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Individual variation in the spatial reference and working memory assessed under allothetic and idiothetic orientation cues in rat

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Abstract. The present study was designed to examine which kind of memory: reference or working, better correlates with individual variation in rats' spatial learning abilities. To answer this question two groups of rats were trained to an arbitrary criterion in a partially baited 12-arm radial maze under two different experimental conditions: with or without allothetic cues. After 10 days break, rats were examined under the same conditions for memory retention. Within- and between-group variation in the length of training to criterion, and in the frequency of reference and working memory errors were analysed. The present experiment confirmed the facilitating effect of the presence of distal visual cues on place learning in rats. Task-dependent (between-group) differences in the rate of learning were attributed to differences in the frequency of reference memory errors. Conversely, within-group variation in the rate of task acquisition reflected individual variation in the frequency of working memory errors. These results were looked upon from an evolutionary perspective. Low correlation between reference and working memory errors confirms that these two types of memory have different mechanisms. The fact that differences in the rate of learning were not paralleled by the differences in the memory retention supports the notion that memory acquisition and memory retention are two independent processes.

Key words: rats, maze learning, allothetic *versus* idiothetic cues, reference *versus* working memory, task acquisition *versus* memory retention

INTRODUCTION

In the wild, spatial learning and memory help animals find locations that provide, among other things, food and safety, and therefore are crucial for survival. Rats displaying extraordinary spatial abilities are commonly used in studies examining animals' cognitive capacity in spatial tasks that comprise a variety of mazes (for review see Hodges (1996) and Cimadevilla et al. (2000)). O'Keefe and Nadel (1978) postulated that rats can solve maze tasks by using at least three different learning strategies, each representing a different learning ability. One of them is referred to as "place learning": spatial learning occurring in relation to all kinds of available distant (allothetic) cues. This type of learning requires formation of a spatial map of the environment. The second possible type of learning is known as a "guidance strategy" and represents an acquired tendency to approach a single salient cue or a set of cues present in close proximity to the goal. The third strategy is sometimes referred to as "response chaining", based on motion-generated (idiothetic) cues. New place avoidance task first introduced by Bures et al. (1997), and employing a modified model of slowly rotating arena allows dissociation of allo- and idiothetic cues used in navigation. It has been demonstrated that during spatial navigation, rats use independently allo- and idiothetic memories (Bures et al. 1998, Bures and Fenton 2000, Fenton et al. 1998). In spatial orientation, place finding may be predominantly controlled either by allothetic cues or proprioceptive idiothetic information. Choice of the strategy is determined by the demands of the task to be solved.

The strategies used by rats to find food in mazes with multiple food locations, such as a partially baited radial maze, are thought to reflect foraging strategies used by these animals in their natural habitat. For efficient task solving, between foraging sessions, rats must employ a win-stay strategy based on a long-term reference memory, and within foraging session, they have to employ a win-shift strategy based on a short-term or working memory. Radial arm maze with a subset of rewarded arms is thus a useful tool to dissociate the spatial reference and spatial working memory components.

It is well known that there is an individual variation in learning capacity among animals belonging to different genetic strains (Nguyen et al. 2000). Similar variation is also observed among animals of the same strain even when they come from the same commercial breeder. The aim of the present study was to examine which kind of memory: reference or working, better correlates with individual variation in a rat's spatial learning capacity examined in two different tasks requiring allothetic or idiothetic memory.

METHODS

Subjects

Experiments were run on 42 male, 3 month old, naïve Wistar rats, obtained from the Rat Breeding Station of Hacettepe University Medical School. Rats were kept in home cages in groups of three or four under natural day/night cycle. In the home cages, rats had free access to water but were subjected to a 23-h food deprivation schedule, with daily food portions given 30 min after the completion of behavioural tests. Prior to the experiments, rats were reduced to 85% of their *ad libitum* body weight. Body weight was recorded every second day. Subjects were trained once a day in the same order, and at about the same time each day.

Apparatus

The apparatus was a twelve-arm radial maze made of plywood, elevated 80 cm above the floor, and painted flat grey to unify intramaze visual cues.

The maze consisted of a circular platform, 40 cm in diameter, and 12 arms, each 60 cm long and 9 cm wide. The arms were flanked by walls 10 cm high and made of transparent plexiglass. Transparent plexiglass guillotine doors were installed at the entry to each arm. Guillotine doors could be moved separately, one by one, or in concert. A hole, 2 cm wide and 2 cm deep, was drilled at the end of each arm to serve as a food cup. To unify through-the-maze food odour traces, a perforated partition was inserted 1 cm from the bottom of all food cups, beneath which two chocolate flavoured rice puffs were placed. The same pellets were used as bait in the course of the training.

Procedure

Rats were trained in two separate groups: A (allothetic $n = 24$) and I (idiothetic, $n = 18$).

Prior to the experiments, for six consecutive days, all rats were daily handled, for five minutes each. At the beginning of the experiments, rats were given 5 days of ha-

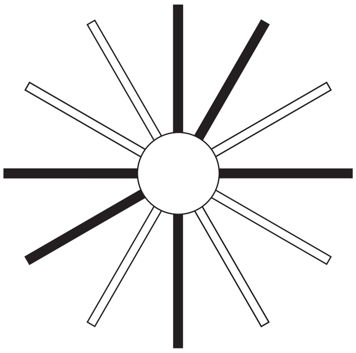


Fig. 1. The scheme of 12-arm radial maze with baited arms painted black.

bituation and shaping trials, in which they were allowed to explore the maze for a 10 min period each day, and eat all the food pellets. During the first day of shaping training, food pellets were scattered throughout the maze. During each successive day, the number of pellets was reduced, and they were placed closer to the ends of the arms. At the end of the shaping, food was placed only in the food cups of pre-selected arms.

During the acquisition training, six out of the total twelve semi-randomly chosen arms (see Fig. 1) were baited with food (2 g chocolate flavoured rice puffs), while the remaining 6 arms (always the same) stayed empty. In a single experimental trial, successful solution to such a task was not to enter the arms that have never been baited, and not to re-enter the arms that have been already visited. On each daily trial, rats were placed on the central platform facing different directions. The guillotine doors were raised and rats were allowed to make their first choice by entering one of the arms. Each time, the rat returned to the central platform after making a choice, the guillotine doors were shut for 5 s, and only then the animal was allowed to make the next choice. Rats were permitted to choose among the arms until they completed the trial by either eating all pellets, or making 12 choices, or when 10 min had passed. The entry to the arm was counted only when the rat crossed the midpoint of the alley with its two forepaws. Within any daily trial, entry to an unbaited arm or re-entry into a baited arm was scored as, respectively, reference or working memory error. All rats were trained to the criterion of three consecutive daily trials with 5 out of the first 6 choices to the baited arms. After reaching the acquisition criterion, rats were given 10 days rest in their home cages. Then, they were subjected to retraining to the same criterion as before. This was considered a memory retention test.

Group A was trained in the presence of many distal cues belonging to the experimental room. In Group I, rats were trained to search for food in the radial maze surrounded by dark plain curtains to eliminate distal cues. The animal behaviour was tracked by a video camera fixed at the ceiling. After memory retention test (Retraining 1), Group I was retrained once more, this time with self-odour trails being removed after each trial (Retraining 2). Then, rats from Group I were given 10 days break and were again tested for memory retention (Retraining 3).

The maze was illuminated with a dim homogenous light. The animal behaviour was monitored by a video camera fixed at the ceiling.

In the course of the experiments the following measures were recorded:

a) number of choices to the acquisition criterion; b) percentage of entries to unbaited arms (reference memory errors, RMEs); c) percentage of re-entries to the baited arms (working memory errors, WMEs); d) number of choices to the retention criterion.

The data were analysed using Students *t*-test and Spearman rank correlation test.

RESULTS

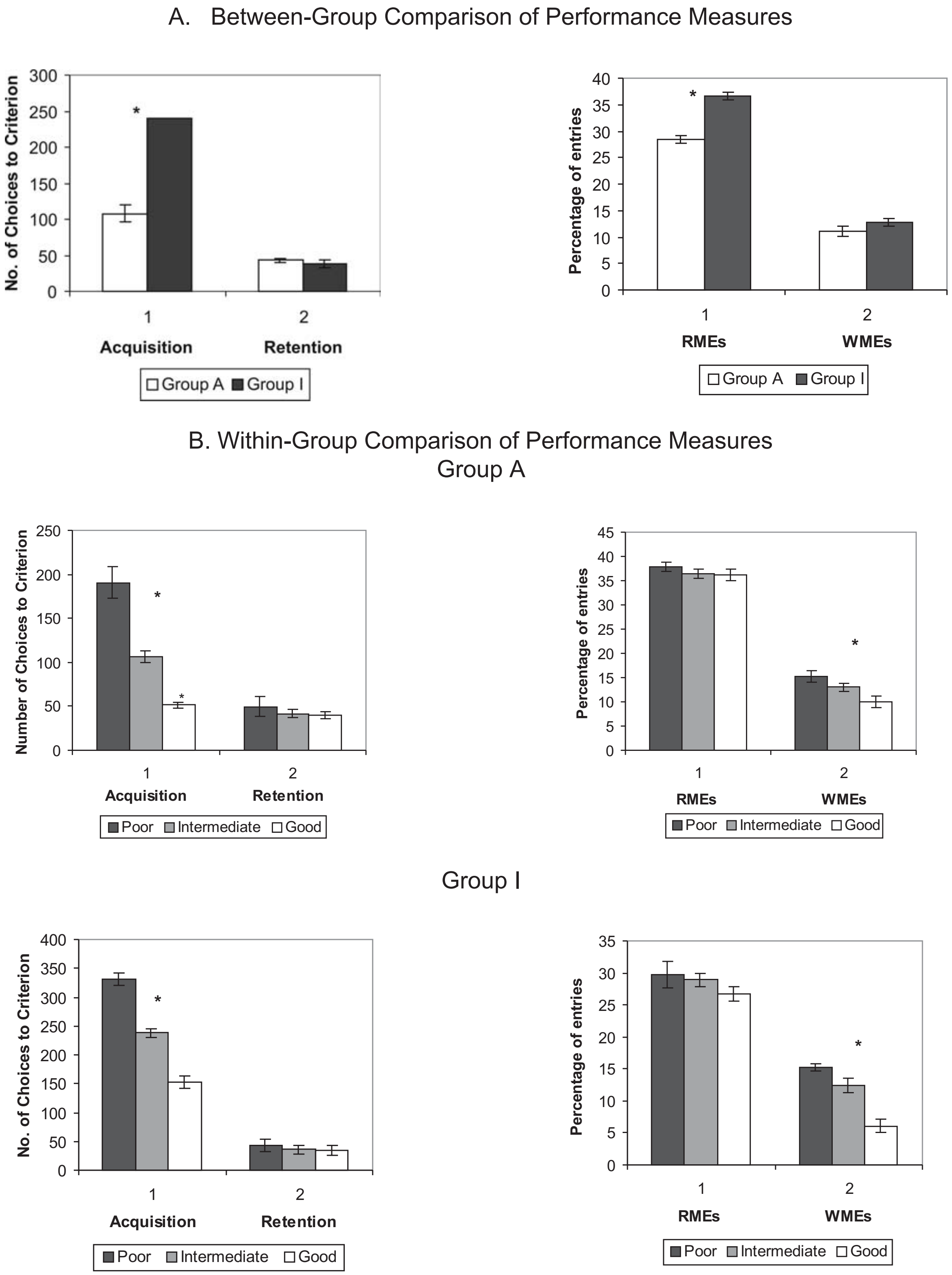
Figures 2A show different learning measures for rats trained either under allothetic (Group A), or under idiothetic (Group I) stimulus conditions. Rats deprived of allothetic cues that could be used in navigation (Group I) required significantly more training to reach the performance criterion compared to rats trained with available distal cues (Group A), $t(40)=6.69$, $P<0.001$. No such difference was observed during the retention test.

The between-group difference in the rate of reaching criterion during the task acquisition corresponded to significant difference in the percentage of reference but not working memory errors, $t(40)=7.54$, $P<0.001$.

In both Groups, the percentage of RMEs was significantly higher than the percentage of WMEs, $t(23)=13.29$, $P<0.001$, and $t(17)=21.24$, $P<0.001$ respectively.

The Spearman rank correlation test did not reveal positive correlation between the percentage of working and reference memory errors under either of the stimulus conditions tested.

In Group I, after reaching the acquisition and retention criterion, additional training with removed self-odour cues was carried out. The results are presented in Table I. Removal of odour trails caused signif-



A. Between-Group Comparison of Performance Measures

B. Within-Group Comparison of Performance Measures
Group A

Group I

Fig. 2. Comparison of different performance measures for rats trained under allothetic (Group A) and idiothetic (Group I) stimulus conditions. Left side figures show the number of choices to the acquisition and retention criteria. Right side figures demonstrate the percentage of entries to unbaited arms (RMEs) and re-entries to baited arms (WMEs). Bars represent mean ± SEM values. The asterisk indicates a significant difference at $P<0.05$ or less as assessed by Student *t*-test.

Table I

Effect of self-odour trail removal on different performance measures (group mean ± SEM) in Group I

Performance measure	Group I: Stage of the experiment I Odour trails present: Training	 Retraining 1	 II Odour trails removed: Retraining 2	 Retraining 3
Number of choices to acquisition criterion	240.1 ± 17.0	38.0 ± 5.0	97.4 ± 11.0	51.4 ± 8.6
REMs (%)	36.7 ± 0.7	10.1 ± 1.8	28.1 ± 1.2	20.7 ± 1.9
WMEs (%)	12.8 ± 0.8	8.6 ± 1.9	7.9 ± 0.9	8.0 ± 1.2

icant impairment in rats' performance. Students *t*-test for pairs yielded highly significant effect of the odour trail elimination on the rate of reaching the retraining criterion, and on the percentage of reference memory errors (*t*(17)=6.29, *P*<0.001, and *t*(17)=9.49, *P*<0.001, respectively, Retraining 1 and 2 compared).

Interestingly, the Spearman rank correlation test did not yield positive correlation between rates of reaching criterion with and without scent cues.

In order to assess the within-group variation in spatial learning, rats from both groups were classified as "poor" (number of choices to the acquisition criterion ≥ group mean + 3 SEM), "good" (number of choices to the acquisition criterion ≤ group mean - 3 SEM), and the remaining "intermediate" learners.

Figures 2B show mean values for different learning measures calculated independently for "poor", "intermediate", and "good" learners in Groups A and I. Consistent with the results earlier described for the whole groups, here too, in all three categories of learners, there were highly significant task-dependent differences in the number of choices to the acquisition, but not to the retraining criterion, with the dramatically worst performance exhibited by rats from Group I (*t*(4)=7.8, *P*=0.002, *t*(7)=19.11, *P*<0.001, *t*(4)=8.51, *P*=0.001 for "poor", intermediate", and "good" learners respectively). As revealed by Students *t*-test for paired comparisons, these between-group differences in the rate of learning mainly corresponded to the differences in the percentage of RMEs (*t*(4)=2.99, *P*=0.04, *t*(7)=5.49, *P*=0.001, and *t*(7)=19.11, *P*<0.001) but not WMEs. Conversely, the differences in the rate of learning between "poor", "intermediate", and "good" learners trained under the same stimulus conditions (Group A or Group I) corresponded to the between-subject differences in the percentage of WMEs rather than RMEs. In both groups, Students *t*-test for pairs confirmed a significant difference in WMEs between "poor" and "good" learners (*t*(4)=4.07, *P*=0.015, and *t*(4)=3.43, *P*=0.027 for Groups A and I respectively), and between "intermediate" and "good" learners in Group A (*t*(6)=3.04 *P*=0.023).

DISCUSSION

In the present experiment employing a partially baited 12-arm radial maze, place learning was significantly faster in Group A trained with allothetic cues, compared to Group I deprived of such cues. This result is consistent with earlier observations by other authors (Hodges et al. 1991, Olton and Samuelson 1976, Stuchlik and Bures 2002, Zoladek and Roberts 1978) and once more indicates that the primary cues used by the animals during foraging in mazes and probably also in the natural habitat are visual in nature.

In contrast to fully baited radial mazes, such as these from the studies by Olton and Samuelson (1976) or Zoladek and Roberts (1978) where the odour trails occurred to be unimportant in performing the task, in the partially baited radial mazes such as ours, self-odour trails proved to play an important role in spatial guiding. In Group I, a significant deterioration in animal's performance was observed due to the removal of odour traces being left by the animals. However, after additional training during which rats could rely only on the idiothetic cues, a stable asymptotic performance level was again reached. These and similar results (Maaswinkel and Whishaw 1999, Stuchlik et al. 2001, Walles et al. 2002) confirm that rats show a hierarchical preference in using visual, olfactory and movement-generated idiothetic cues, and, according to the task demands, are able to shift from one strategy to another.

Different rates of spatial learning with different strategies (Group A *versus* Group I), and lack of correlation between rates of learning observed for the same subject under different task conditions (Group I trained with and without odour trails) constitute indirect evidence that different tasks require involvement of different memory systems.

Despite the significant task-dependent differences in the rate of maze learning, there were no between-group differences in the memory retention test. This observation supports the notion that memory acquisition and memory retention are two independent processes.

The aim of the present study was, however, to show how the variation in the reference and working memory errors affected group and individual rates of spatial learning. In the course of the experiments it was noted that the between-group, task-dependent differences in the rate of learning were attributed to differences in the frequency of reference rather than working memory errors. Conversely, variation in the rate of spatial learning observed among rats trained under the same, allothetic or idiothetic, stimulus conditions reflected between-subject differences in the emission of working rather than reference memory errors. Within groups, reference memory operated at a stable level independent of the individual variation in the rats' capacity to load the working memory information. The relative invariability of the reference memory for cues important in spatial navigation can be the result of natural selection. Like other traits, memory and learning abilities in animals can be considered as adaptive specializations that are shaped by natural selection to solve specific problems animals encounter in their environment. Natural selection would be expected to favour memory systems that permit efficient exploitation of available food resources. In this way such systems become stronger (Sherry and Schacter 1987). However, intense selection for a trait may reduce the amount of genetic variance that can be detected. Working memory seems to be less stable and as such may be more susceptible to impairment. Indeed, it has been reported that neuroactive and/or neurotoxic substances have a predominant effect on working memory (Gustilo et al. 1999, Hodges et al. 1991, Markowska et al. 1996). It is also known that it is the working memory that first undergoes deterioration in different forms of amnesia including senile dementia and Alzheimer's disease.

The dissociation of the reference and working memory systems is also confirmed by the lack of correlation between the frequencies of reference and working memory errors. It confirms the notion that these two types of memory have different mechanisms (for review see Izquierdo et al. 1999).

To determine whether the individual variation in working memory observed among subjects trained under the same task conditions has indeed a genetic basis, further experiments should be done examining the changes in working memory occurring over few generations in inbred rat lines of "poor" and "good" learners.

CONCLUSIONS

Present experiment demonstrated that rats show a hierarchical preference in using visual, olfactory and movement-generated idiothetic cues. The animals' learning capacity changes with the task to be solved. Task-dependent differences in the rate of learning are attributed to species-specific variation in the strength of reference memory for different types of cues. Conversely, differences in the rate of spatial learning observed among rats trained under the same stimulus conditions reflect individual variation in the working memory.

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