

Research report

# Massed but not spaced training impairs spatial memory

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## Abstract

The Morris water maze and the object displacement task are two popular tools used to investigate spatial learning and memory. Research has focused mainly on the acquisition of spatial tasks while little attention has been given to the retention phase. We examined the effects of different training procedures on retention of the water maze and also reactivity to spatial change in the object displacement task 7 days post-acquisition. We found that massed-trained animals were impaired on retention of the water maze compared to those animals that had received spaced-training. We also found that the massed-trained animals habituated readily to their environment in the object displacement task while the spaced-trained group did not. Furthermore the massed-trained group did not react to spatial change 7 days post-habituation compared to the increased reactivity displayed by the spaced-trained group. Results are discussed in terms of poor encoding of the environment leading to poor retention.

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## 1. Introduction

One popular tool described 20 years ago to investigate spatial learning and memory is the Morris Water Maze (MWM) [9,10]. Its simplicity and the possibility to differentiate between the spatial learning (invisible platform) and non-spatial (visible platform) conditions is one of its main advantages [2]. Much of the research has focused on the acquisition phase of such a task [2]. There is, however, variation in the training schedules employed by different laboratories during acquisition. Brandner et al. [1] for example used 36 acquisition trials over 5 days, others only use 20 trials over 4 days (for example [19]). These different training procedures may highlight different encoding and consolidation processes [21]. Rats can acquire spatial tasks with massed training, that is training on a certain number of trials with short inter-trial intervals [24]. This training is considered to involve an encoding process based on spatial working memory [11,24]. Rats can also acquire spatial tasks with spaced training, that is training with a certain number of

trials with large inter-trial intervals [16]. This procedure may involve an encoding process also based on spatial working memory but with the addition of long-term memory [11] and consolidation processes [21]. Comparisons of massed and spaced training have produced conflicting results. Goodrick [4] for example, demonstrated that rats that were spaced-trained on the t-maze learned better than massed-trained animals. In the water maze task rats that received massed training had higher number of errors when compared to the spaced-trained group [6]. However, Spreng et al. [21] found no difference between training procedures on acquisition of the water maze. It has also been demonstrated that massed training impairs acquisition of the water maze only under certain conditions. These conditions may include the age of the animal [17]. Therefore, whether impairments observed are due to poor encoding of the environment or fatigue as in the case for aged animals or a stressful task such as the water maze [7,17] remains controversial.

In contrast to acquisition and encoding, retention of long-term spatial memories has received little attention. This is despite the importance of these processes in models of spatial navigation and spatial memory [12,13]. It has been demonstrated that different training sche-

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dules not only affect acquisition but also retention of spatial tasks. Spaced training for example facilitates retention of spatial navigation in the water maze [21]. This effect, however, is observed only on long-term and not short-term retention. The effect is also age-related affecting adult and not adolescent rats. We will use the water maze task to determine the effects of massed-trained animals compared to spaced-trained animals on retention 7 days post-acquisition. We hypothesise that animals that receive massed training will be impaired on retention of the location of the platform. The spaced-trained animals will encode the environment better leading to better retention.

In a second experiment we will further test spatial ability of animals by using an object-displacement task [14]. This task examines exploration, spatial encoding and reactivity to spatial novelty. It is generally thought that this task is designed to estimate the ability of animals to encode spatial relationships among stimuli [14,22,23]. Furthermore, renewal of exploration after spatial rearrangement implies that the second arrangement is compared with an internal representation of the first arrangement and indicates the ability of animals to acquire and retain spatial knowledge [5]. We will use the object displacement task to determine the effects of massed-trained animals compared to spaced-trained animals on retention of an environment. An increased reaction to a spatial change should indicate a better encoding of the initial environment leading to better retention. This task will also eliminate any fatigue effects that might confound the water maze task. Finally this task will examine the differences in exploratory and encoding processes in massed compared to spaced-trained animals. We hypothesise that in the object displacement task both groups will habituate to the environment, although the spaced-trained group may habituate at a slower rate than the massed-trained group. We also hypothesise that the spaced-trained animals will react to the changed environment stronger than the massed-trained group.

## 2. Method

### 2.1. Experiment 1

#### 2.1.1. Animals

Male Wistar rats (200–300 g; Bioresources Unit, University of Dublin, Trinity College) aged approximately 3 months were used as subjects. Rats were housed three per cage and were kept in a temperature-controlled room which was maintained on a fixed light–dark cycle. All rats were given free access to food and

drink. All rats were well handled before experimentation and testing was carried out during the light phase.

#### 2.1.2. Apparatus and procedure

The watermaze was a black circular pool (1.7 m diameter; 35 cm deep; water  $20 \pm 1$  °C) filled to 31 cm. Rats can escape the water by climbing on to a hidden platform (29 cm  $\times$  9 cm). The hidden platform was placed in the North Eastern quadrant of the pool and submerged 2 cm below water surface so it was invisible at water level; the location of the platform was fixed during the experiment. A curtain surrounded the water maze at a distance of 50 cm from the pool wall. Distal cues included two (60 W) lights suspended from the ceiling. One light was suspended from the NE corner, and the other from the NW position. Both lights were at a distance of 75 cm from the pool wall and at angles of approximately 60°. A rectangular sheet of black paper (55 cm  $\times$  81 cm) stuck to the curtain on the eastern side of the pool was also used as a cue. A computerised digital tracking system (EthoVision) recorded escape latencies and velocity during each trial (Noldus Information Technology, Wageningen, Netherlands).

Rats were randomly assigned to one of two groups:

Group 1: massed trained group ( $n = 6$ ). Rats were placed into the water maze for 16 consecutive trials. Rats were allowed 60 s to find the platform; otherwise the rat was led to the platform by the researcher. The rat remained on the platform for 20 s. The inter-trial interval was 10 s. All rats entered the maze from a pseudo-random starting position (North, South, East or West).

Group 2: spaced trained group ( $n = 6$ ). Rats received 4 trials/day for 4 days. Rats were again allowed 60 s to find the platform and allowed to remain on the platform for 20 s. The inter-trial interval again was 10 s. All rats entered the maze from a pseudo-random starting position (North, South, East or West).

Various behavioural criteria were used to measure acquisition including escape latency and velocity. Retention was assessed 7 days after the end of the acquisition phase. Retention for both groups was assessed by removing the platform. The rat was then placed into the water maze from the South starting position and allowed to swim for 60 s. Percentage time spent in the platform area (on total time spent in the pool, i.e. 60 s) was used to measure retention. This was defined by a circular area centred at the platform with a radius of approximately 27 cm (body length of a rat). Percentage time spent (again on total time spent in the pool) in three other equivalent locations (NW, SW and SE) was also calculated.

## 2.2. Experiment 2

### 2.2.1. Animals

Male Wistar rats (200–300 g; Bioresources Unit, University of Dublin, Trinity College) aged approximately 3 months were again used as subjects. Rats were housed three per cage and were kept in a temperature-controlled room which was maintained on a fixed light–dark cycle. All rats were given free access to food and drink. All rats were well handled before experimentation and testing was carried out during the light phase.

### 2.2.2. Apparatus

The apparatus consisted of a black circular fiberglass arena (diameter = 130 cm, height = 38 cm) resting on a table 70 cm above the ground and illuminated from above by a single lamp. Curtains surrounded the entire arena. During testing the experimenter, wearing a white laboratory coat stood at the side of the arena. Four objects were placed in a square formation at the center of the arena approximately 40 cm apart (see Fig. 1). The four objects included a rectangular plastic box (13.5 cm height  $\times$  27 cm length  $\times$  11 cm width), a concrete pillar (18.5 cm height  $\times$  12.5 cm diameter), a wooden tree stump (12 cm height  $\times$  8 cm diameter) and a plant in a glass vase (33 cm height  $\times$  10.5 cm diameter).

### 2.2.3. Procedure

Initially, each rat was allowed 1 min to explore the empty arena. The experimenter did not stand with the curtains during this phase. The four objects were then positioned (see Fig. 1).

Rats were randomly assigned to one of two groups:

Group 1: massed trained group ( $n = 6$ ). Rats were placed in the center of the arena and given four consecutive trials to explore the arena. Each trial

lasted 3 min. The inter-trial interval was approximately 20 min.

Group 2: spaced trained group ( $n = 6$ ). Rats received 1 trial/day for 4 days. Each trial again lasted 3 min.

Various behavioural criteria were used to assess exploratory behaviour. The experimenter recorded the number of nose contacts each rat made with the individual objects. The path length of each rat was also recorded by a computerised digital tracking system (EthoVision). Retention was assessed 7 days after the end of acquisition. This consisted of a single trial (3-min duration) where the wooden block was moved towards the side of the arena (see Fig. 1). To eliminate the possible biasing factor of any olfactory cues, the experimenter handled every object.

### 2.2.4. Statistics

A series of repeated ANOVAs were used. Where appropriate independent and/or dependent  $t$ -tests were also used. All statistics were carried out using SPSS (version 10).

## 3. Results

### 3.1. Experiment 1

#### 3.1.1. Water maze acquisition

Both the massed-trained group and the spaced-trained group acquired the water maze. Fig. 2a demonstrates that the mean escape latencies decreased over the 16 trials (averaged over four trials) for the massed-trained group. Fig. 2a also displays a decrease in escape latency for the spaced-trained group. A two-way repeated measures ANOVA with training condition (massed vs. spaced) as the between-group measure and acquisition session as the within-group measure revealed

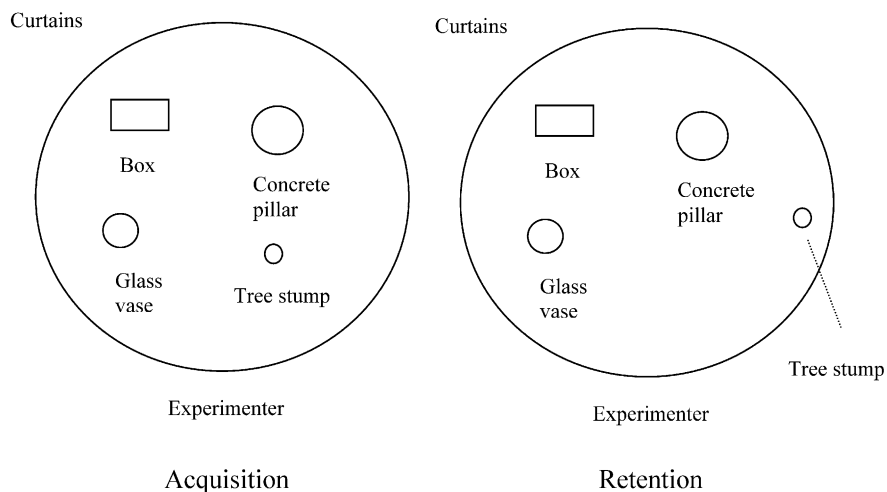
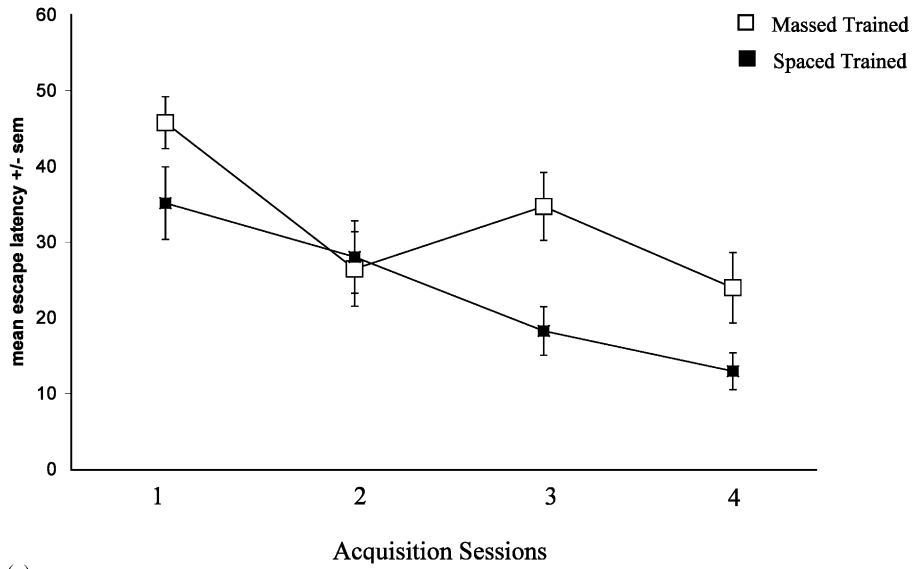
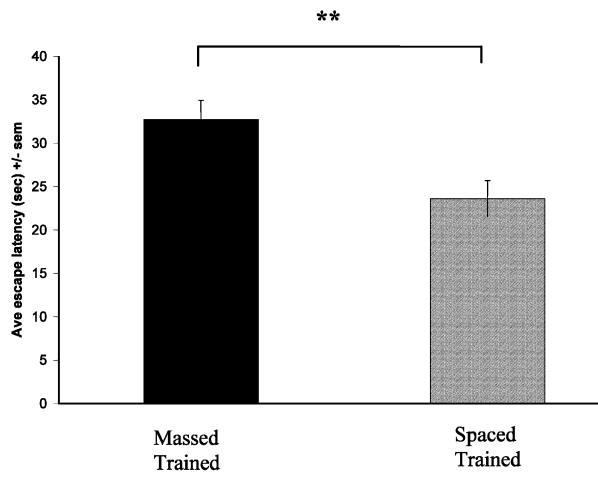


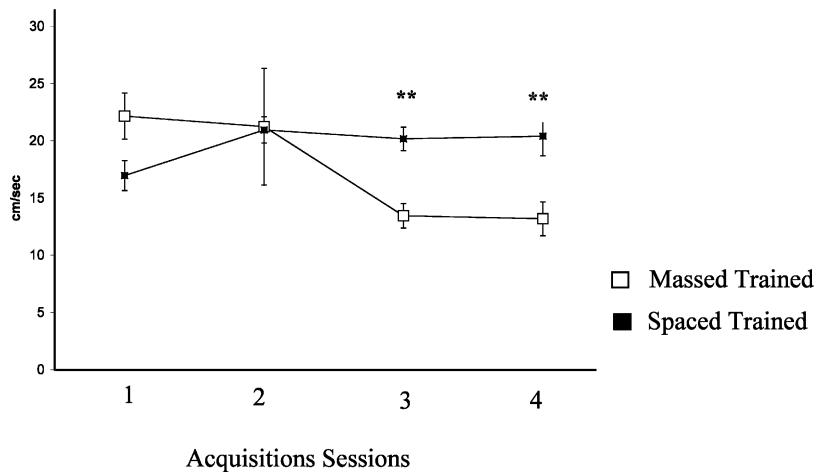
Fig. 1. Layout of the environment in experiment 2 during habituation (acquisition) and retention phase.



(a)



(b)



(c)

Fig. 2

a significant effect of training condition (see Fig. 2b;  $F = 9.191$ ,  $df = 1$ ,  $177$ ,  $p < 0.01$ ). This demonstrates that the spaced-trained group had lower escape latencies than the massed-trained group. There was also a significant effect of acquisition session ( $F = 8.919$ ,  $df = 3$ ,  $177$ ,  $p < 0.01$ ). There was, however, no interaction effects.

Fig. 2c demonstrates a decrease in velocity over the 16 trials (averaged over four trials) for the massed-trained group. The mean velocity for the first four trials was  $22.15 \pm 2.01$  cm/s. This decreased to  $13.18 \pm 1.46$  cm/s for the final four trials. In comparison, the velocity of the spaced-trained group on the first day of acquisition was  $16.95 \pm 1.3$  cm/s. This increased to  $20.39 \pm 1.7$  cm/s on the final day of acquisition. A repeated-measures ANOVA with training condition as the between-group measure and acquisition session as the within-group measure revealed a non-significant effect of training condition ( $F = 1.648$ ,  $df = 1$ ,  $177$ ,  $p > 0.05$ ), and a non-significant effect of acquisition session ( $F = 1.687$ ,  $df = 3$ ,  $177$ ,  $p > 0.05$ ). However, there was a significant Condition  $\times$  Session interaction ( $F = 3.295$ ,  $df = 3$ ,  $177$ ,  $p < 0.05$ ). Subsequent *t*-test analysis for between conditions revealed that the massed-trained group was significantly slower than the spaced-trained group (see Fig. 2c) on acquisition sessions 3 and 4 ( $t = 4.54$ ,  $df = 46$ ,  $p < 0.01$ ;  $t = 2.881$ ,  $df = 46$ ,  $p < 0.01$ , respectively).

### 3.1.2. Retention of water maze

Fig. 3a reveals that animals that were spaced-trained spent significantly more time swimming in the platform area (NE,  $17.57 \pm 3.7\%$ ) than animals that were massed-trained ( $6.6 \pm 2.6\%$ ). An independent *t*-test confirmed this finding;  $t = -2.39$ ,  $df = 10$ ,  $p < 0.05$ . There were no significant differences in the amount of time spent swimming by either group in any of the other 3 equivalent areas ( $t = 0.452$ ,  $df = 10$ ,  $p > 0.05$ ;  $t = -0.691$ ,  $df = 10$ ,  $p > 0.05$ ,  $t = 0.432$ ,  $df = 10$ ,  $p > 0.05$ ; SW, SE, NW areas, respectively). Fig. 3b shows representative swim paths taken by both massed- and spaced-trained groups during the retention trial.

## 3.2. Experiment 2

### 3.2.1. Habituation

Fig. 4a shows that animals that were given 4 sequential trials (massed-trained) habituated rapidly to the environment. The number of nose contacts made with the 4 objects decreased from  $35.5 \pm 3.7$  on the first trial to  $16 \pm 1.9$  on the fourth trial. This habituation was confirmed by a one-way ANOVA. There was an overall

significant difference between the trials ( $F = 11.522$ ,  $df = 3$ ,  $20$ ,  $p < 0.001$ ). Subsequent post-hoc tests (Tukey,  $p < 0.05$ ) revealed that there were fewer nose contacts with the objects on trial 4 compared to trials 1 and 2. There were also significantly fewer nose contacts on trial 3 when compared to trial 1. In contrast animals that were exposed to the environment for one trial for each of 4 days (spaced-trained) did not habituate (see Fig. 4a) to the environment. The number of nose contacts with the objects went from  $22.16 \pm 2.57$  on day 1 to  $18.33 \pm 2.1$  on day 4. Again a one-way ANOVA was used to compare the number of nose contacts across days. No significant difference was found between days ( $F = 0.404$ ,  $df = 3$ ,  $20$ ,  $p > 0.05$ ) suggesting an absence of habituation.

In order to compare both groups across habituation trials a two-way repeated measures ANOVA was carried out with training condition (massed vs. spaced) as the between-group measure and trial number as the within-group measure. This revealed a non-significant effect of training condition (see Fig. 4b;  $F = 3.708$ ,  $df = 1$ ,  $10$ ,  $p > 0.05$ ). This suggests that the average number of contacts made by either group was similar over the habituation period. There was a significant effect of trial number ( $F = 9.201$ ,  $df = 3$ ,  $30$ ,  $p < 0.01$ ) and also a significant Condition  $\times$  Trial effect ( $F = 5.261$ ,  $df = 3$ ,  $30$ ,  $p < 0.01$ ). Subsequent *t*-test analyses for between-group differences revealed that the massed-trained group made significantly more nose contacts with the objects on trial 1 than the spaced-trained group ( $t = -2.909$ ,  $df = 10$ ,  $p < 0.05$ ). No other differences were noted.

Further analysis revealed no significant difference ( $t = -0.705$ ,  $df = 46$ ,  $p > 0.05$ , see Fig. 4c) between the groups on the average path length over the habituation period ( $1627.4 \pm 77$  vs.  $1708.3 \pm 84.9$  cm massed and spaced groups, respectively).

### 3.2.2. Reaction to a displaced object

Reaction to a displaced object (spatial novelty) was assessed 7 days post-habituation. Fig. 5a reveals that animals that were spaced-trained made a significantly higher number of nose contacts with all objects when compared to the animals that were massed-trained ( $t = -3.045$ ,  $df = 10$ ,  $p = 0.01$ ). This is despite similar path lengths recorded by the two groups ( $t = -0.830$ ,  $df = 9$ ,  $p > 0.05$ , data not shown).

We then compared the number of nose contacts made with all the objects on the final day of habituation to those on the retention trial. A repeated measures ANOVA was conducted for the massed-trained animals.

Fig. 2. (a) Mean escape latency ( $s \pm$ S.E.M.) for massed-trained group and spaced-trained group during acquisition of the water maze task. Averages of four trials are plotted for the massed-trained group. (b) A bar chart displaying the mean escape latencies for both massed- and spaced-trained groups over the total acquisition period. (c) Total velocity (cm/s  $\pm$ S.E.M.) of each group within 60 s allowed across the acquisition period.

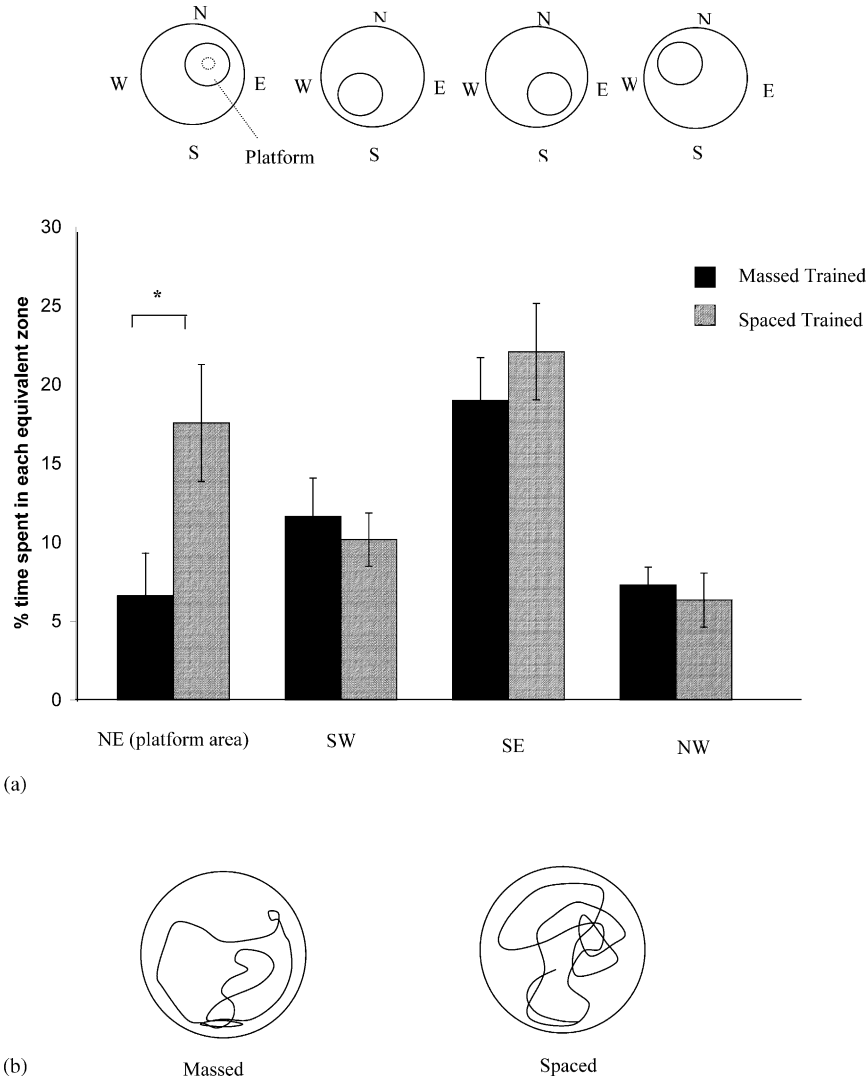


Fig. 3. (a) A bar chart displaying the mean percentage time  $\pm$  S.E.M. spent in the platform area (NE) and the three other equivalent areas (SW, SE, NW) for both massed- and spaced-groups on the retention phase. (b) Representative swim paths for both groups are also displayed.

Object type (wood, plant, box and pillar) was used as the between-group measure and day (final day vs. retention) as the within-group measure. This revealed a non-significant effect of day (Fig. 5b,  $F = 0.244$ ,  $df = 1$ ,  $20$ ,  $p > 0.05$ ). There was a significant effect of object type ( $F = 4.274$ ,  $df = 3$ ,  $20$ ,  $p < 0.01$ ). There was also a significant Object  $\times$  Day interaction effect ( $F = 3.833$ ,  $df = 3$ ,  $20$ ,  $p < 0.01$ ). However, subsequent  $t$ -test analyses revealed no significant between-group differences (see Fig. 5c). A further repeated measures ANOVA was conducted for the spaced-trained animals. Object type was again used as the between-group measure and day as the within-group measure. This revealed a significant effect of day (Fig. 5b,  $F = 6.64$ ,  $df = 1$ ,  $20$ ,  $p < 0.01$ ). There was a non-significant effect of object type ( $F = 0.734$ ,  $df = 3$ ,  $20$ ,  $p > 0.05$ ). There was also a significant Object  $\times$  Day interaction effect ( $F = 5.006$ ,  $df = 3$ ,  $20$ ,  $p < 0.001$ ). Subsequent  $t$ -test analyses for between-

group differences revealed that the number of nose contacts made by the spaced-trained group with the displaced object (wood) significantly increased (Fig. 5d,  $t = -4.842$ ,  $df = 5$ ,  $p < 0.001$ ) on the retention trial when compared to the final day of habituation. No other differences were noted.

#### 4. Discussion

The experiments presented in this paper demonstrate that massed training impairs the ability of animals to retain a spatial memory of an environment. We have shown that animals that received massed training in the water maze subsequently spent significantly less time in the platform area compared to the animals that received spaced training. We have shown that the massed-trained animals were also slower to acquire the task both in

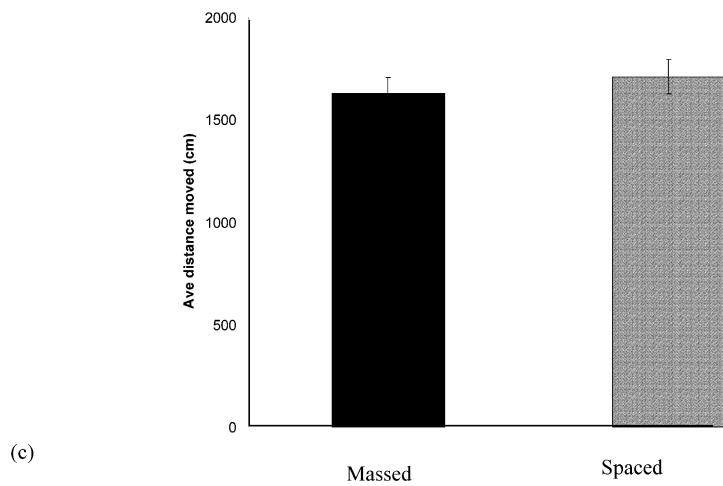
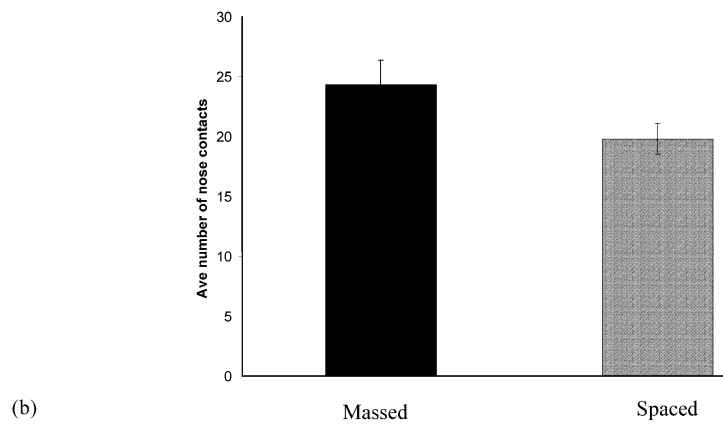
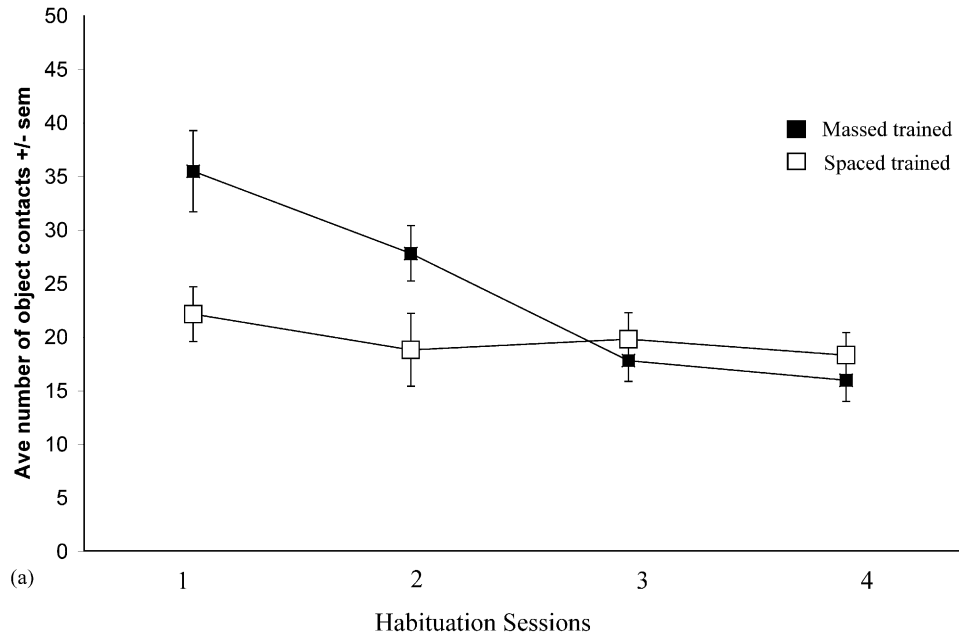


Fig. 4. (a) Exploration and habituation patterns for massed- and spaced-trained groups. (b) A bar chart displaying the average number of nose contacts made with all objects over the total habituation period. (c) A bar chart displaying average distance moved for both groups over the total habituation period.

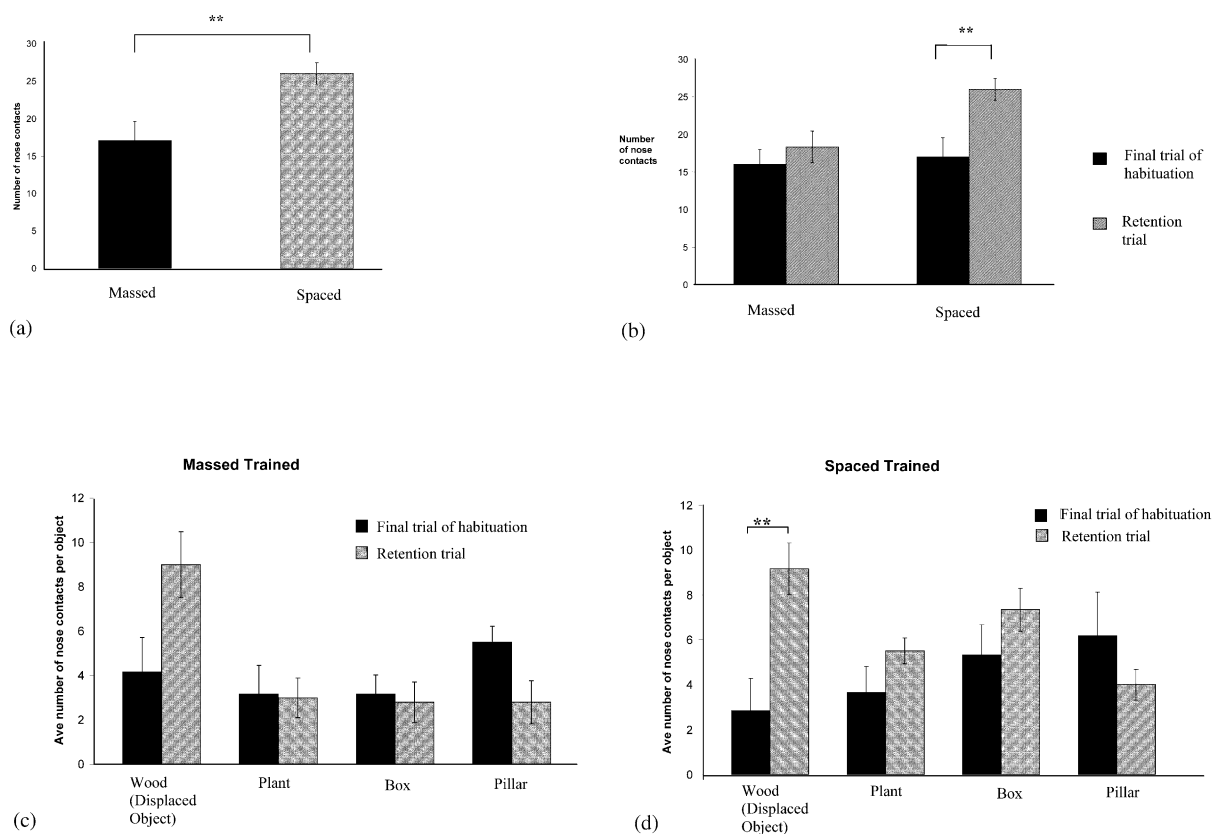


Fig. 5. (a) A bar chart displaying the reactivity to all objects by massed- and spaced-trained groups during the retention phase. (b) A bar chart displaying average number of contacts made with all objects by both groups on the final day of habituation compared with the retention trial. (c) A bar chart displaying reaction to each object by the massed-trained group on the final day of habituation compared to the retention trial. (d) A bar chart displaying reaction to each object by the spaced-trained group on the final day of habituation compared to the retention trial.

terms of escape latency and velocity. Fatigue problems may account for poor retention. Although fatigue has been shown to be a source of behavioural impairment in some animals [7,17], Spreng et al. [21] did not detect any such manifestations. Instead these authors suggest that long-term retention impairments result from the lack of occasions for consolidation processes. This suggestion is similar to that provided by Morris and Doyle [11] who accounted for the differences between massed and spaced-trained animals in terms of working and long-term memory differences. These suggestions may indeed contribute to the poor retention performance of the massed-trained animals but we would also attribute the impairment to poor encoding of the initial environment. This can be seen in the object displacement task.

The object displacement task requires animals to retain the spatial arrangement of the environment. Exploratory behaviour is a natural activity during which animals acquire spatial information about their environment [5]. When an animal is placed into an environment they explore it and any objects that may be present [14,15]. This exploration decreases over time as long as no change occurs [5]. We demonstrated that animals exposed to an environment with short inter-trial intervals (massed trained) habituate normally and their

exploration of the environment decreases over trial number. However, animals that had 24 h between trials (spaced-trained) did not habituate and renewed their exploratory activity each day. Although the massed-trained animals made more nose contacts on the first trial of habituation compared to the spaced-trained group, this did not affect the overall habituation pattern. The habituation pattern still was evident from the second trial where no group differences were noted. Similarly Espejo [3] reports that repeated exposure to the elevated plus-maze does not lead to habituation of exploratory or locomotor responses if tested either weekly or daily. We suggest that as the spaced-trained animals did not habituate, they renewed their exploration and built up a better spatial representation. This suggestion can be seen in studies involving aged animals. Shukitt-Hale et al. [20] for example have demonstrated that old rats explored objects less than young rats and have decrements in the ability to build spatial representations of the environment and to use this information to detect changes in new spatial arrangements. The opposite also seems to hold true. 5-HT1B knock-out mice for example, show increased exploratory activity and also increased memory performance in the water maze [8]. It should be noted however that exploratory



activity is strain-dependent [18] and care should be taken when comparing studies.

This paper also demonstrates that animals that were spaced-trained displayed an increased reaction to the new spatial environment compared to the massed-trained animals. We observed that there is a general increase in reactivity to all objects in the spaced-trained animals. Furthermore, this group displayed an increased reaction to the object itself that was displaced. Lack of exploration has been found to correlate with a failure to react to spatial change in old animals [25]. These authors argue that lack of spatial reactivity shown by an absence of exploration renewal occurs when there is no discordance between the two arrangements (before and after the object is displaced). We would also argue that better encoding of the environment leads to better retention and therefore an increased reactivity to a new spatial arrangement.

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