

# **Light Deprivation-Related Changes of Strategy Selection in the Radial Arm Maze**

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## **Summary**

During the early postnatal age environmental signals underlie the development of sensory systems. The visual system is considered as an appropriate system to evaluate role of sensory experience in postnatal development of sensory systems. This study was made to assess the effect of visual deprivation on strategy of arm selection in navigation of radial arm maze. Six-week-old light- (LR, control) and dark-reared (DR) rats were trained for correct choices and adjacent arms tasks. Our results showed that both the LR and DR animals equally selected correct arms. In the adjacent arms task, however, the control group significantly outperformed the DR animals. While the LR males and females displayed some differences in performing the tasks, no sex dependency was found in the performance of the DR group. These findings indicate that the lack of visual experience is likely to influence the strategy selection as well as sex differences. Thus the difference in the performance of LR and DR animals seems to be due to the male rather than female behavior.

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## **Key words**

Learning and memory • Radial maze • Sensory experience • Sex difference • Strategy selection

## **Introduction**

It has been demonstrated that the hippocampus is involved in some aspects of learning and memory. This area receives sensory inputs indirectly, converged on the entorhinal cortex (Lavenex and Amaral 2002), as well as directly from the neocortex (Yukie 2000). Development of cortical sensory systems is influenced by environmental experience during a critical period before the onset of behavioral function (Waters *et al.* 1997). During this period, neural circuits across several systems display remarkable plasticity to environmental stimulation (Fagiolini and Hensch 2000). In a variety of

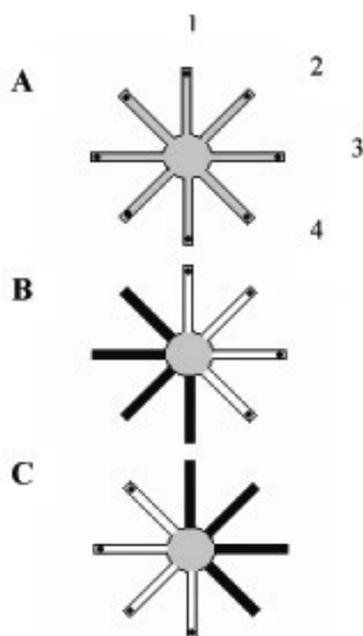
learning and memory tasks visual cues play an important role in conducting animals to access their proper path. Like the sensory cortices, the hippocampus undergoes a period of postnatal development that is thought to underlie the emergence of behavioral function (Dumas and Foster 1995). The visual system has been considered as an appropriate system for assessment of the involvement of sensory experience in development of neural circuits. Hippocampal-cortical interactions lead to strong and persistent memories for events and their constituent elements and interrelations, together with a capacity for flexibly producing memories across a wide range of circumstances (Eichenbaum *et al.* 1994). Using

different approaches, sex differences in performance of spatial tasks have intensively been studied. Although controversial, a growing body of evidence suggests superiority of male over female in a variety of tasks which require the use of visuospatial cues in both human (Saucier *et al.* 2002) and rodents (Roof and Havens 1992). This study was designed to address effect of visual experience on the selection strategy as well as sex-dependent performances of rats during a life period, which is critical for postnatal development of the brain.

## Methods

### Apparatus

The apparatus was a radial maze placed 70 cm above the floor. It was made of Plexiglas and consisted of 8 arms ( $70 \times 15$  cm) radiating from a central platform (30 cm in diameter). The radial maze is well suited to assess strategic behavior because different strategies can be used by rats to efficiently solve the task (Janis *et al.* 1998). To test the strategy of animals in searching the maze no door separated the arms and the central platform. Food rewards were placed in a small cup at the end of each arm (Fig. 1).



**Fig. 1.** Representative graphs illustrating different status of the radial maze during different phases of the experiments. **A**, all trials in phase 1. **B**, all trials in phase 2 and first daily trials in phase 3. **C**, second daily trails in phase 3. The baited arms are indicated by spots at the end of the white arms.

### Experimental subjects

Subjects (acquired from the Pasteur Institute of Tehran) were Wistar male and female rats at 40 days of age (on arrival day to the testing room). The animals were divided to two groups, each containing 12 rats. Control group was litter reared on a 12 hr light/dark cycle throughout life (light-reared rats, LR). The other group was litter born and housed in complete darkness since birth till the end of experiments (dark-reared rats, DR). A dim red light (5 Watt) was used for daily care of the DR animals. Each group, in turn, consisted of subgroups of 6 males and 6 females kept in separate cages. The animals were reared with their mother until 38 days of age. They had free access to food and water through the rearing period. During the experiments, however, water was available *ad libitum* but a restricted feeding schedule to maintain body weight of approximately 85 % of free feeding levels. One hour daily feeding took place after the second trial (see below).

### Behavioral procedures

The maze was located in a large test room ( $4 \times 6$  m), surrounded by many visual cues external to the maze (e.g. the experimenter, lights, computer, rack, pictures, etc.), which were visible from within the maze and could be used by the rats for spatial orientation. Locations of the cues were unchanged in all phases of the experiment. Shaping and trainings were carried out in dark period of the light/dark cycle of housing. The testing room was illuminated with a dim light, as described above for animal housing. The rats were shaped with the maze for two days prior to testing. In two sessions of the first day they were placed in the maze so that could obtain food rewards that were scattered throughout the maze. On the second day, in both trials, the animals were placed in the maze and they had to find food rewards placed only in the food cups at the end of the arms. Then the animals were subjected to three phases of experiments lasting for 16 days. Experiments were designed to test strategy of maze searching using extramaze cues (phase 1), and, fixed (phase 2) and variable (phase 3) intramaze cues. In the first phase the rats were trained on days 1-8 with the full baited arm maze in a room with numerous visible cues. In this phase the animals had to visit each of 8 baited arms once within a session (Fig. 1A). A trial started that the animal was placed in the central platform and could navigate the maze freely. The session continued until all the baited arms were entered or 10 min had elapsed. Rats were given two trials/day with 3-4 h

intertrial interval. Experiments continued at least until the animal entered all 8 baited arms in 8 or maximum 9 selections over two consecutive sessions. In the second and third phases the rats were tested in the maze with four baited arms and trained to perform the tasks based on the intramaze cue (white color). Four adjacent arms were painted white while the other arms were painted black. Only the white arms were baited and the animals were required to enter only these arms. The animals were allowed to have a maximum of 5 selections over a total time of 5 min. On days 9-12 (phase 2) the white arms (numbered 1, 2, 3 and 4) were fixed in all sessions (Fig. 1B). Then, to confirm further the searching is based on the intramaze cues, the rats were introduced to variable intramaze cues on days 13-16 (phase 3) where place of the white arms changed between the two daily trials; the baited arms were those numbered 1, 2, 3 and 4 (Fig. 1B) and 5, 6, 7 and 8 in the first and the second trials, respectively (Fig. 1C).

#### Data analysis

The performances were considered for 1) number of the correct entries during each session and 2) whether the maze arms were selected randomly or on the basis of a special strategy.

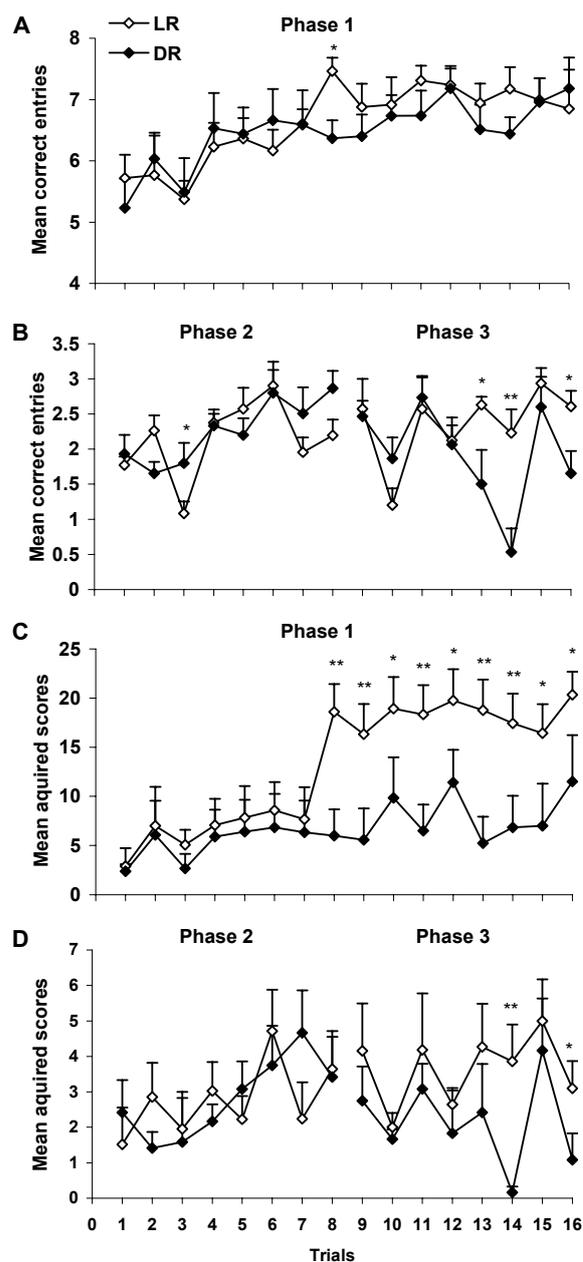
For measuring strategy selection the animals' choices were scored in the following way. The first entry was scored 0. If the second entry was an adjacent arm (regardless of the direction) the animal received score 1. For next adjacent arm selections scores were 2, 3 and so forth. Therefore, if the animal entered all arms adjacently without re-entries it was scored 28 in the phase 1 and 6 in the phases 2 and 3. Random entries to nonadjacent arms or return to revisited adjacent arms set back scoring to 0 so that the next entry to a neighbor arm was scored 1.

Unpaired t-test was applied to the data to compare performances between the LR and DR rats in the correct entries and strategy selection tasks. The comparisons were also made between male and female animals in the different groups using analysis of variance (ANOVA) followed by Tukey's *post hoc* test. The results are presented as means  $\pm$  S.E.M and  $P < 0.05$  value was considered significant.

## Results

#### Correct choice task

Training the animals in the full baited maze showed that in the correct arm choice task the two groups



**Fig. 2.** Graphs to show the mean correct choices (**A** and **B**) and scores (**C** and **D**) for each trial and group. The trials were conducted over a 16-day period with 2 trials/day using extramaze cues (phase 1) on days 1-8, fixed intramaze cues (phase 2) on days 9-12 and variable intramaze cues (phase 3) on days 13-16. Graphs **A** and **B** indicate that early exposure to dark rearing does not affect entering correct arms. Instead, in the strategy of arm selection (graphs **C** and **D**), the LR group acquired significantly further scores than the DR group in using either extra or intramaze cues as training progressed. \*  $P < 0.05$ , \*\*  $P < 0.005$

improved their performances in parallel (Fig. 2A). Then, the rats were tested for selecting only white baited arms in terms of intramaze cues. Whereas both groups slightly declined the number of errors over 8 trials of the second phase no improvement was observed in the performances

across the third phase (Fig. 2B). In general, statistical analysis indicated no considerable difference between performances of the LR and DR animals in this task. Although a significant difference was apparent between LR and DR rats in the third phase of training (Fig. 2B), it was due to a poor function of the DR group rather than due to an improvement in the LR animals' behavior.

#### Strategy selection task

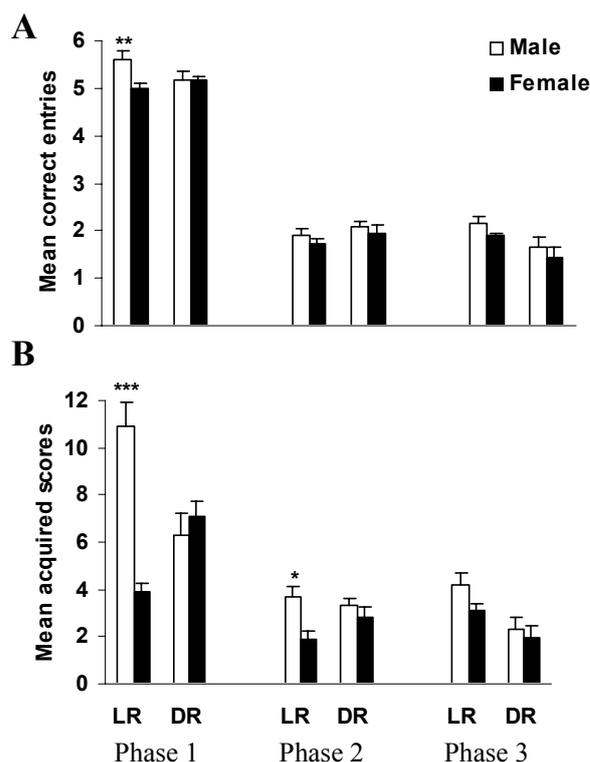
The data from the first phase of strategy selection experiments reveal a marked superiority of the LR rats over their DR counterparts. However, the higher performance of the control group appeared in the middle of the first phase, when they obtained scores of about 3 times higher compared to the DR group (Fig. 2C). In the second phase with fixed intramaze cues, the rats had to find the rewards in four neighbor baited arms. In this phase the behavior of LR and DR animals was slightly improved and both groups performed equally (Fig. 2D). No progress was observed in the phase 3 when the animals steered the maze with variable baited arms; further, intertrial alternation of cued arms disturbed performances of both (especially the DR) groups so that the animals confused the baited adjacent arms. However, there were some differences between function of the LR and DR animals in the third phase.

#### Sex differences

In this study we also considered sex differences in searching the radial arm maze.

Concerning the correct choice task in the phase 1, ANOVA revealed differences in behavior of males and females in both groups where the males outperformed the females ( $F_{3,60} = 4.322$ ,  $P=0.008$ ). Nevertheless, superiority of the males was significant only in the LR group ( $P<0.01$ ). We observed no difference between the two sexes in either the LR or DR animals in the second ( $F_{3,36} = 0.891$ ,  $P=0.456$ ) and the third phase ( $F_{3,28} = 2.99$ ,  $P=0.05$ ) (Fig. 3A).

Data analysis indicated a substantial sex-dependent performance in the first phase of the strategy selection tests ( $F_{3,60} = 13.205$ ,  $P<0.0001$ ). Here again, this sex dependency was noticeable in the control group where the male LR rats searched the full baited maze with higher scores than did the females ( $P<0.001$ ). The difference between the two sexes in the DR group was not significant. ANOVA also shows that performance of males and females varies in the second ( $F_{3,36} = 3.501$ ,  $P=0.025$ ) and third ( $F_{3,28} = 4.757$ ,  $P=0.008$ ) phases, but



**Fig. 3.** Sex differences are solely evident in the control group. **A**, in the LR group the males outperformed the females in the phase 1 of the correct choice task. The DR males and females behaved similarly across all phases. **B**, in three phases of the strategy selection task the LR males acquired greater number of scores than did the LR females. The difference is significant in phases 1 and 2. \*  $P<0.05$ , \*\*  $P<0.01$ , \*\*\*  $P<0.001$ .

*post hoc* test indicated that the sex difference was significant only in the LR animals with an obvious superiority of the males on the females in the phase 2 ( $P<0.05$ ) (Fig. 3B).

## Discussion

Two general strategies can be used by rats to solve the eight arm radial maze: highly variable patterns of arms or selection of adjacent arms. Adult animals typically use the random choice of arms when solving the radial maze, and young rats tend to adopt the adjacent arm choice strategy (Berman and Hannigan 2000). In the present study young adult rats were used for assessment of correct arm choice and strategy selection tasks.

Our results revealed that the two groups of animals similarly perform the correct choice task. Freely entering arms is a strategy which does not require memory (Gresack and Frick 2003). Thus, an analogous behavior of the LR and DR rats would be expectable when the subjects do not rely on their spatial memory.

Nevertheless, the data of the present work can not rule out a probability of variation between normal and visually deprived rats in selection of correct arms. It is worthwhile to note that in a series of experiments on a radial maze with gated arms we observed different performances on the working and reference memory tasks between the DR and LR animals (unpublished data). On the other hand, in the strategy selection task, the LR rats displayed a higher performance than the DR ones. This indicates that lack of visual inputs deteriorates the animals' performances in this task. It has been reported that the stereotypic behavior is not produced but revealed by hippocampal lesions, which remove the memory-guided behavior masking of the stereotypic behavior in the intact animal (Devenport and Merriman 1983, Lanke *et al.* 1993). From these considerations one possibility could be underlying the hippocampal function by the light deprivation. Another possibility might be a direct influence of the sensory deprivation on the visual system so that the DR animals weakly perform the strategy selection task on basis of visual cues.

Some studies have shown a sex-related performance in radial maze favoring males (van Haaren *et al.* 1987). However, inconsistent results have also been reported demonstrating no difference between sexes in performance of radial arm maze task (Waters *et al.* 1997). As illustrated in Figure 3B the present findings point out that in both, particularly in the strategy selection, tasks the males outperform females only in the normal reared rats. This strongly suggests that the visual deprivation considerably underlies capability of the animals in adjacent arms selection. The present data led us to conclude that, in fact, the discrepancy in the LR and DR representations is due to the difference between the males rather than the females of the two groups. Thus, our results suggest that the dark rearing negatively influences non-random behavior in the males. Sex differences in the use of an adjacent arm strategy have been reported. While Roof (1993) believes that on radial maze males performed better than females, Juraska *et al.* (1984) reported no gender differences in adjacent arm strategy.

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Sex difference in rats has partially been attributable to gonadal steroid differences in males and females (Luine and Rodriguez 1994) where testosterone *via* its conversion to estradiol enhances visuospatial ability (Roof and Havens 1992). Altogether, it may be concluded that the visual experience affects the hormonal function so that the LR and DR animals differently perform the radial maze tasks.

A hormone that fluctuates with cycles of darkness and light is melatonin, darkness stimulates it and light inhibits it. Melatonin is proposed to modulate cognitive plasticity, independent of the effects of sex steroids (Yun *et al.* 2004). It was reported that melatonin has a positive effect on water maze performances (Gonenc *et al.* 2005) and decreased melatonin involves in Alzheimer-like spatial memory impairment (Zhu *et al.* 2004). On the other hand, Feng *et al.* (2002) reported that application of melatonin in rats significantly inhibited spatial learning and memory. However, because of nature of the present procedure (a non-random selection of maze arms rather than a pure spatial representation), the role of melatonin may not be pertinent to the data presented here.

In conclusion, the present study suggests that early exposure to light deprivation impairs the strategy selection in the visually deprived animals. Also, a sex difference is evident only in the light reared rats. Our findings propose that the difference between the LR and DR animals' performances comes from the behavior of males. Whether alteration of maze solving phenomena in visually deprived animals is a direct effect of decreased visual inputs to the involved brain areas or change in function of hormones requires further clarification.

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### Reprint requests

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