall 7:3) studied whether the animals remember it after the same total time but by presenting the cue for shorter periods of time. Also, this will allow us to have data comparable with similar data being gathered in the laboratory at the same time (Fitchett, unpublished data). The animals' recall performance confirmed the results obtained in the previous condition that led to the conclusion that the animals did remember after ten minutes. Also, there was evidence that they performed well when the cue was presented for only three minutes after a delay of seven minutes. It was observed that the recall performance was significantly better compared to previous 5:5. Although the delay between the second sample phase and the test was the same that is, ten minutes, the cueing during only three minutes may have improved the remembering of what-where-which. However, it is worth noting that this could also be an order effect (i.e. learning). However, there was no evidence for a difference in the familiarity judgements expressed by the D2 scores. Therefore, next condition for cued recall 9:1 studied whether the animals might perform correctly the task by exploring the cue object for shorter periods of time with the same 10 minute delay between sample and test phases. Specifically, cued recall 9:1 showed the rat the cue object for exclusively 1 minute previous the test phase. The rats did remember the information they encoded after periods of time of 10 minutes which confirmed what had been concluded before. In addition to that, it was also demonstrated that this cued recall task where only 1 minute cue produced really good levels of recall performance. At the present cued recall task, it indicated that the level of accuracy for recall processes was high and therefore the task in the presence of the complete novel object C was successful in increasing the results obtained in the what-where-which task which was reflected in high level recall performances such as 76% (cued recall 7:3).

The animals demonstrated remembrance of the events being held in the E maze at the sample phases after 20 minutes delay also (cued recall 19:1). In summary the "percent correct first turn" was good showing that the animals turned towards novelty the 66% of the time and a strong preference to the complete novel object by having an average of D2 scores of 0.61. Also, these results confirmed the animal's remembrance by using cue time of 1 minute exclusively. Studies from previous work (Zinkivskay, 2006) showed the animals′ ability to remember after similar delay of 15 minute. However, the "percent correct first turn" was much poorer (about 60%) revealing a decrease in the recall accuracy. The reason that the recall performance was better here relies on the incorporation of an object at test that the rat had never seen before that day at sample phases. This fact increased the novelty attributes of that object over a relatively novel object that had been seen before that day. Apart from this, the novel object was made to be very interesting contributing an additional preference or motive to explore that object. These explanations were reflected on the high preference to explore the novel object where high values of the D2 scores (0.61) were obtained. This rate was produced to a much greater extent than when only relatively novelty was present in the E maze at test (i.e. experiment 6, chapter 3) where this value was close to 0.13.

4.5: Experiment 4: Probe; A three-level repeated study

4.5.1: Introduction

This experiment explored the recall performance on the what-where-which task, objects hidden by introducing some modification at the test phase. This experiment was based on the task previously described with complete novelty for cued recall. The aim of this experiment was to test whether the animals used unintended cues to perform the task rather than memory when these modifications were present in the E maze at test when they made a choice. In this regard, this experiment included three probes or repetitions studying this aspect. The three probes were run in such a way that there were no objects (neither relatively novel nor completely novel objects), identical copies of the complete novel objects in the two arms and two identical copies of the relatively novel objects in the two arms of the E maze. Other possibilities or designs for probe experiments might be; 1) empty arm and exclusively one copy of the relatively novel object at test, 2) one empty arm and exclusively one copy of the complete novel object C at test and 3) no cueing and objects at test.

For the first probe whereby there were no objects at test, the following expectations of the performance were suggested:

Hypothesis 1: The rats identify, either at the start arm or when turning towards one arm and before the empty arm is visible for the animal, the absence of the objects (completely novel and/or relatively novel) in the E-maze by using unintended cues. The expected recall performance of rats behaving like this would be at chance level.

Hypothesis 2: They do not know about the absence of the objects (complete novel and/or relatively novel) in the E-maze by using unintended cues, relying instead on memory. The expected recall performance of rats behaving like this would be above chance level, as on the non-probe trials in the previous experiments.

For the second repetition of the probe whereby two identical copies of complete novel object were in the two arms of the E maze at test, the following expectations were suggested:

Hypothesis 1: The rats identify, either at the start arm or when turning towards one arm and before the object is visible for the animal, the presence of complete novelty in the arms of the E-maze by using unintended cues. The expected recall performance is at chance level.

Hypothesis 2: They do not know where the complete novelty is in the E-maze by using unintended cues. The expected recall performance is above chance level as they should turn on the basis of memory of where they expect to find the novel object.

In regard to the third and last repetition probe, these were the hypothesis suggested:

Hypothesis 1: The rats identify, either at the start arm or when turning towards one arm and before the object is visible for the animal, the presence of relatively novelty in the arms of the E-maze by using unintended cues. The expected recall performance is at chance level.

Hypothesis 2: They do not know where the relatively novelty is in the E-maze by using unintended cues. The expected recall performance is above chance level because they are relying on memory.

4.5.2: Behavioural Methods

The conditions at the test phase in the probe trials were different from a normal training schedule where the two objects explored during the sample phases are shown in their specific locations in a context. The first probe type (probe 1) studied the performance when there were no objects in the arms of the E maze at test. The second probe type (probe 2) studied it when two identical copies of the complete novel object C were in the arms of the E maze at test. The last probe type (probe 3) was characterized by using two identical copies of the relatively novel object in the arms of the E maze at test. Each probe trial was performed just after four normal training trials and two probe trials were done for each probe type. Therefore, there were two consecutive phases that included 4 training trials followed by one probe trial each. Thus, each probe type was formed by a complete set of eight training trials and 2 probe trials. The conditions at test phase in the training trials remained the same as in the sample phases for the cued recall task. This means that at the test phase of a training trial, the relatively novel object and complete novel object C were present in their specific locations within a context, as it had been shown in the sample phases of that training trial. The procedures were the same as in experiment 3 and therefore between sample and test phases there was a delay followed by the presentation of the cue object. The criteria for which delay to use from the range explored in previous experiment 3 for cued recall was distinct for the first phase of probe 1 as opposite to second phase of probe 1 and the other probe types 2 and 3. In the first phase of probe 1, the criterion was selecting the cued recall task that produced the better results by cueing for only 1 minute and therefore delay of 19 minutes followed by cueing time of 1 minute (19:1) was used (see experiment 3). In the second phase of probe 1 and for the subsequent probes 2 and 3, the decision was made on the basis of using shorter periods of training by cueing also for 1 minute and therefore the delay of 9 minutes followed by 1 minute cueing was selected (9:1) (see experiment 3). The analysis of the data was based on the "percent correct first turn", first turn made towards the arm whereby the complete novel object C would be as it had been shown in the sample phases of a normal training schedule.

4.5.3: Results

The training trials on the complete novelty test for cued recall showed that the rats turned on average over 16 days the 66.4% of the time correctly and completely significant above the level expected at chance performance (t (15) = 4.63, p = 0.00, 2-tailed). A repeated measures ANOVA was performed to study whether there was any difference between the total of six probe trials and it was found that there was not significant difference between them (F (5, 75) = 0.60, p = 0.70). Then, the average of correct turns throughout the 6 probe trials was found to be at chance level (M = 51.0%, SE = 3.5) not significantly different from 50% (SE = 3.7) (t (15) = 3.00, p = 0.77, 2 tailed).

The first probe included two phases of training for cued recall 19:1 and 9:1 and therefore the results were split on the basis of these two delays. The "percent correct first turn" at both training phases were significantly above chance (19:1: M = 66.4%, SE = 6.7, t (15) = 2.44, p = 0.03, 2-tailed; 9:1: M = 60.9%, SE = 4.8, t (15) = 2.27, p = 0.04, 2-tailed). The "percent correct first turn" on the probe trials was not significantly different from chance (19:1 M = 40.6%, SE = 12.3, t (15) = -0.76, p = 0.46, 2-tailed; 9:1 M = 56.3%, SE = 12.8, t (15) = 0.49, p = 0.63, 2-tailed). A paired t test was performed to compare the "percent correct first turn" between the four training trials and the probe trial for each of the two phases for cued recall 19:1 and 9:1. It was found that there was a marginally significant difference between the two types of trials in the 19:1 cued recall phase (t (15) = 2.04, p = 0.06, 2-tailed). There was not significant difference between the two types of trials in the 9:1 cued recall phase (t (15) = 0.37, p = 0.72, 2-tailed) (figure 4.15).

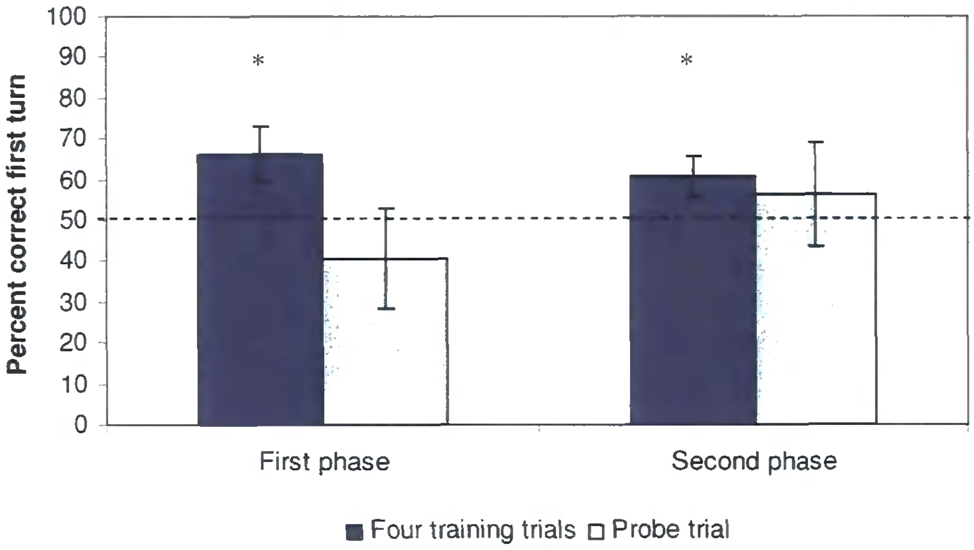


Figure 4.15: Recall performance compared between the training and probe blocks in relation to the two phases (19:1 and 9:1), probe 1. The bars represent means and standard errors. Asterisk (*) represents statistical significance of the "percent correct first turn" means compared to chance, 50% ($p < 0.05$).

The "percent correct first turn" of the training block in probe 1 was significantly above chance (M = 63.7%, SE = 4.1, t (15) = 3.35, p = 0.004, 2-tailed). The "percent correct first turn" on the probe trials was not significantly different from chance (M = 48.4%, SE = 8.1, t (15) = -0.19, p = 0.85, 2-tailed). A paired t test was performed to compare the "percent correct first turn" over the two types of trials and it was found not significant difference between them (t (15) = 1.57, p =

0.14, 2-tailed) (figure 4.16). Following the finding of a possible effect on the recall performance on the probe trial (i.e. there was a marginally significant difference between the training and probe trials in the first phase of probe 1), it was performed the study of a second probe or probe 2 (cued recall 9:1). The "percent correct first turn" of the training block was significantly above chance (M = 67.6%, SE = 4.6, t (15) = 3.82, p = 0.002, 2-tailed). The "percent correct first turn" on the probe trials was not significantly different from chance (M = 56.3%, SE = 9.0, t (15) = 0.70, p = 0.50, 2-tailed). A paired t test was performed to compare the "percent correct first turn" over the two types of trials and it was found not significant difference between them (t (15) = 1.17, p = 0.26, 2-tailed) (figure 4.16). In order to explore the apparent significant difference on the recall performance between the two types of trials found in probe 1, a third probe or probe 3 (cued recall 9:1) was then studied. The "percent correct first turn" of the training block was significantly above chance (M = 65.2%, SE = 5.0, t (15) = 3.04, p = 0.01, 2-tailed). The "percent correct first turn" on the probe block was not significantly different from chance (M = 48.4%, SE = 8.1, t (15) = -0.19, p = 0.85, 2-tailed). A paired t test was performed to compare the "percent correct first turn" between the two conditions and it was found a marginal significant difference between them (t (15) = 2.01, p = 0.06, 2-tailed) (figure 4.16).

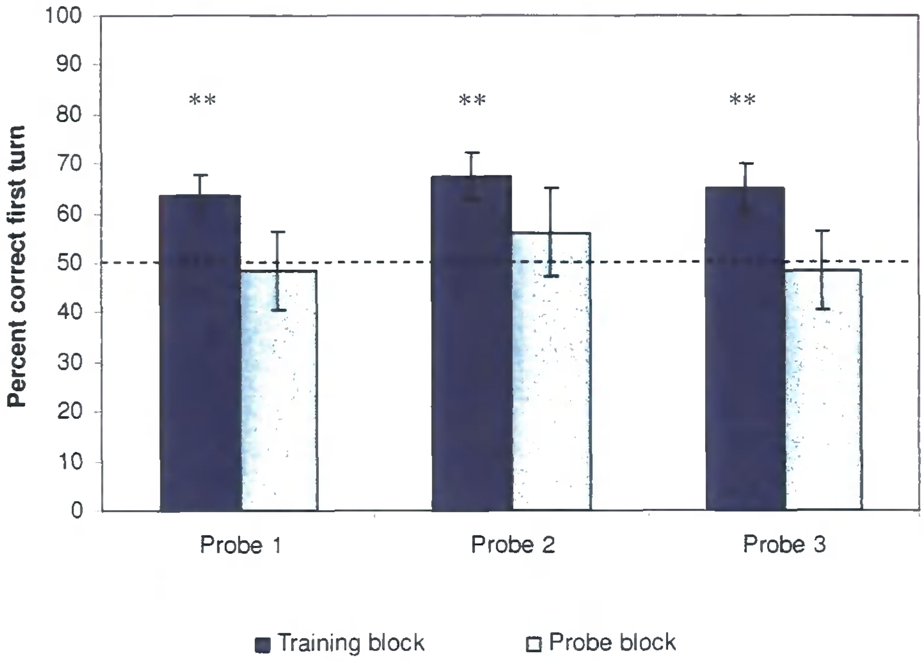


Figure 4.16: Recall performance compared between the training and probe blocks in relation to probes 1, 2 and 3. The bars represent means and standard errors. Asterisks (**) represent statistical significance of the "percent correct first turn" means compared to chance, 50% ($p \leq 0.01$).

An analysis was undertaken whereby the sample size or number of trials for each condition were increased. In this sense, a total of sixteen training trials (training block) were studied that corresponded to the probes 2 and 3 for cued recall 9:1 and its corresponding four probe trials (probe block). The "percent correct first turn" of the training block was significantly above chance (M = 66.4%, SE = 3.5, t (15) = 4.63, p = 0.00, 2-tailed) whereas the probe block did not differ from chance (M = 52.3%, SE = 5.6, t (15) = 0.42, p = 0.68, 2-tailed) .A paired t test was performed and a significant difference was found (t (15) = 2.31, p = 0.04, 2-tailed) (figure 4.17).

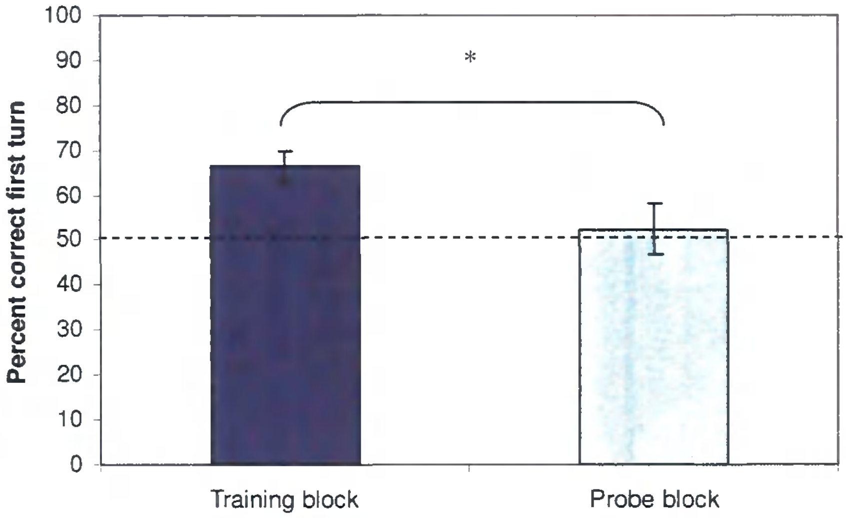


Figure 4.17: Recall performance compared between the training block (eight trials) and the probe block (four trials). The bars represent means and standard errors. Asterisk (*) represents statistical significance of the "percent correct first turn" means between training block and probe block ($p < 0.05$).

4.5.4: Discussion

It was initially found in the first probe run an apparent difference between training trials or object present trials compared to the probe trials whereby there were no objects at test (i.e. there was a marginal significant difference). Therefore, they may be able to use unintended cues which presumably would be olfactory to smell the absence of objects and know something is different. Therefore, we tested probe 2 with two identical copies of the complete novel object to control for this. There was not significant difference between the training trials and the probe block. However, confirming what was shown in probe 1, the recall performance of the probe trials did not differ from chance as opposite to the training block which showed significant values above chance. Since there was an apparent difference between the two types of trials in probe

1 and the recall performance on the probe block did not differ from chance, another probe 3 with two copies of the relatively novel object was carried out to study further these aspects. Again, the recall performance of the probe block was not different from chance as opposite to the recall performance in the training block. Besides, an apparent difference between these two conditions on the recall performance was found. Further analysis with increased sample sizes showed a difference in the recall performance between the probe and training trials by comparing sixteen training trials with a total of four probe trials. Therefore, it may be the case that the animals were using odour cues to solve the task.

4.6: Discussion

Experiment 1 showed that the absence of the habituation object or non novel one at test did not improve the preference to explore the relatively novel object. By contrast, there was a preference for the empty arm expressed by a significant D2 score below no preference level. It was suggested that this preference for the empty arm may rely on the empty arm being novelty at test in the animal's experience and therefore the animals would show a tendency to explore this arm. With the aim of improving the performance of the task, the animals' response at test (that showed a preference to explore the empty arm) was then reinforced by incorporating complete novelty on that arm (complete novel object C present). There was a preference for this completely novel object expressed by high positive D2 scores values and significant turns made to this object at test. The fact that complete novel object C was made to be as much interesting as it may be may have had an effect on this performance but should not affect the ability for the cued recall. Experiment 2 constituted a cued recall task whereby the object present in the holding box between sample phase 2 and test phase was a cue for the rat since it was associated with the arm where the complete novel object C was located. Based on the good results observed in this experiment, the complete novelty task was found to be a reliable task to study cued recall in the rat. With the aim of developing it, various conditions for the cued recall task were then explored in experiment 3. These conditions studied the performance on the task by using shorter periods of cue object presentation in the holding box. Also, these conditions explored the capacity of the rats for remembering after prolonged periods of time. All these conditions for the cued recall task showed good results expressed by significant preference for the complete novel object C and significant turns made to this object. By this study, it was shown that the animals could remember the events previously experienced in the sample phases during prolonged periods of 20 minutes and that they could perform correctly when the cued for only 1 minute. The last experiment 4 showed that these animals may have been using unintended cues when performing the probe trials apparently done by odour. Therefore, if they did use odour in the probes we had to assume that they used odour in the test phases. In that case, the good recall performance observed in the cued recall task for complete novelty may be a result of odour detection rather than memory. Previous work (Zinkivskay, 2006) compared the recall performance of the task between the two conditions or object present trials and probe trials. This study found that there was not a significant difference between the two conditions and that the recall performance on the probe trials did not differ from the overall performance. Also, animals showed significant turnings towards the arm where the more novel object would be present. Whether non-odour trained animals may solve the task the same way or differently, may be explored with a complete naïve and non-experienced group of animals by studying their performance on the cued recall task with complete novel objects in the two conditions of normal training and probe trials.

Chapter 5: General discussion

5.1: Discussion

Previous findings in the open field task (Eacott and Norman, 2004) showed that the fornix group animals were impaired for recognition memory of a what-where-which configuration. It is worth noting that fornix animals were found to perform at above chance. However, somewhat below the sham group, in an object-context recognition task whereby the position (where) of the objects was irrelevant (Norman and Eacott, 2005). This finding pointed out that the fornix would be somehow related to tasks requiring the memory for the context but it would not be essential since the animals still showed to perform correctly the task that did not require spatial information but contextual, still slightly below the normal group. The impairment on recognition memory might be due to impairment in recall and/or familiarity judgments. However, the fornix was found to be impaired specifically in recall although the familiarity processes remained intact (Eacott and Easton, 2007). Since memory for a what-where-which combination was found to be impaired by the fornix interruption in these studies, it was interesting to investigate this aspect further and to test whether the fornix would contribute to the memory for the whole triad what-where-which or it would be more related to spatial memory. The fornix disrupts the function of the hippocampus and the hippocampus is involved in other types of memory such as spatial memory. Therefore, the impairment observed in a what-where-which task by the fornix lesion might be caused by impairment in spatial memory for what-where. In other words, it could be possible that the animals with fornix damage might recall which object they are looking for and the occasion on which they showed it, but they cannot remember where they have seen it. Therefore, it was interesting to investigate the performance on a task that has a spatial component but which is not impaired in the animals with an episodic-like memory impairment. Eacott and Norman (2004) managed to design this task in the open field. This control task is very similar to the what-where-which task except that there is no second sample phase in a different context since the contextual information is irrelevant to perform correctly this task. At this task, both groups of sham and fornix lesion animals were shown to have good memory for what-where at both delays of two minutes and five minutes. However, the open field task relies on recognition and not on recall. Therefore, we needed to test the performance of the animals in the what-where task in the E maze to investigate specifically recall.

The what-where task with objects visible did not work since there was no preference for either object at test phases and the recognition memory measures were poor. Either the sham lesion or the fornix lesion animals did not perform significantly differently from chance in their turns to the relatively novel object. The performance in the what-where task with objects visible did not predict the performance with objects hidden for individual animals (Zinkivskay, 2006). It was then studied how the same group of animals would perform in the objects hidden task. Results from previous work with unoperated rats (Chapter 2; Zinkivskay, 2006) showed that animals′ recall performance on the what-where task with objects hidden was significant above chance averaging 61% over 16 days. It may be possible that the objects hidden task would be more interesting for the animals and therefore there may be observed an increase on the individual's motivation and performances of the task. The task did not work and either the sham or the fornix groups did not performed differently from chance. It is worth remembering that the fornix lesion animals still showed an object preference expressed by the familiarity measures and although there was no evidence of a preference throughout all the experiments, it may suggest that the fornix damage did not disrupt the familiarity processes according to previous findings (Easton et al, 2006; Eacott and Easton, 2007).

The poor performance in the task could be because the animals found the what-where task too difficult since it does not include contextual information. This hypothesis was not supported since these animals were not able to perform the what-where-which task, a task that has been reported to work well (Eacott et al, 2005, Zinkivskay, 2006). This study (Eacott et al, 2005) showed that animals performing the objects visible and hidden versions of the task turned towards the non habituated object significantly above chance. Specifically, when objects were out of sight, animals showed recall of the what-where-which combination averaging 65% over 16 days significantly above chance. Also, previous unpublished work (Chapter 3; Zinkivskay, 2006) showed that over 16 days when objects were out of sight recall performance was also significant above chance showing an average of 66%.

Summarizing, the results obtained in the what-where-which task, a task that has been shown to work out, suggested that these animals might be now older and experienced or that they may not habituate in the same way to the objects. However, it was found that a task similar to that in the open field (Eacott and Norman, 2004), which did not require the habituation to the object, did not work properly either. The performance was at chance and therefore lowers than In the task performed in the E maze that included habituation.

In summary, sham group showed poor first turn performance on the recognition task and they also showed poor results when recollection of the event was needed to perform correctly. This observation may support that the effect of aging on memory is partly reflected in a sensible aspect of memory that is, the retrieval or recall of past events that shows a significant decline with age (Small et al, 1999; Davis et al, 2003). On the other hand, previous published demonstrations of good performance in the task took animals that were naïve (Eacott et al, 2005; Zinkivskay, 2006) and it may be the case that these animals would be now too experienced.

In summary, the results obtained on these tasks pointed to the fact that the animals performance were possibly affected by the animals being older than in previous training experiences or/and that they were too experienced or/and they were not trained for a long time between previous experimenter and actual training and possibly small differences in the procedure. It was then worth studying the animals behaviour of a complete naïve and young group of animals to explore whether any small detail on the procedure may have influenced those results and in relation to the preference to novel objects and the tendency to turn towards those objects.

The what-where task with objects visible did not work in this normal, young and naïve group of animals. The objects were interesting since the animals showed a significant exploration of them. It was hypothesized that the rates of exploration for individual animals would be correlated to first turn performance. The hypothesis could not be observed in this sample of animals, however, relatively small in size and in the number of pairs of data points gathered. Therefore, it would be interesting to study this issue further. The what-where recognition task in the open field was demonstrated (Eacott and Norman, 2004). However, the task differed mainly from the E maze task on that it did not include the habituation to an object. In previous experiments (Eacott et al, 2005; Eacott and Easton, 2007) first turn performance on an objects visible version of the what-where-which task has not always predicted performance when objects are hidden from view. Therefore, it was then tested the recall performance of these same young naïve animals on an objects hidden version of the what-where task. The what-where task did not work with objects visible nor with objects hidden and the animals did not show an object preference. Also, there was a significant exploration of the objects that was not different from previous experiment with objects visible. Therefore, it could not be the case that the objects were not interesting enough to perform these tasks as the animals showed motivation. It was then analyzed any biasing factor on the performance of the task, such as a turning preferred side and the type of context at test. Studies where the contextual information was irrelevant on the performance of the task, odour recognition occurred in a different but familiar context showing a lack of context specificity (Burman and Mendl, 2002). However, whether the test phase occurs in the same context as the most recently explored one or different (i.e. learning sequence) might have an effect on the memory processes being held in the test phase. There are various studies investigating the lateralization or lateral asymmetry related to a right-left response differentiation (Zimmerberg et al, 1978; Glick and Ross, 1981; Noonan and Axelrod, 1989; Uziel et al, 1996; Andrade et al, 2001; Schwarting and Borta, 2005). In general, it appears that there were behavioural biases showed by a right preferred side of turning in rats. Therefore, a turning bias would have relevant implication in tasks with a spatial component. None of the factors from the analysis was found to have had an effect on the performance. Previous work in the what-where-which task (Chapter 3; Zinkivskay, 2006) did not find evidence that the contextual background within test phase took place would affect event recollection. However, in that work a side bias to the left was observed over 18 days but it did not have an effect on the choice for novelty. The what-where task could be a difficult one and to explore the performance on the what-where-which task was worth doing. The what-where-which task with objects hidden did not work in this group of young and naïve animals.

At that point, the procedures used were reviewed to find any possible small difference on the procedure that did not match previous published work with good performance of the task. Based on that the animals showed evidence of a preference to the habituation object it was definitively studied any possible factor at habituation driving this performance. In this sense, the sawdust present in the habituation box was identified as a potential aspect that may have affected the poor performance. The sawdust in the habituation box accumulated the odour scents or odour marking from the con specifics.

The olfactory information in mammals plays an important function and a number of social odours are described to play a role in the social communication. The social information is passed between individuals on the form of odour signals that give knowledge about the individual identity, ownership, sex, reproductive status, competitive ability and health status (Brennan and Kendrick, 2006). This information is relevant for the reproductive success, mate choice and territorial marking. In rodents, it plays a significant role as they can use information about specific individuals to adjust their behaviour for future interactions. This social recognition is associated with hormonal, neural and genomic mechanisms (Cheetham et al, 2007) in rodents and is produced in the form of chemical signals including genes from the Major Histocompatibility Complex (MHC) and Murine Urinary Proteins (MUPs). The territory marking behaviour in individual mammals or groups is performed by depositing urine, faeces or marks produced by the specialized scent glands throughout their territory but overall at the boundaries or access routes. In this sense, it has been demonstrated in rodents that sensory chemical signals present in the urine can be used to convey information about the individual identity of the con specifics (Beynon and Hurst, 2004). Males can use this olfactory communication to know the competitive capacity of other males and to adjust their behaviour for possible future interactions (Rich and Hurst, 1998) as it has been identified a broad range of odours associated with dominance in male mice (Novotny, 1990). For example, countermarking consists of the marking on the neighbourhood of a scent mark from a con specific. These scent marking and countermarking provide information about the ability of a con specific to dominate or defend an area to other competitors and potential mates (Rich and Hurst, 1999). In this sense, dominant male mice deposit urine marks in their territory to countermark the marks from intruder males (Hurst et al, 2005). This ownership signature is advertised by the urinary scent marks that are in-volatile and the use of individual specific patterns which has proved to last long periods of time without loosing the olfactory properties (Nevison et al, 2003). In that sense, the MUPs from male mice urine are a source of information and interest to other males and females that is preserved in dried urine marks (Hurst et al, 1998). Females can use these odours for mate selection and to distinguish between parasitized and non-parasitized males (Kavaliers and Colwell, 1995; Willis and Poulin, 2000; Ehman and Scott, 2002; Kavaliers et al, 2005) and there is a specific urinary pattern depending on the social rank (Desjardins et al, 1973). The role of odour in social communication plays an essential role on maintaining the social relationships. It has also been reported that the bedding containing the scent marks consists of a conditioned place preference in subordinate male mice (Fitchett et al, 2006). The social interaction has been demonstrated to have a valuable rewarding outcome as isolated pups choose the social interaction instead of food rewards (Ikemoto and Panksepp, 1992).

As it has been reviewed briefly before, the sawdust in the habituation box accumulates the scent marking from the con specifics since all the animals are transferred to the same holding cage and consequently this serves as a social recognition source. To test the involvement of the olfactory social recognition in the task, it was studied the effect of the sawdust in the habituation box by taking it out in the following experimental approach. The hypothesis was supported and the performance improved in the absence of the sawdust in the habituation box by showing preference to the relatively novel objects. However, overall recall performance did not differ from chance in both conditions. Other factors at habituation that were changed were the timing for exploration phases that was prolonged to 3 minutes and the position of the habituation object alternated between one of four positions. It may be the case that the increase of exploration times since it may allow to better learning processes, may lead to better recall. The alternation of the object in the habituation box might have had an effect on the motivational state to perform the task and it is suggested that it may improve the accuracy of results.

Following this result, the continuation of testing on the task was appropriate to reach a good level of performance. In doing so, the holding in the home cages of the animals between the two events exploration was not found to affect the performance. When the conditions were as much similar to published work (Eacott et al, 2005), there was some evidence of recall and significant preference to the non habituated object. There was a negative correlation between the rates of exploration at the test phases and the recall data by showing that the greater the exploration was the preference to either object was increased reaching recall performances at chance level. This correlation was found to be an exponential one. However, the correlation observed between recall measures and exploration time of the objects was found exclusively for the last set of 8 days but no correlation was found in previous 16 days of the study. In previous work on the what-where-which task (Chapter 3; Zinkivskay, 2006), it was observed that the rate of objects′exploration showed a progressive decrease for the subsequent object presentation phases. Nevertheless, the time the animals spent exploring objects in the first exploration phases did not predict the turn towards the non habituated object. That study may be slightly different from the present one in the sense that the time exploring the objects was calculated for the sample phases instead of the test phase as used here. Good results on the task were obtained only for 8 days over the total 24 days of performance at the present experiment.

When the procedures were reviewed to match as much as possible those from previous work (Eacott et al, 2005) and good results were obtained in the task, we aimed to improve the performance by introducing some modification of the base task. In this sense, to increase the preferred turnings to explore the relatively novel object, the habituated object was not any longer present at test phases. As opposite, this modification turned into a preference for an empty arm. This preference was novelty based and would rely on the natural behaviour the animals have to explore the novel aspects of the environment (Ennaceur and Delacour, 1988) since an empty arm is a novel event for the experience of these animals in the E maze. It was found that the exploration ratios the empty arm as novelty were increased and the performance as the turnings to either direction drop to chance. The animals were then reinforcing in their turning towards to the empty arm by including a complete novel object in that arm replacing the habituated object. This object was made to be as much interesting as it could be with the aim of reinforcement. The exploration rated of the complete novelty was high and the recall performance was also good. Therefore, the task was found reliable to perform further studies for cued recall. These studies for cued recall incorporate a modification since there was not a habituation to the object but instead it showed a cue (object from previous experiences) after a delay. Increasing delays were tested along with shorter cueing times which all showed good recall performances of the task.

Previous work studied memory retention in the rat when performing the what-where-which, objects hidden version of the task (Chapter 4; Zinkivskay, 2006). This study compared the effect on recall by three conditions differing at the habituation phase by using different delays before and thereafter the habituation to an object for 5 minutes. The first experiment, when the rat was habituated to an object followed by a 15 minutes delay, there was no recall and the preference for either the non habituated or the habituated object was the same. Second experiment, when a delay of 15 minutes preceded the habituation to an object, the performance was affected since recall accuracy was poorer compared to no delay condition although the preference to the non habituated object was significant. The third and last experiment, when an increased delay of 1 hour preceded the habituation to the object, there was no recall of what-where-which combination showing a correlated decrease of the recall measures as the delay between exploration and testing phases before habituation were increased. It was observed that total amount of time the animals spent exploring objects during the first exploration trials was positively correlated with the preference to the non habituated object when there was no delay between first exploration trials and the test phase. In other words, it was observed that as the rate of time spent exploring the objects increased so did the tunrs toward the non habituated object. In other cases, it was found either no correlation (15 minutes delay post-habituation) or a positive trend (15 minutes and 1 hour delay pre-habituation). Furthermore, there was a positive significant correlation when grouping together the data corresponding with no delay and delays pre-habituation. In summary, the delay of 15 minutes after the habituation was found to be sufficient to the objects become equally relatively novel. On the other hand, the increase on the delay previous habituation was found to affect the recall of the past events by decreasing it. This data supported the results observed on the recognition task described by Eacott and Norman (2004) which showed that at delay of 15 minutes the animals were still able to recollect the experiences. The rats were not able to remember the past events after a delay of 1 hour and it was suggested that this observation might have been due to the interference between the two episodes. The rats were not able to remember the past events after a delay of 1 hour and it was suggested that this observation might have been due to the interference between the two episodes. When comparing these data with that obtained by Eacott and Norman (2004) after 1 hour delay, the animals were able to remember the past event which may be explained by the task being simpler. Based on those results which showed that animals were able to remember when a delay of 15 minutes previous habituation, although somehow poorer compared to no delay condition, and that they were not able to remember what-where-which after a delay of hour, it was then interesting to study the performance after delays of up to for example 19 minutes followed by 1 minute cued. It was demonstrated that the animals recalled the past experiences after longer periods of 20 minutes and that cueing for 1 minute produced also good results. Therefore, the cued recall task was demonstrated to be a reliable task to test this aspect of memory. It was interesting to develop this task since it would serve a based task to study memory retention capabilities in situations whereby it is sensibly affected such as the natural decline with age neurodegenerative diseases. Therefore, it may serve as a reliable task to study recovery in an animal model these diseases.

On the basis that these animals would have received an odour-training experience, the hypothesis was then tested that they may have been guided by unintended cues when performing the task. For that purpose, the conditions at test were slightly altered in probe trials in which there were no objects at test, two identical copies of the complete novel object or two same copies of the relatively novel object at test. Evidence was found that these animals may now know that something is different in the E maze at test. In this sense, the recall performance was found to be significantly different from the overall recall performance on a normal training schedule. The same probe trials were run in previous work (Zinkivskay, 2006) and it was then reported that they did not differed from the overall recall performance and that they were significantly above chance although showing lower significance values. Specifically, this study was carried out for the what-where task with objects hidden (Chapter 2; Zinkivskay, 2006). Two probe trials were then performed to control for any odour cues in the E maze. The probe trials were the same as a normal day or training session except for there were no objects at test in the E maze. This study showed that the animals turned toward the non habituated object significantly above chance averaging 61% in the training group (16 trials), the same as in the probe trials. The same test was performed in the what-where-which task for objects visible and hidden (Chapter 3; Zinkivskay, 2006). In this sense, a total of 16 trials were performed for each of the two conditions that was, objects visible or out of sight from the start arm and two probe trials after trials 8 and 17 with objects hidden whereby the objects were not present at test. The performance of 18 trials with objects hidden including the tow probe trials indicated that the animals turned towards the non habituated object significantly above chance showing an average of 66%. There was no difference in the performance between the training group and the probe trials. Therefore, it would be interesting to test the recall performance of non odour trained animals on the task to see whether the recall performance may or may not be different.

5.2: Conclusion

Various aspects of the behaviour have been described that could have affected the related performance to an episodic-like memory paradigm such as the effect of the age or the experience of the animals. The episodic-like memory task was found to be affected by the fornix damage specifically in recall of what-where-which (Eacott and Easton, 2007). However, since the fornix is a main afferent to the hippocampus it may be possible that impairment in memory could be due to a dysfunction in memory for what-where or spatial memory. With the aim of understanding the cognitive contribution of the fornix to memory aspects, the performance on a control task was studied that was very similar to the what-where-which task (Eacott et al, 2005) except that the contextual background was irrelevant and only what-where memory was studied. With the aim of understanding the neural basis of episodic memory, the control task for spatial memory was performed and it did not work. Therefore, we had to return to the what-where-which task which has been demonstrated to work well. However, this task did not work either, what brought us to the understanding of the aspects in the procedure that did not match previous published work and may affect the poor performance. Removal of sawdust may have improved the performance on the task. With the aim of developing the results of the task, it was found that an empty arm was a preference site of exploration since, based on the natural tendency to explore the novel aspects of the environment (Ennaceur and Delacour, 1988), the animals would seek this arm and that the incorporation of a complete novel object in this arm would show good results. The evolving of a cued recall task based on this paradigm showed the rats capability to remember the episodes after periods of 20 minutes and when the cue was present for 1 minute exclusively. However, this performance may be the result of odour detection rather than memory. Therefore, it would be interesting to test the recall performance on the task by animals that have not been habituated in the presence of sawdust which may lead to the animals being more interested in smells. When non odour trained animals solve the task by using memory for what-where-which, it would be interesting to study the contribution of the fornix and to compare with the control spatial task. It would be interesting to investigate in the task other related areas to episodic memory such as the mamillary bodies and medial thalamus and/or to study how the prefrontal cortex may be somehow related to the efficient encoding of information for subsequent recall.

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