A new test of object-recognition memory for rats

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Abstract

Object-recognition memory has been assessed in rats using the delayed non-matching-to-sample (DNMS) task and the novel-object preference (NOP) test. The DNMS task provides an accurate measure of rats' object-recognition abilities, however, the conventional procedures are not practical because rats require extensive training to reach peak performance and object-recognition memory can only be assessed using retention intervals of up to a few minutes. The NOP test does not require rats to be trained in advance, and for this reason it has become widely popular as a test of object-recognition memory. Recent findings, however, question the internal validity of the NOP test, namely its assumption that the strength of novelty preference corresponds directly to the strength of the memory for an object. The goal of the present study was to develop a new test of object-recognition memory. The new method incorporates the appetitive-reward and explicit choice aspects of the DNMS task and it employs a circular-track apparatus that has been previously used in a modified NOP test. Rats' performance on the new task was similar to the levels of accuracy reported on conventional DNMS tasks but were achieved in far fewer trials than conventional DNMS tasks. When the delay was increased, performance decreased slightly but remained significantly above chance. Additionally, we compared rats' performance on the new task to their scores on the NOP test and did not find a consistent linear relationship. The results from this new task confirm its utility as a test of object-recognition memory in rats, while challenging the assumption that the strength of novelty preference on the NOP test corresponds to the strength of memory for an object.

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Introduction

Visual-recognition memory consists of the ability to discriminate previously encountered stimuli from novel ones. This type of memory is impaired in human amnesic patients. The refinement of visual recognition tests for nonhuman animals has played a fundamental role in developing animal models of human amnesia (Mumby, 2001). Two prominent tests of object recognition developed for nonhuman animals are the delayed matching-to-sample (DMS) task and the delayed nonmatching-to-sample (DNMS) task. A trial on each task consists of two phases—a sample phase and test phase. On the sample phase, the animal is presented with an object (referred to as the sample) and is provided a food rewarded for displacing it. Following a brief retention interval (lasting seconds) the animal receives the choice test. On the test, the sample is presented alongside a novel object. On the DMS task, the animal is rewarded for displacing the sample object; conversely, on the DNMS task, the animal is rewarded for displacing the novel object. On both versions of the task, a performance criterion is set at the beginning of training to ensure the animal learns the reward contingency. The dependent variables are the number of trials required to reach a performance criterion, and the mean percent of correct choices across trials. On both versions of the task, accurate performance on the test relies on the ability of the animal to recognize the sample object.

Research conducted in the 1950's and 1960's revealed that humans with damage to the medial temporal lobes suffered from amnesia (Scoville & Milner, 1957). Patients had impairments in the conscious recollection of information relating to facts and events—a type of memory that is now referred to as *explicit* memory. Conversely, memory for information that did not require conscious recollection, *implicit* memory, was

spared in these patients. Examples of this type of memory are: procedural learning, priming, and classical conditioning. Moreover, one of the features of medial temporal lobe amnesia was a sparing of short term memory; whereas memory for information over longer retention periods was impaired. (Duva, Kornecook, & Pinel, 1999, p. 198; Squire & Zola-Morgan, 1991).

The major structures in the medial temporal lobe are the hippocampus (HPC), amygdala, and rhinal cortex (i.e., entorhinal and perirhinal cortices). Early attempts at developing animal models of human amnesia focused on surgical lesions made to either the HPC or the HPC in conjunction with the amygdala (Duva, Kornecook, & Pinel, 1999, p. 200). The focus on the HPC was due to findings that indicated there was a correlation between the extent of HPC loss and severity of memory impairments in patients suffering from medial temporal lobe amnesia (Scoville & Milner, 1957; Milner, Corkin, Teuber, 1968).

In the 1970's the first successful animal models of brain-damage-produced amnesia emerged as the result of changes made to the procedures used on tests of visual-recognition memory in nonhuman primates (Gaffan, 1974; Mishkin & Delacour, 1975; Mishkin, 1978). Formerly, only small sets of object stimuli were used on the DMS and DNMS task, which over several trials, all became familiar. Thus, the design of the tasks was ultimately an assessment of the ability to discriminate between the recency in presentation of familiar objects, which was much more difficult than recognizing a previously encountered item (Mumby, 2001). As a result, normal performance on the tasks was poor, and it therefore provided an insensitive baseline against which to compare the effects of experimental brain lesions.

Implementing the use of trial-unique stimuli (i.e., a different sample and novel object on each trial) revealed that nonhuman primates could in fact perform at very high levels of accuracy and required significantly fewer trials to master the task at brief delays. For example, nonhuman primates trained with trial-unique stimuli reached an average score of 90% correct choices in 90 trials as compared to 62% correct choices in 1000 trials for those trained with a single pair of objects (Mishkin & Delacour, 1975).

The introduction of longer delays between the sample phase and test phase, as well as using trial-unique stimuli, revealed that nonhuman primates could perform well at delays lasting up to several minutes as compared to only a few seconds when using recurring objects on the previous version (Gaffan, 1974). One experiment assessed nonhuman primates' performance on a DMS task using trial-unique stimuli. Nonhuman primates were trained to reach a performance criterion of 81 correct choices (selecting the sample) out of 90 trials at a short delay (10 s). Following training, they received additional testing at a 70-s and 130-s delay. Nonhuman primates were able to reach an average number of 94% correct choices on the task at both the 70-s and 130-s delay (Gaffan, 1974).

Comparing nonhuman primates' performance on the DNMS and DMS tasks revealed that the *non*matching-to-sample principle rather than the matching-to-sample principle, led to more successful performance on the task. For example, one group of nonhuman primates trained on a delayed nonmatching-to-sample (DNMS) task, reached the performance criterion in half the number of trials that it required a different group to reach that were trained on a DMS task (Mishkin & Delacour, 1975). The innate tendency of nonhuman primates to select the novel object when presented alongside a familiar one

(the sample) likely contributed to the faster acquisition of the task (Mishkin & Delacour, 1975).

In 1978, Mishkin developed the first animal model of human amnesia using trial-unique stimuli on a DNMS task. Nonhuman primates with combined surgical lesions to the HPC and amygdala, but not separate lesions made to each structure, were found to be severely impaired on the task. Additionally, this observed impairment was delay-dependent. Nonhuman primates with combined lesions to the HPC and amygdala were able to reach a performance criterion when the delay between the sample and test phase was short (10 s) but they were impaired when the delay was increased to 30, 60, and 120-s (Mishkin, 1978). The results from this study, along with results from other experiments, led to the conclusion that the HPC and amygdala equally contributed to object-recognition memory (Murray & Mishkin, 1984; Zola-Morgan & Squire, 1985).

The observed delay-dependent impairment on the object recognition tests for nonhuman primates was similar to the recognition impairments observed in human patients who had suffered from medial temporal lobe damage; patients' performance was normal on recognition tests when the retention interval was a few seconds, but decreased when the retention interval increased to several minutes (Squire & Zola-Morgan, 1991; Zola-Morgan & Squire, 1985). The tasks developed for nonhuman primates appeared to assess the similar types of memory abilities affected by temporal lobe damage, while sparing memory for procedural learning, which was not affected by damage to the medial temporal lobe (Squire & Zola-Morgan, 1991).

In order to confirm that patients with medial temporal lobe damage would be impaired on the same recognition tasks provided to nonhuman animal models of amnesia,

one group of researchers compared amnesic patients' performance on the same object recognition task that was given to nonhuman primates. Amnesic patients and control participants were tested using the nonrecurring items delayed-nonmatching-to-sample task. Following extensive training, amnesic patients' performed similarly to control participants on the task at a 5-s delay but were impaired at delays lasting 15 and 60 s (Squire, Zola-Morgan, & Chen, 1988).

Research conducted over the next decade revealed that the observed impairments in nonhuman primates on the DNMS task following combined lesions made to the HPC and amygdala were the result of incidental damage made to the rhinal cortex (i.e., the entorhinal cortex and perirhinal cortex) during the surgical removal of the HPC and amygdala (Duva, Kornecook, & Pinel, 1999, p. 204). Nonhuman primates that received lesions to the rhinal cortex (i.e., entorhinal and perirhinal cortices) were found to be impaired on the DNMS task (Meunier, M., Bachevalier, J., Mishkin, M., & Murray, E.A., 1993; Suzuki, W.A., Zola-Morgan, S., Squire, L.R., & Amaral, D.G., 1993), whereas select lesions made to the HPC and amygdala did not result in impairments (O'Boyle, Murray, & Mishkin, 1993).

Research conducted on rats confirmed that only mild impairments were observed on the DNMS task following combined HPC and amygdala lesions (Mumby, Wood, & Pinel, 1992), and that lesions made to the rhinal cortex produced delay-dependent impairments similar to those observed in nonhuman primates (Mumby & Pinel, 1994). Today, most researchers agree that the HPC plays a limited role in object-recognition memory (Mumby, 2001) whereas, the rhinal cortex, particularly the perirhinal cortex, plays an essential role in object-recognition memory (Murray & Richmond, 2001).

The first successful adaptation of object recognition tasks used with nonhuman primates for use with rodents began in the 1980's. One of the first tasks developed was a DNMS task employing a Y-maze apparatus (Aggleton, 1985). The three arms of the Y-maze apparatus were separated by a guillotine-like door placed in the center of the maze. One arm was designated the start box, while the other two were designated goal boxes. The stimuli used on the task were forty different pairs of goal boxes. These boxes differed in their visual and tactile properties, each pair containing an identical object. A hole at the back of each goal box made it possible to deliver a food reward to the rodent after it had made its choice.

A session in the Y-maze consisted of placing the rat in the start box and raising the guillotine-like door. Two identical goal boxes were presented (e.g. A1 and A2) and the rat was rewarded with food pellets for selecting one of the two. Selection of a goal box was defined as the rat placing all four paws in the arm of the maze containing the goal box. The rat was then contained in the goal box it had selected (e.g. A1) for 20 s while the experimenter removed the start box and the second goal box, attached a novel goal box (B1) to one arm and re-attached the second copy of the sample goal box (A2) to the other arm. The location of the second sample goal box (same arm vs. different arm) was randomized. When the guillotine-like door was raised, the rat was rewarded only if it chose the novel goal box (B1).

Unlike conventional procedures used to test nonhuman primates, the trials in this DNMS task proceeded sequentially such that the novel goal box on the first trial became the sample goal box on the second trial (e.g. B1 now became the sample object). Rats received ten trials per day accordingly to the method described earlier. The performance

criterion was 80% correct choices on five consecutive days (more specifically, at least 40 correct choices out of 50). Rats required a mean number of 130 trials to reach the criterion with no delay. When the delay was increased to 20 and 60 s, rats' performance did decrease but remained significantly above chance. This study provided evidence that rats, like nonhuman primates, could perform well on a DNMS tasks.

Rothblat and Hayes (1987) developed a rodent-based DNMS task that more closely matched the tasks used for nonhuman primates (see Figure 1). Their apparatus consisted of an elevated platform with two recessed food wells at one end of the apparatus and a start area at the other. Objects could be placed over the food wells, and could easily be displaced by the rat. At the beginning of a trial, the rat was retained in the start area by a door that could be raised up and down by the experimenter. Once the door opened, the rat could run down the platform and displace a single object (referred to as the sample object) placed over a food well for a food reward. Following the displacement of the sample object, the experimenter returned the rat to the start area where it remained during the retention interval which lasted either 10, 30, or 70 s. Following the delay, the door was opened and the rat began the choice test. The rat was presented with the sample object and a novel object, each over a food well, and the rat was rewarded if it displaced the novel object first.

Rats' mean level of accuracy on this task at a 10-s delay was 75%. When the delay in the start area was increased to 30 and 120 s, accuracy decreased to 70% and 63%, respectively (Rothblat & Hayes, 1987). This delay-dependent decrease was similar to the results obtained in previous studies with nonhuman primates; however, the mean level of correct responses for the rats was much lower.

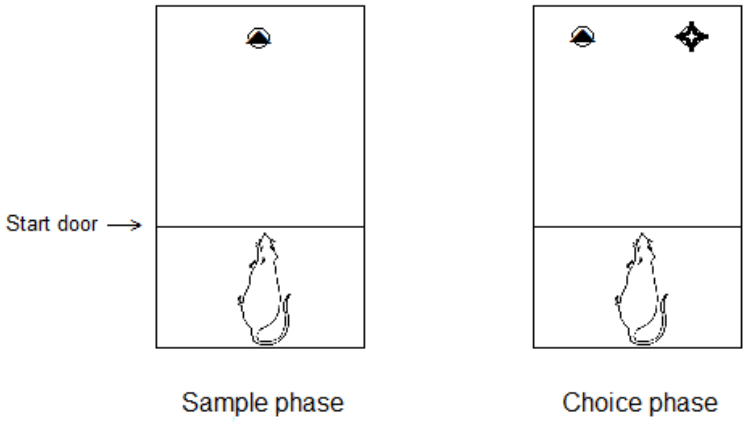

Figure 1. The apparatus developed by Rothblat & Hayes for testing rats on delayed nonmatching-to-sample. On the sample phase, a single object was placed over one food well and the rat was rewarded for displacing it. On the choice phase, the sample object and a novel object are each placed over a food well. The rat is rewarded for displacing the novel object.

To achieve still more comparable levels of accuracy with nonhuman primates on DNMS tasks using rats, researchers modified the training procedures and design of the apparatus (Kesner, 1993; Mumby, Pinel, & Wood, 1990). The DNMS paradigm developed by Mumby et al. (1990) implemented the use of a redesigned apparatus referred to as the 'nonrecurring-items delayed nonmatching-to-sample' apparatus. The apparatus consisted of an elevated rectangle-shaped platform with two recessed food wells located at each end of the platform (see Figure 2). An opening next to the food wells provided the experimenter with access to place objects over the food wells and to remove them. Two guillotine-like doors were located in the middle of the platform. The purpose of the doors was to allow the experimenter to control the rat's access to different parts of the apparatus by manually raising and lowering them. Additionally, implementing the use of doors removed the need for the experimenter to handle the rat within and between trials.

A trial consisted of two phases—a sample phase and a choice phase. At the beginning of a trial, the experimenter places two different objects over food wells at opposite ends of the apparatus. The researcher opens one of the doors and the rat is rewarded for displacing the object. This object is referred to as the sample for the trial. The rat returns to the middle of the apparatus and the second door remains closed for the retention delay (which can last several seconds to several minutes). During this period, the researcher places the sample object next to the other object at the opposite end. At the end of the retention interval, the experimenter opens the second door, and the rat chooses an object. If the rat displaces the novel object, it receives a food reward.

A required feature of the DNMS task is a strict performance criterion; in this case 84% correct choices (i.e., choosing the novel object at choice) on two consecutive sessions (21 correct trials out of 25). These higher performance criteria are essential as they make it possible to detect even slight impairments in performance due to a treatment (e.g. surgical lesion; Mumby, personal communication, July 2012). Predictably, these performance levels are achieved only through extensive and time-consuming training.

Following the modifications made to existing DNMS tasks for rats, they were able to reach high levels of performance on the DNMS task at short delays comparable to those achieved with nonhuman primates. For example, in some experiments, rats were able to reach average scores of 90% at a 4 and 15-s delay (Mumby et al., 1990; Mumby & Pinel, 1994). Although performances dropped when the delay was increased to 60 s (81%) and 120 s (77%), they were still above the average for previous findings in rats.

Results from experiments assessing performance on the DNMS task following lesions made to structures implicated in object recognition memory demonstrated that the task could be used to detect impairments in object recognition memory in rats. In one experiment, rats were trained on the DNMS task and were then tested at several delays ranging from 4 to 600 s. Afterwards, rats received rhinal cortex lesions (i.e., entorhinal and perirhinal cortex) and were tested again at the same delays. Rats' performance at the 4-s delay was similar to their performance prior to surgery, whereas their performance at longer delays was significantly impaired (Mumby & Pinel, 1994).

Although the DNMS task provides a relatively precise measure of rats' object-recognition abilities, the current DNMS procedures for rats each have drawbacks. Rats require several weeks, and hundreds of trials, to reach peak performance. For example,

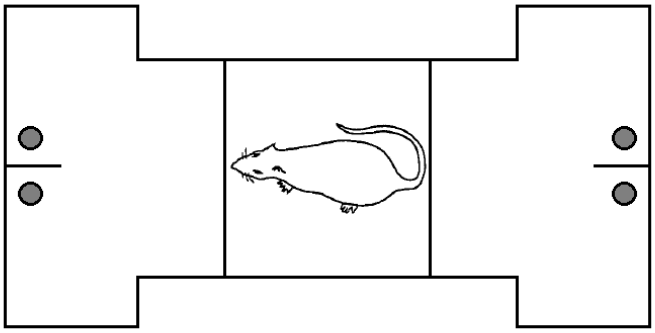

Figure 2. The nonrecurring-items delayed nonmatching-to-sample apparatus developed by Mumby, Pinel, & Wood, 1990.

the rats in the Rothblat & Hayes (1987) study required an average of 177 trials to reach the 75% correct performance criterion over three consecutive days using a 10-s delay. Moreover, the modified versions of the DNMS tasks, which have led to improvements in the levels of accuracy on the task, require giving rats hundreds of trials, ranging from 200-400, to reach these high levels of performance at short delays (Mumby et al.,1990; Mumby, Pinel, Kornecook, Shen, & Redila, 1995; Clark, West, Zola, & Squire, 2001). Even at peak performance, rats do not perform accurately when the retention interval is more than a few minutes. In one study rats received extensive training at a short delay and were able to reach very high levels of accuracy (90%). When the delay was increased to 10 min, performance dropped to an average of 57% correct choices on the test (Mumby et al., 1990).

DNMS tasks are difficult to administer and require experienced experimenters. So close to the test subjects, the experimenter must be mindful of making any movements and sounds that could distract the animal. Without realizing it, an experimenter could also unknowingly deliver cues to the rat as to which object will be rewarded on the choice test (e.g. a slight body movement in anticipation of the rat making a correct choice on the test or leaving odour cues on the objects as a result of touching them between the sample phase and choice phase) (Mumby, 2005, p.385).

Due to the challenges faced employing the DNMS task for rats, researchers have generally abandoned it in favour of the *novel-object-preference* (NOP) test. The NOP test takes advantage of rats' spontaneous bias to explore novel objects more than familiar ones when both are presented in a familiar environment (Ennaceur & Delacour, 1988; Besheer & Bevins, 2000). On conventional versions of the NOP test, a rat is presented

with two identical copies of a sample object in an open field arena and is allowed to explore them for a designated amount of time. The rat is then removed for a retention delay. When the rat is placed back into the arena for the test, the arena contains a copy of the sample object and a novel object. Rats tend to spend more time exploring the novel object relative to the sample object. This bias towards the novel object suggests that the rat recognizes the sample object. Normal rats display novel-object preferences on tests following delays lasting several minutes to hours (Clark, Zola, & Squire, 2000; Ennaceur & Delacour, 1988 Ennaceur & Aggleton, 1994).

When a rat does not display a novelty preference on the test, it is interpreted that the rat has an object-recognition memory impairment. However, a treatment may eliminate a novelty preference for reasons unrelated to memory failure. For example, the treatment may alter or suppress a rat's natural exploratory bias for novel objects (Mumby, 2001). The number of potential factors is compounded by the fact that the NOP test does not involve a goal, and thus rats are not required to make an explicit choice response based on memory. One study examined rats' performance on the NOP test following lesions made to the perirhinal cortex (Mumby, Glenn, Nesbitt, & Kyriazis, 2002). On the test, rats with perirhinal cortex lesions did not display a novelty preference, and surprisingly showed a significant preference for the sample object. The results suggested that rats with perirhinal cortex lesions were capable of recognizing the sample object, because if they were unable to do so, then they should have exhibited an equal preference for both objects on the test because both would have been equally unfamiliar.

The type of objects used in the study can also influence the behaviour of the rat. For example, a discrepancy in the features of the sample and novel objects may result in a

biased preference for one over the other. Rats may prefer a shorter object simply because they can climb on it. If objects are not properly counterbalanced between groups, it can produce observed preferences unrelated to recognition memory that are in fact merely a result of object features (Ennaceur, 2010).

There is no general consensus on the method used to report results obtained on the NOP test. When two or more groups of rats are tested, the dependent measure can be reported in two ways. One method is to compare a group's average score to what would be expected by chance. Given there are two objects on the test, 50% of time spent with an object is considered chance. The dependent measure is a score based on the difference in amount of time spent investigating the novel object relative to total time spent investigating both objects on the test. A group score significantly above chance indicates that, on average, the group spent more time investigating the novel object (indicating they recognized the sample object). Another method is to compare the average score of each group to one another (e.g. treatment group vs. control group). If a significant difference is observed between group scores, then the group with the lower mean score is presumed to have an object-recognition memory impairment. However, reporting results using the latter method can be misleading. For example, in one study (Clark, Zola, & Squire, 2000), researchers assessing the effects of hippocampal damage on object recognition memory reported the HPC damage group was impaired on the test relative to the control group. Although the HPC damage group had lower scores, they were still significantly different from chance, indicating the rats had successfully discriminated between the novel and sample object. Furthermore, reporting scores on the NOP test as values indicative of the strength of a memory may not be appropriate because there is a lack of

evidence that higher novelty preference scores are necessarily an indication of better strength in memory of the sample object.

Presuming that rats encode object features while investigating them, one would predict an increase in time spent investigating objects should indicate greater encoding of object features. Therefore, the amount of time spent investigating objects on the sample phase should be a predictor of strength in memory for an object. One study examined the relationship between time spent investigating objects and NOP test performance (Gaskin et al., 2010). Rats were allowed to investigate a sample object for different amounts of time: 5, 30, 60, 90, or 120 s, and were tested 3 hours later. The rats in the first three groups failed to show a novel-object preference on the test, whereas the latter two groups displayed a significant preference. The lack of a linear relationship between the amount of time spent investigating sample objects and subsequent novelty preference suggests the latter may not truly represent strength in object recognition memory. This finding raises concerns about the validity of assuming that stronger preferences on the NOP test are indicative of stronger memory for the sample object.

Considering the drawbacks of the current procedures used on DNMS tasks and the interpretational problems of the NOP test, the goal of the present study was to develop a new method for testing object-recognition memory. The new method incorporates the appetitive reward and explicit choice aspects of the DNMS task, and makes use of a circular-track apparatus previously used in a modified NOP test (Piterkin, Cole, Cossette, Gaskin, & Mumby, 2008). The circular-track apparatus was designed with the aim to reduce constraints on natural exploratory behaviour when testing rats on their novel-object preference. When rats explore in their natural environment they travel

from one location to another, with a tendency to proceed to new locations rather than revisit ones they have recently investigated. The design of the circular track provides rats this opportunity, unlike conventional open field arenas used on the NOP test which may constrain rats' natural exploratory behaviour (Mumby, 2005, p.389). Considering the NOP test and DNMS task capitalize on rats' innate exploratory bias, it is essential this behaviour not be constrained or prevented.

The current paradigm, in brief, is similar to conventional DNMS paradigms. Objects are placed over food wells in different areas of the circular track. A session on the task consists of two phases—a sample phase and a test phase. On the sample phase of a session, the rat traverses the track, encountering four different pairs of identical objects (sample objects) and is provided a food reward for displacing them from over food wells. The rat is free to investigate the objects as much or as little as it chooses. For the test, one sample object in each pair is replaced with a novel object, the rat again traverses the track, now it receives a food reward each time it displaces a *novel* object from over a food well. The dependent measure is the relative performance (selecting the novel object first in each pair of objects) across trials. Thus, like the conventional DNMS tasks, this procedure provides an estimate of a rat's recognition abilities based on several trials, each of which involves an explicit choice response.

An essential feature of this new task, the *Circular-track delayed nonmatching-to-sample* (DNMS) task, is that the experimenter does not need to be present in the room while testing the rat. This removes the issues of distracting the rat or providing unintentional cues to it, both of which are a possibility when using conventional DNMS apparatuses. Additionally, on the sample phase, the rat encounters two identical sample

objects rather than the one object encountered on conventional versions of the DNMS task. This increases the opportunity to investigate the sample object, and greater encoding of the sample object.

The design of conventional DNMS apparatuses may hinder natural behavioural responses to novelty. Considering novelty preference is an exploratory bias necessary for good performance on the DNMS task, it may be one factor as to why rats require so many trials to learn the task and to reach high levels of performance. Other procedural factors such as having the experimenter in the room distracting the animal during the delay and providing the rat with only a brief exposure to the sample object on the sample phase may contribute to the marked decrease in performance as the delay increases.

The present DNMS paradigm addresses the confounds in the current procedures, and thus may lead to rats acquiring the task faster and maintaining higher levels of performance as delays increase. We tested rats' performance on the Circular-track DNMS task at three retention intervals: 90, 180, and 300 s. We predicted rats to reach a similar level of performance as those observed on conventional DNMS tasks; however, we predicted rats would reach this level of performance in far fewer trials.

We also tested rats' performance on the NOP test using a 180-s delay. We predicted rats' performance on the test would be significantly above chance based on previous research using the NOP test at short delays. Additionally, assuming performance on the NOP test is a valid and reliable indicator of rats' object recognition memory, and given the Circular-track DNMS task accurately measures strength in memory for a familiar object, we predicted a positive linear relationship to exist between scores on the

NOP test and scores on the Circular-track DNMS task. In order to test this hypothesis we calculated Pearson correlation coefficients on the scores obtained on both tests.

Method

Subjects

Subjects were 6 male Long-Evans rats (Charles River, St. Constant, Quebec), approximately 32 weeks old at the beginning of the experiment. Rats were housed in pairs under a 12:12 light-dark cycle, with light onset at 8:00 p.m. Rats received a daily ration of food (20-25g) and had continuous access to water. All procedures were approved by the Concordia University Animal Care and Use Committee, and were in accordance with the guidelines of the Canadian Council on Animal Care.

Apparatus

Circular-track delayed nonmatching-to-sample task. The apparatus had a diameter of 270 cm with a floor width of 45 cm. The height of the inside and outside walls of the track was 40 cm. The floor of the circular track was covered with woodchip. The circular track was separated into nine compartments by divider walls, with seven equal-sized compartments and two smaller compartments designated as the "start" and "stop" compartments (see Figure 3). The divider walls had small doors (10 x 10 cm) which opened such that the rats could only circumnavigate the apparatus in a counterclockwise direction. Once a rat entered a new compartment it could not return to the previous compartment. All the divider walls had a door, except for the one dividing the start and stop compartments. Once a rat reached the final compartment, it was removed from the apparatus.

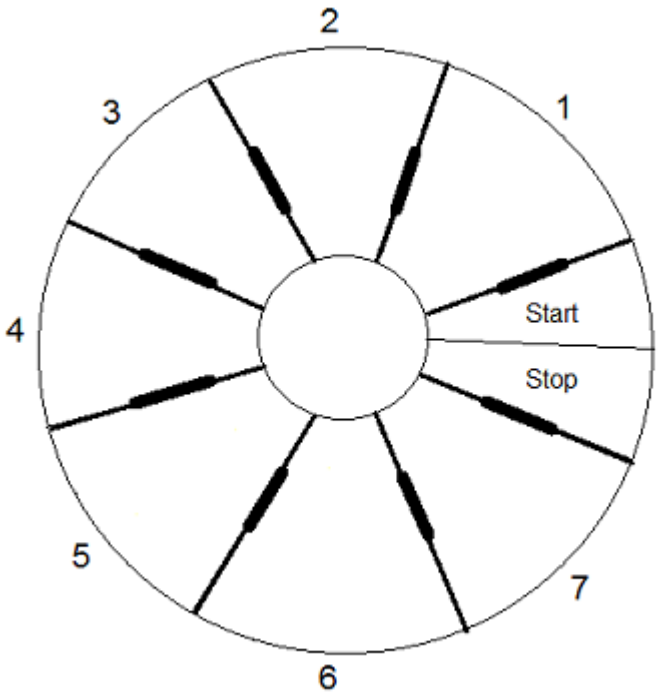

Figure 3. The circular-track apparatus. The circular track was separated into nine compartments by divider walls; seven equal sized compartments and two smaller ones designated as the “start” and “stop” compartments. The divider walls had small doors which opened such that the rats could only traverse the apparatus in a counterclockwise direction.

A removable rectangular platform (30 cm x 12 cm x 1 cm) which contained two recessed food wells (20 cm apart) was placed in the center of each compartment, with the exception of the "start" and "stop" compartments. Objects were placed over the recessed food wells on the platform. A total of 176 different objects were used as stimuli in the DNMS task. Objects were made of plastic, metal, glass, or glazed ceramic. Each object was large enough to cover the food well but light enough to be easily displaced by a rat. The objects ranged in size from 4 to 18 cm in height, and between 4 and 13 cm in width. There were three identical copies of each object; two were used during the sample phase and one was used during the retention test as a copy of the sample object. The sample and test objects were randomly paired together. At the end of each day, the objects were cleaned with a 70% ethanol solution. A video camera was positioned above the apparatus to record the sample phase and retention test.

Novel-object-preference test. The NOP testing was conducted in an open field arena (60 cm ×70 cm ×70 cm) constructed of gray PVC plastic. A stainless-steel tray covered with woodchip served as the arena floor. A video camera was placed over the arena. The familiarization and test phases were videotaped for later analysis.

A total of 6 different objects were used as stimuli and were made of glass or glazed ceramic. They ranged from 5 cm to 15 cm in height, and between 6 cm and 10 cm in width. There were three copies of each object which were used interchangeably. Two were used during the familiarization phase and one was used during the retention test. A small glass jar (6 cm high) was attached to the bottom of each object with epoxy. The glass jar could then be screwed into lids which were attached to the stainless-steel tray. The objects were positioned 27 cm from opposing corners of the rectangular arena. The

objects were washed with a 70% ethanol solution at the end of the day. Each object pair had been previously screened for preference by a different group of rats in a non-choice test.

Behavioural Procedure

Circular-track delayed nonmatching-to-sample task

Habituation. The rats were handled daily for ten minutes for a two week period prior to habituation. During the habituation sessions, a rat was placed in the track apparatus and allowed to circulate the track in one direction while being provided the opportunity to collect sunflower seeds from the unobstructed food wells in each compartment. On the first three days of habituation, the rat was required to make at least one trip around the track or spend a minimum of ten minutes in the apparatus. Following the first three days, rats were required to make two trips around the track. No objects were placed in the track during habituation. Rats required a total of sixteen habituation sessions.

Training stage 1. A training session consisted of two phases: a sample phase and a test phase. Both the sample and test phase consisted of one trip around the track. Objects were encountered in six of the seven compartments (compartment 2 through 7). Thus, each compartment was treated as a single trial. On the sample phase, the rat traversed the track encountering twelve identical copies of a sample object, two in each compartment. A seed was placed in each food well, and the rat was allowed to displace the object in order to retrieve the seed until it reached the "stop" compartment. On the test phase, one sample object from each compartment was replaced with a novel object. This time, a seed was placed in the food well under the novel object only (see Figure 4).

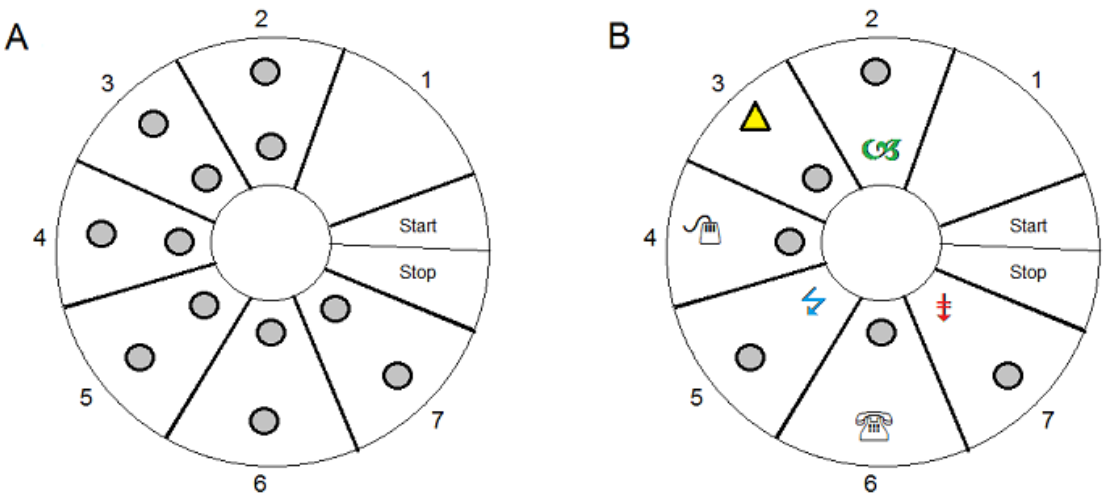

Figure 4. Training stage 1 of the Circular-track delayed nonmatching-to-sample task. On the sample phase, rats encountered twelve identical copies of a sample object (A). On the test phase, one copy in each compartment was replaced with a novel object (B).

Different sample and novel objects were used for each session. For the first training session, the objects partially covered the food well (the seed was exposed) and as sessions continued the object gradually covered the food wells. A correct choice was scored as displacing the novel object first on the test phase. By the fourth training session, objects completely covered the food wells. From this session onward, a rat moved to the second stage of training once it reached a performance criterion of at least 83% of trials correct in four consecutive sessions (20 out of 24 trials correct).

Training stage 2. The second training stage was similar to the first, except the rat encountered two distinct sample objects while traversing the track. In compartments 2, 3, and 4 the rat encountered pairs of one sample object and in compartments 5, 6, and 7 the rat encountered pairs of a second sample object. On the sample phase, one seed was available to the rat in each compartment, placed randomly under one of the sample objects. On the test phase, the seed was placed under the novel object in each compartment. The performance criterion remained the same as the first training stage; at least 83% of trials correct in four consecutive sessions (20 trials out of 24 correct).

Acquisition of delayed nonmatching-to-sample. During the final training stage, rats encountered four distinct sample objects while traversing the track. Four of the seven compartments contained objects, with every second compartment being empty. Objects were only encountered in compartments 1, 3, 5, and 7 (see Figure 5). A session consisted of a sample and test phase. On the sample phase, a rat made one trip around the track to familiarize itself with four distinct sample object pairs. One seed was placed under one of the sample objects in each compartment. During the test phase, both sample objects that were in the compartments during the sample phase were replaced with a copy of the

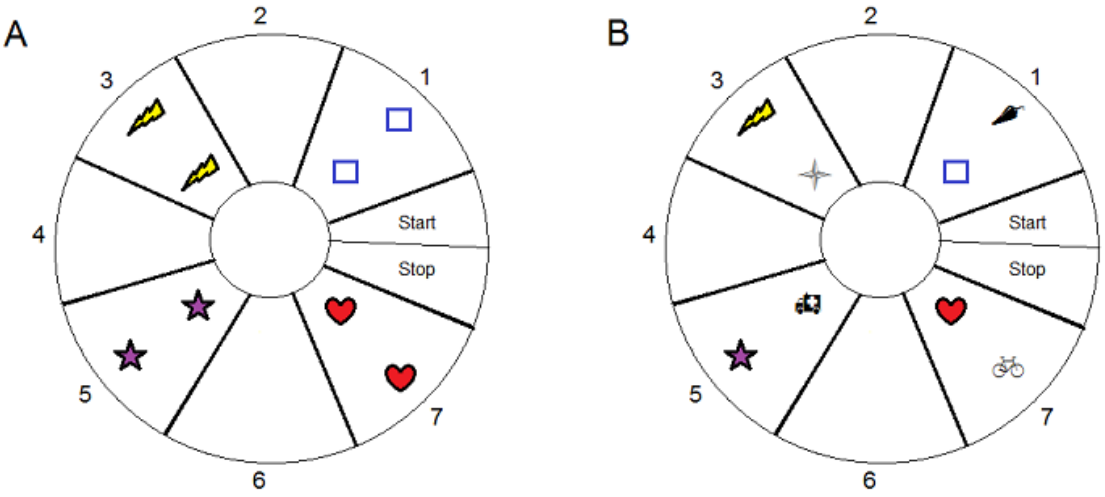

Figure 5. Acquisition phase of the Circular-track delayed nonmatching-to-sample task. On the sample phase, a rat encountered four distinct sample objects (A). On the test phase, both sample objects in each compartment were replaced with a copy of the sample object and a novel one (B).

sample object and a novel object. A seed was placed under the novel object in each compartment.

Each session consisted of four trials (as there were four distinct sample objects in the apparatus). A rat was required to reach a performance criterion of at least 80% of trials correct in five consecutive sessions (16 trials correct out of 20). The shortest delay between the sample and retention test was 90 s (the minimum amount of time necessary to replace each object and re-bait the food wells in the apparatus). During the delay, the rat was placed in a large bin (75 cm x 48 cm x 37 cm) located outside the testing room.

Once a rat met the performance criterion at the 90-s delay, the delay between the sample and test phase was increased to 180 s and then 300 s. At the longer delays, the rat was required to reach the same performance criterion as the 90-s delay (16 trials correct out of 20 trials) or receive a maximum of twenty five sessions at the 180-s and 300-s delays. The same objects served as the sample objects and novel objects for all rats. The location of the seed in each compartment on the sample phase and test phase was counterbalanced in a pseudorandom order. Rats received one to two sessions per day and no fewer than five days per week.

Rats that did not reach the performance criterion at the 180-s or 300-s delay received additional sessions at the 90-s delay or 180-s delay, respectively. The purpose of the additional sessions was to remove side preferences (i.e., consistently selecting the object on the left or on the right) that appeared during training at the longer delays.

Testing the retention function. Testing consisted of measuring rats' recognition abilities at each delay, in a mixed fashion following the completion of training at the three delays. This additional phase of testing was added to account for practice effects

which may have occurred during the training phase. Additionally, it was necessary to ensure that any observed deficit for an individual rat at a longer delay was the result of the task becoming more cognitively demanding and not simply because the animal did not remember the non-matching rule. Rats received seven sessions at each delay in the following types of order: (90, 180, 300, 300, 180, 90, 90, 180…) and (90, 180, 300, 90, 180, 300…). Rats received two sessions per day with an inter-session interval of two hours.

Novel-object-preference test

Rats were habituated to the open field arena for ten minutes a day for two consecutive days. Two identical objects were present in the open field arena during habituation. These objects were not used on subsequent experimental trials. Twenty four hours following the last habituation session, rats received their first trial. A trial consisted of a familiarization phase and a test phase. For the familiarization phase, a rat was placed in the open field arena and allowed to explore two identical sample objects for five minutes. Following a 180-s retention interval, the rat was returned to the arena which then contained a copy of the sample object and a novel object, and the rat was allowed to investigate for five minutes. One of the objects in each pair was designated the sample for half the rats and the other object was the sample for the other half of the rats. The side in which the novel object appeared on was counterbalanced between rats and across trials for an individual rat.

Each rat received three trials at the 180-s retention interval. Trials were conducted on different days during a three week period. Different object pairs were used for each trial, but the same object pair was used for all rats on corresponding trials.

The rats were considered to be investigating an object if their head was 4 cm away from the object and oriented towards the object, or away from the object at no more than a 45º angle. A rat standing on its hind legs and touching the object with at least one forepaw was also considered to be investigating. Climbing or sitting on top of an object was not considered investigation. The main dependent measure was the investigation ratio. This ratio compares the total object investigation time to the time spent with the novel object during the test phase (Ratio = [Tnovel/ (Tnovel + Tsample)]. To determine whether rats' discriminated between the objects, a one-sample *t*-test ($p < .05$) was used to compare mean investigation ratios to chance level of investigation (i.e., a ratio of 0.50). A ratio that was significantly above 0.50 indicated the rat spent more time investigating the novel object.

Testing for each behavioural task was performed in a counterbalanced fashion. NOP trials were conducted during the same period as testing for the DNMS retention function sessions. Half of the rats received an NOP trial first while the other half received a session on DNMS first. Testing on both the NOP test and DNMS tasks never occurred on the same day for any individual rat.

Results

Circular-track Delayed Nonmatching-to-Sample Task

Training stage 1. The mean number of sessions rats' required to reach the performance criterion of at least 83% of trials correct in four consecutive sessions (20 trials correct out of 24) was 3.3 sessions (*SEM* = .71) (excluding criterion sessions). The range of the number of sessions was 2-6.

Training stage 2. The mean number of sessions rats' required to reach the performance criterion of at least 83% of trials correct in four consecutive sessions was 2 sessions (*SEM* = .73) (excluding criterion sessions). The range of the number of sessions was 0-5.

Acquisition of delayed nonmatching-to-sample. Figure 6 represents the mean level of performance during the first and last five sessions at each delay during the acquisition phase. The mean score on the first five sessions at the 90-s delay was 69% (*SEM* = 3.00%). This mean score was significantly above chance, $t(5) = 6.38$, $p < .05$, 2-tailed. The mean score on the last five sessions of the 90-s delay was 83% (*SEM* = 1.71%) and was significantly above chance, $t(5) = 20.00$, $p < .05$, 2-tailed. The average number of sessions required to reach the performance criterion was 25 (*SEM* = 3.91), or 100 trials (excluding criterion sessions). The range of number of sessions was 13- 37. Average scores on the last five sessions at the 90-s delay were statistically significantly higher than scores on the first five sessions, $t(5) = 4.63$, $p < .05$, 2-tailed. This significant improvement in scores suggests rats learned the nonmatching rule.

As indicated by Figure 6, rats' performance was transiently disrupted as the delay increased, but improved over sessions at the new delay. Rats received an average of 7.16 sessions (*SEM* = 3.64) at the 180-s delay. A total of five rats reached the performance criterion at the 180-s delay within the maximum 25 sessions. Of the five rats, the mean number of sessions required was 3.6 (*SEM* = 0.87) (excluding criterion sessions). The range of number of sessions was 1-6.

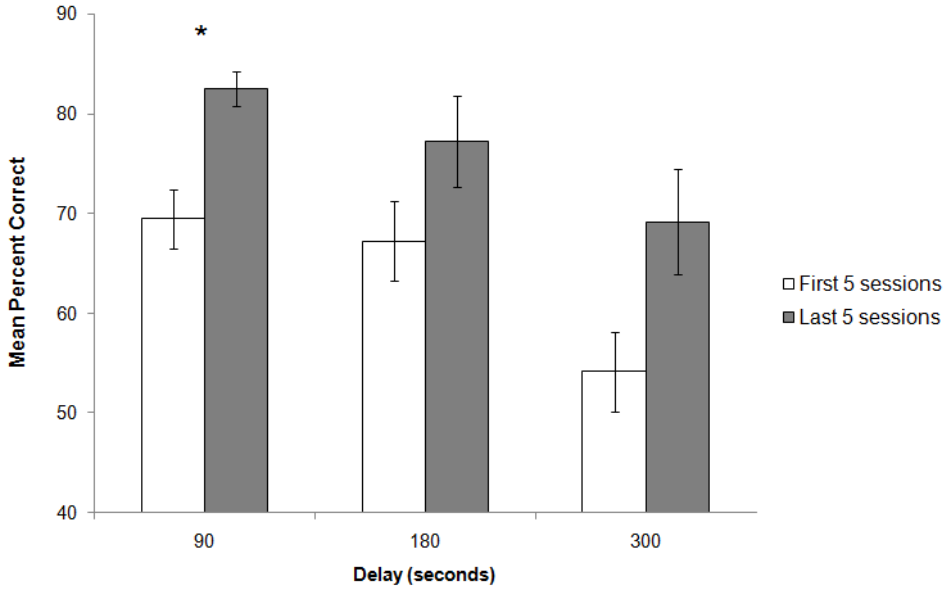

Figure 6. Mean level of performance during the first and last five sessions of acquisition of delayed nonmatching-to-sample at the 90-s delay and performance during training at the longer delays. Asterisk indicates a statistically significant difference between mean scores on the first and last five sessions ($p < .05$).

Rats received an average of 16.83 sessions (*SEM* = 4.21) at the 300-s delay. Only three of the six rats reached the performance criterion at the 300-s delay within the maximum 25 sessions. Of the three rats, the mean number of sessions required was 8.7 (*SEM* = 4.70) (excluding criterion sessions). The range of number of sessions was 3-18. The three rats that did not reach the performance criterion had five consecutive sessions that were close to reaching it. One rat reached five consecutive sessions twice with a score of 75% (one by session 2 and the other by session 16). Another rat reached five consecutive sessions with a score of 75% by the fourth session.

Testing the retention function. Figure 7 illustrates the mean level of performance at each delay during the mixed-delay sessions. The mean score at the 90, 180, and 300-s delay was respectively 74% (*SEM* = 2.58%), 64% (*SEM* = 3.15%), and 67% (*SEM* = 1.52%). A repeated measures ANOVA revealed there was an effect of delay $F(2, 10) = 6.53$, $p < .05$ (partial $\eta^2 = 0.57$). Bonferroni corrected pairwise comparisons revealed that scores on the 90-s delay were significantly higher than scores obtained on the 180-s delay, $t(5) = 3.63$, $p < .05$, $d = 1.46$.

One sample *t*-tests (2-tailed) revealed that scores at the 90, 180, and 300-s delay were significantly above chance, $t(5) = 9.16$, $p < .05$, $d = 3.85$, $t(5) = 4.43$, $p < .05$, $d = 1.81$ and $t(5) = 11.47$, $p < .05$, $d = 4.50$ respectively.

Novel-Object-Preference Test

The mean investigation ratios are based on the first minute of the test (*M* = 0.62, *SEM* = 0.05). A decrease in exploration of the novel object over time on the test is typically observed because the novel object becomes more familiar as the animal

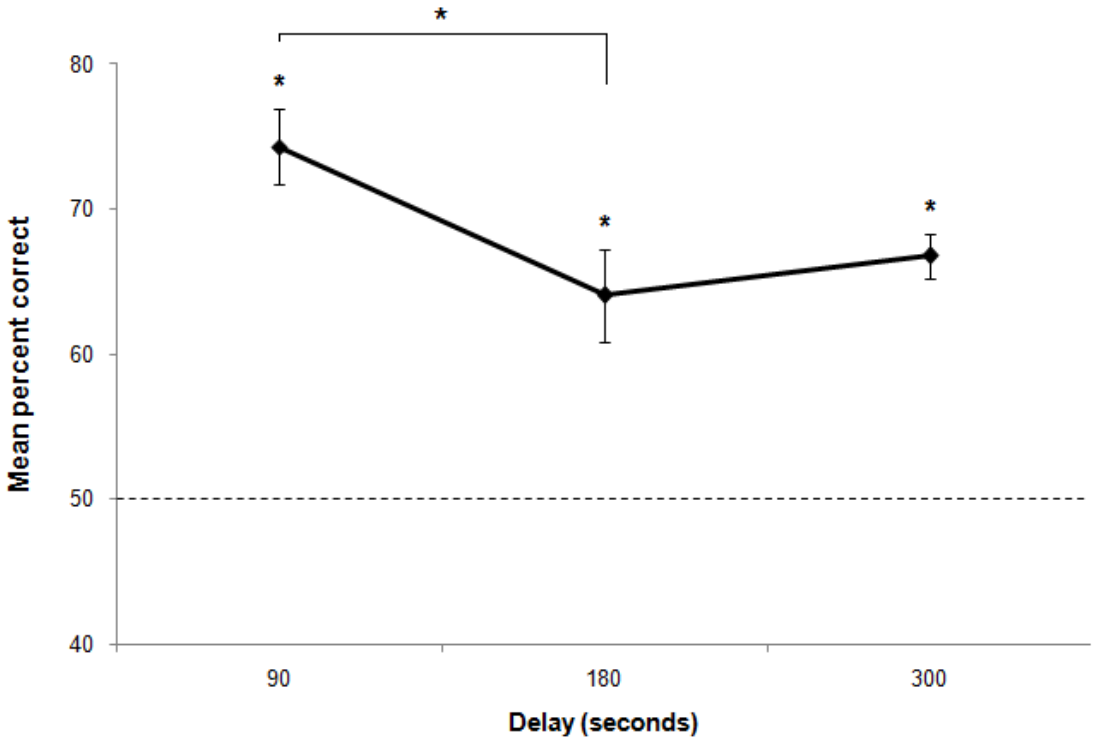

Figure 7. Mean level of performance during testing of the retention function at each delay on the Circular-track delayed nonmatching-to-sample task. Asterisk above the connector lines indicates a statistically significant difference between mean scores ($p < .05$). Asterisk above mean scores indicates a significant difference from chance ($p < .05$). The dashed line represents the chance level of performance (i.e., a ratio of 0.50). Error bars represent SEM.

investigates it (Dix & Aggleton, 1999). One-sample *t*-tests (1-tailed) revealed that rats' mean investigation ratios were significantly above chance level of performance, $t(5) = 2.17$, $p < .05$, $d = 0.88$.

Correlational Analyses

Pearson product-moment correlation coefficients were calculated in order to determine whether a relationship existed between the mean level of performance on the Circular-track DNMS mixed-delay sessions and investigation ratio scores during the first minute of the NOP test. The correlation coefficient obtained for scores on the NOP test and scores on the DNMS task at the 90 s delay was $r(4) = 0.49$, $p > .05$ ($R^2 = 0.24$), at the 180-s delay was $r(4) = -0.32$, $p > .05$ ($R^2 = 0.10$), and at the 300 s delay was $r(4) = -0.44$, $p > .05$ ($R^2 = 0.19$). The lack of statistically significant correlations was likely a result of the low observed power of the study. The observed power at the 90, 180, and 300-s delay were respectively, $\beta = 0.19$, $\beta = 0.11$, and $\beta = 0.16$.

Discussion

Rats were able to reach an average score of 83% correct choices following a 90-s delay between a single presentation of four distinct sample objects and a choice test. Importantly, this level of accuracy was reached following a mean number of 25 training sessions (100 trials). Testing the retention function following training revealed that rats maintained a very good level of performance at the 90-s delay, and although it decreased as the delay was increased, performance at the 180-s and 300-s delays remained significantly better than chance levels. It is therefore likely that rats are able to perform this new task with retention intervals beyond the 300-s maximum that was used in this study.

Rats' mean investigation ratios on the NOP test were significantly above chance following a 180-s retention interval. This significant novelty-preference demonstrates that rats were able to detect the familiarity of the sample object on the test. The results of the correlational analyses performed on the scores on the NOP test and the DNMS task at each delay revealed a moderate positive linear relationship for scores obtained at the 90-s delay, a weak negative linear relationship for scores obtained at the 180-s delay, and a moderate negative linear relationship for scores obtained at the 300-s delay. The lack of any consistent relationship between NOP and DNMS scores across these three conditions casts doubt on the likelihood that the two tasks engage the same cognitive functions.

The significant improvement in rats' performance from the first five sessions to the last five sessions at the 90-s delay during the acquisition phase indicates that rats learned the nonmatching rule. The decrease in performance between the 90-s delay, 180-s and the-300 s delays suggests that this task taxes memory as the delay increases. However, unlike some conventional DNMS tasks on which the reward was delivered only *after* the correct choice has been made (e.g. Mumby et al., 1990), in the present study, the reward was placed under the novel object *prior* to the choice phase. This raises the possibility that rats could determine the location of food reward by detecting its odor. To determine whether this might be happening, we examined the rats' behaviour during the sample phase during the acquisition phase. Both objects in each compartment were identical, and the location of the seed was determined in a pseudo-random order, so if a rat was choosing objects on the basis of where it smelled food-odor cues, this should be evident in the proportion of occasions on which the rat displaced the baited sample before displacing the unbaited sample. Rats' mean choice for selecting the sample object during

the sample phase was not statistically different from chance at any of the three delays. This confirms that rats did not rely on smell to locate the reward. Additionally, if rats' were choosing objects on the basis of detecting the bait, this ability would not be expected to produce delay-dependent changes in the rats' performance.

We are confident that once the rats acquired the delayed nonmatching-to-sample task, their behaviour on the test was guided by the visual properties of the object, rather than its tactile or odour properties. First, in most instances, rats directed their movement towards the novel object immediately after entering a compartment. Second, rats rarely made contact with an object without also displacing it. Although some rats did occasionally make contact with the sample object first, before eventually turning to displace the novel object, this behaviour was observed most frequently in rats that had strong side preferences. Third, we used a separate copy of the sample object on the test to ensure the rats could not solve the task via odours they had previously left on the sample objects.

It is clear the Circular-track DNMS task takes advantage of rats' spontaneous exploration of novel stimuli (Berlyne, 1950). This is suggested by the rats' mean score of 69% during the first five sessions of the task. This above average initial mean score has also been reported in a previous study. Rats' performance on the first two sessions during the acquisition phase of the DNMS task was 59% (Mumby et al., 1990). The rats' level of initial performance in the present study may be a result of reduced constraints on exploratory behaviour due to the design of the apparatus.

Additionally, the training rats received prior to acquisition of the delayed nonmatching-to-sample phase (Training stage 1 & 2) may have been a contributing factor

to this level of initial performance. On conventional DNMS tasks, pre-training consisted of repeatedly presenting the rat with the same two distinct sample objects (Mumby et al., 1990; Kesner et al., 1993). One of the sample objects is always rewarded (S+) whereas the other sample object is never rewarded (S-). The purpose of the training was so that rats could learn the instrumental-response requirements (displace objects for food), while simultaneously learning that the visual/tactile object features were the key to predicting food location. Compared to conventional DNMS tasks, the training procedure involved in this study incorporated those features in addition to providing rats the opportunity to learn that displacing the sample object on the choice phase would provide no reward. Moreover, learning this relationship may have been facilitated even further, because we presented multiple copies of the same sample object.

The results from the present experiment indicate that the Circular-track DNMS task can accurately measure rats' object recognition abilities and good levels of performance can be achieved in fewer trials compared to traditional DNMS tasks. For example, a previous experiment which assessed the effects of preoperative training on performance on the DNMS task following partial dorsal hippocampal lesions showed that the control group required a mean number of 264 trials to reach the performance criterion of 85% correct choices on two consecutive days at a 4-s delay. Following training at the short delay, rats then received 100 training trials at the following three delays: 60, 120, and 300 s. When rats were tested, their mean level of accuracy at the 60, 120 and 300-s delay was approximately 74%, 66%, and 68% respectively (Duva, Floresco, Wunderlich, Lao, Pinel, & Phillips, 1997). The rats in the present experiment reached almost identical levels of performance in far fewer trials; 100 trials at the 90-s delay, 28 trials at the 180-s

delay and 64 trials at the 300-s delay. Additionally, the comparable performance levels were achieved following a single presentation of four distinct sample objects, as compared to only one sample object in the previous study.

Rats' ability to retain information for several sample objects over the delay was better than rats' performance in previous studies. In a previous experiment, rats were presented with lists of three sample objects and were given a choice test 75 s after the presentation of the third sample object. Rats' mean level of performance on the test was 70% (Mumby, Pinel, Kornecook, Shen, & Redila, 1995). In the present experiment, rats' mean level of performance in recognizing lists of four sample objects after a 90-s delay was 74%. Moreover, in the previous experiment when rats were shown lists of five sample objects and required to retain the information for those objects over a 75-s delay, their mean percent correct choices on the test was approximately 67%. This score is comparable to the rats' mean score in the present experiment following a 300 s delay.

Although the rats in the present experiment performed at levels comparable to rats that had received extensive numbers of trials in previous experiments, there may yet be an opportunity to improve the procedure. For example, there was no cost to the rat for displacing the sample object before the novel object; that is, rats were still able to retrieve the reward if their first choice was incorrect. Increasing the negative consequences of incorrect first-choices might accelerate acquisition of the nonmatching rule that underlies performance. One possible way to do this might be to bury the seed so the rat has to dig for it. This simple change may reduce a rat's tendency to make hasty choices during training.

Giving rats additional opportunities to encode object features might increase their rate of task acquisition, as well as increase the level of post-acquisition performance. The addition of two sample phase trips will increase the amount of opportunities the rat has to investigate the sample object, and presumably object feature encoding. In a previous study using a conventional DNMS task, one group of rats was provided 90 s with the sample object after displacing it from over a food well, and a second group was given only 10 s with the sample object. The group of rats that were provided with 90 s of additional time with the sample object performed more accurately on the task, and more of the animals reached the performance criterion in the designated number of trials (Beck & Kalynchuk, 1992).

The inconsistency in the direction of the correlation coefficients when comparing the scores on the NOP test to scores on the DNMS task at each delay suggest NOP test scores may not be a reliable predictor of performance on the Circular-track DNMS task. We observed a moderate positive linear correlation between scores on the NOP test and scores on the DNMS task at the 90-s delay and a negative linear correlation between scores on the NOP test and scores on the DNMS task at the 300-s delay. These results indicate that good performance on the NOP test predicts good performance on the DNMS task at a 90-s delay, however, when the delay is increased to 300 s, poor performance on the NOP test is a predictor of good performance on the DNMS task.

The results from the present experiment do not provide any evidence which would indicate that the strength in a rat's novelty preference is a reliable indicator of the strength of the memory for the sample object. Furthermore, a failure to exhibit a novel object preference on the test may not accurately reflect a rat's ability to detect the

familiarity of the sample object. The present experiment provides evidence counter to the assumption that a higher novelty preference is indicative of stronger object-recognition memory and thus, one should be cautious when interpreting results from the NOP test.

Further studies are needed to determine if rats' good level of performance on this new task can be maintained using delays lasting several hours to days. This is an important next step as rats' object recognition memory has only been assessed at long delays using the NOP test. Moreover, the effects of various pharmacological agents and surgical lesions on performance at long delays have only been assessed using the NOP test. The interpretational problems of the NOP test and increasing amounts of evidence indicating it may not accurately measure rats' object recognition memory, make it apparent that a new task is needed to clarify results obtained using the NOP test.

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