Early Dark Rearing Influences Spatial Performances in the Radial Arm Maze

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This study evaluates the effect of light deprivation on spatial working and reference memory. Light and dark reared rats at the age of 40 (P40) and 60 (P60) postnatal days were trained in a radial maze. Present results indicated that in the working tasks, the P40 dark reared rats outperformed their light reared counterparts. No difference in performance was observed between the P40 light and dark reared rats. In both the light and dark reared groups, the P60 animals outperformed the P40 ones on the working memory experiments. In contrast, the P40 light and dark reared groups showed superiority to the P60 animals in the reference memory tasks. Time was found to drastically undermine the effects of sensory experience by eliminating the difference between the light and dark reared rats' performances.

Key words: Age, reference memory, sensory experience, visual deprivation, working memory

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INTRODUCTION

The nervous system is able to remodel its connections in order to adjust the organism’s response to lasting changes in experience. This process is known as experience-dependent plasticity. This plasticity appears primarily during early development when neuronal circuits are originally being made. Early in development, the basic connections that define these circuits are determined genetically (Crowley and Katz, 1999; Goodman and Shatz, 1993; Tessier-Lavigne and Goodman, 1996) and the developing brain relies primarily on spontaneous activity. With the maturation of the sense organs, the developing brain relies less on spontaneous activity and more on sensory experience. The sequential combination of spontaneously generated and experience-dependent neural activity endows the brain with an ongoing ability to accommodate to dynamically changing inputs during development and throughout life (Katz and Shatz, 1996).

Experimental manipulations of sensory experience can have significant effects on the functioning of the resulting circuits (Daw et al., 1992; Hubel and Wiesel, 1970; Stern et al., 2001). Sensory experience-dependent development has been extensively studied in the visual system. Vision, in fact, is critical for the functional and structural maturation of connections in the mammalian visual system. The refinement of these connections during later development is strongly influenced by neuronal activity (Desai et al., 2002). Visual experience, however, is a subset of a more general requirement for neural activity which transforms immature circuits into the organized connections that subserve adult brain function (Katz and Shatz, 1996). The first several months of an animal’s life is a critical period for the development of the central visual pathways. During this period, response properties of neurons in the visual cortex are modifiable in response to changes in visual inputs (Hubel and Wiesel, 1970). The critical period for susceptibility to environmental influences is one of the central concepts to emerge from studies of visual system development (Lee and Nedivi, 2002). The critical period, however, is not a simple age-dependent maturational process, as visual inputs play a role in controlling the time period of visual cortical plasticity (Mower, 1991).

Like the sensory cortices, the hippocampus, a well-known area of the brain involved in spatial memory, undergoes a period of postnatal development (Dumas and Foster, 1995). Recent studies indicate that, via changes in hormonal functions, circadian rhythms influence hippocampal frameworks (Yun et al., 2004; Gonene et al., 2005). Hippocampal-cortical interactions lead to strong and persistent memories of 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). A characteristic of animals and particularly of humans is the ability to alter behaviour on the basis of experience. Learning is acquisition of the information that makes this possible and memory is the retention and storage of the information. Spatial representation, including working and reference memory, is one aspect of cognition in which the abilities of humans and animals have been extensively compared (Pouset, 1993). Working memory is memory for (task-specific) information that does not change over time while reference memory is memory for (trial-specific) information that changes over time (Gruenack and Frick, 2003). The present study was designed to explore if spatial working and reference memories are influenced by light deprivation and age during a critical period for postnatal development.

MATERIALS AND METHODS

Apparatus: The apparatus was a custom-made radial maze, elevating 70 cm off the floor. It was made of Plexiglas and consisted of 8 arms (70×15 cm) radiating from a central platform (30 cm in diameter). Vertically moving guillotine doors were located at the entrance of the arms. A small food cup was placed at the end of each arm.

Animals: Twenty-nine male and female Wistar rats, which arrived to the testing room at 40 (P40) and 60 (P60) postnatal days of age, were used in this study. The experiments were carried out on two groups of rats. One group was housed in a 12 h light/dark cycle (light reared, LR) and the other group was reared in complete darkness from birth to the end of the experiments (Dark Reared, DR). A dim red light (5 Watt) was used for daily care and maintenance of the DR animals. Each group consisted of P40 and P60 subgroups. The rats in each group were selected randomly from at least two mothers (from Pasteur Institute of Tehran, Iran) and were housed in separate 22×44×18 Plexiglas cages by sex (not more than 6 per cage). The animals had ad libium access to drinking water and food (small pellets). During the experiments, however, water was available ad libitum but a restricted feeding schedule was administered to maintain weight of the animals to approximately 85% of their initial body weights. In addition to receiving food from the cups at the end of the maze arms over testing the animals took one h daily feeding after the second trial. All efforts were made to minimize suffering of the animals used.
Procedures: The experiments were designed for evaluation of working and reference memory. The maze was located in a large test room (4.6 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 throughout the testing period. The animals were acclimatized to the testing room two days prior to the experiments.

Shaping: The rats were shaped for two days prior to the experiment. On the first day, two trials were conducted by placing the rats in the maze to obtain food rewards scattered throughout the maze. The rewards were pieces of pellets weighing about 100 and 200 mg for the P40 and P59 rats, respectively. In the first trial performed during the second day, the rats were placed in the maze and could freely explore the maze, but the food rewards were placed only in the food cups at the end of the arms. In the second trial of performed during the second day, the doors controlling the entrance to the arms were closed and opened regularly to accustom the rats with the movements of the doors.

Working memory procedures: During the working memory experiments, the animals learned to visit each of the eight baited arms once within a trial (Fig. 1A). Rats were given two trials/day with 3-4 h intertrial intervals. A trial started with the animal placed in the central platform with all doors closed. After 30 sec, all doors were opened simultaneously and the animal was free to choose an arm to enter. Then, all doors but that of the selected arm were closed. Once the rat explored the selected arm and returned to the central area, the door of the arm was closed, confining the animal for 10 sec in order to test spatial memory without interference with stereotypic behaviours. Then, all doors were opened again and the same procedure was repeated. The session continued until all the baited arms were entered or 10 min had elapsed. Experiments for each animal continued until a consecutively session in which the animal entered all 8 baited arms in 8 or a maximum of 9 selections (the criterion for the working memory tasks).

Reference memory procedures: When the animals acquired the criterion for the working memory experiments they were introduced to the reference memory experiments. Four of the 8 arms were painted white in a plus like form (Fig. 1B, C). The other 4 arms were painted black. The colour white was used as an intramaze cue to test reference memory. The same procedure described for the working memory experiments was conducted in the reference memory tests. The only exception was that the white arms were baited and the animals were trained to enter only these arms. The criterion was entering all white arms in 4 or a maximum of 5 selections. The animals were
allowed to navigate the maze for a maximum of 5 min. The reference memory experiments were performed in two phases. In the first phase, the animals were trained to acquire the criterion while the white baited arms were those numbered one, three, five and seven (Fig. 1B). In the second phase, to exclude the involvement of extramaze cues in searching the maze, the black arms were replaced for the white arms (now the arms numbered two, four, six and eight) and these arms were baited (Fig. 1C).

**Statistical analysis:** The performances were evaluated in terms of the number of errors during each trial and the number of required trials to reach the criterion as described before. The working memory errors were re-entries into the arms. The reference memory errors were entries into the black arms. Two-way analysis of variance (ANOVA) followed by the Tukey test was applied to the data to compare the results in terms of rearing condition and age.

**RESULTS**

**Spatial working memory findings:** Training the DR and LR rats for the working memory tasks revealed that, in selecting the correct arms, the DR group outperformed their LR counterparts. As shown in Fig. 2A, B the DR and LR rats performed differently in this task, $F_{1,39} = 16.48$, $p<0.0001$. The post hoc test revealed a significant difference between the P40 LR and DR rats ($p<0.0001$). The normal and visually deprived animals at P60 showed a parallel tendency in the selection of correct arms over the experiment. Concerning the criterion statistics demonstrated a significant difference between the DR and LR performances, $F_{1,3} = 6.98$, $p<0.001$). While the P40 DR animals showed superiority on LR rats ($p<0.05$). Both groups at P60 acquired the criterion within similar trials (Fig. 3).

A remarkable feature of the rats’ behaviour at P40 and P60 was a steadier performance of the DR animals than the LR ones throughout the trainings. While the DR groups, particularly at P40, began with higher performances than the LR groups, the latter displayed a better improvement as training proceeded.

**Fig. 2:** Comparison of the LR (open diamond) and DR (closed diamond) rats behaviour over the working memory experiments. A, The P40 DR rats (n = 8; 5 males and 3 females) committed fewer errors than the P40 LR rats (n = 8, 4 males and 4 females). And B, The P60 DR (n = 7; 6 males and 1 female) and LR (n = 6; 3 males and 3 females) animals declined number of the errors in a parallel way over the experiment. Each point represents the mean±SEM of each group for a single trial.

To examine the persistency of working memory, the LR and DR animals at both ages were subjected to another set of experiments (as a memory persistence test) with the same protocol 5 days after finishing the working memory experiments. During the interval period, the LR and DR animals were kept in their relative conditions and both food and water were freely available. Analysis of the data gathered from the memory persistence experiments on the LR and DR rats of different ages indicated no statistical difference. For instance, the P40 LR and DR rats achieved the criterion in 3.00±0.33 and 2.50±0.18 trials, respectively. The values for the P60 LR and DR animals were 3.85±0.59 and 4.92±1.04 trials, respectively.

**Fig. 3:** Mean number of trials required to reach the criterion during the working memory experiments. The P40 DR rats obtained the criterion in fewer trials than the LR animals at the same age. No difference was evident between the performances of the LR and DR animals at P60 *p<0.05.

We observed no sex dependency in the performances of both groups. For example, in the P40 group, the LR males and females acquired the working memory criterion in 18.80±0.57 and 17.66±2.02 trials, respectively.
Fig. 4: A comparison of the LR (open diamond) and DR (closed diamond) rats performances over the reference memory experiments. In the first phase the baited arms were the arms numbered 1, 3, 5 and 7. In the second phase the baited arms were the arms numbered 2, 4, 6 and 8. (A), While the P40 DR rats are superior to the P40 LR group during the first phase the latter make fewer errors during the second phase and (B), both groups of animals at P60 slightly improved their behaviour over the experiment. Note, however, that no variation is evident between performances of the P40 and P60 animals.

Fig. 5: Mean number of trials required to hit the criterion during phases 1 (RM1) and 2 (RM2) of the reference memory experiments. No significant difference was observed between performances of the animals over two phases of the reference memory experiments. The values for the DR males and females are 13.80±1.35 and 17.75±1.03, respectively.

Fig. 6: Age dependency of the LR and DR behaviour. The performance of the animals in the working memory (WM) tasks was improved with age. Conversely, the P40 rats were superior to the P60 animals in either the first (RM1) or second (RM2) phases of the reference memory tests.

Spatial reference memory findings: General analysis of performances in the first phase of the reference memory tasks indicated that the animals behaved differently in choosing the correct arms, F_{3,27} = 4.92, p<0.003. Similarly, the animals needed a different number of trials to acquire the criterion, F_{1,21} = 9.35, p<0.001. However, the post test revealed that, regardless of age, the differences in performing the task were not significant in either selecting correct arms or hitting the criterion.

Analysis of the data taken from the second phase of the reference memory tests demonstrated that the DR and LR groups were not different in number of errors (Fig. 4A, B). Also, the LR and DR rats showed no differences in achieving the criterion for the task in the second phase (Fig. 5).

Comparison of the 40 and 60 days old animals' performances: We found that the differences between the 40 and 60 days old rats were more comparable based on the criterion. Thus, focusing on the criterion, comparisons were made between the LR and DR performances. The working memory tasks demonstrated that both P60 LR and DR animals outperformed their counterparts at P40, F_{3,16} = 6.98, p<0.001. The post test indicated that the difference was significant between the P40 and P60 LR rats (p<0.008). Alternatively, comparison of the data pooled from the first phase of the reference memory tests showed that the younger rats outperform the older ones in this task, F_{3,16} = 9.35, p<0.0001. During this phase of the reference memory experiment, the P40 rats show superiority to the P60 LR rats (p<0.002). Also, within the DR group, the P40 rats outperformed the P60 animals (p<0.0001). Analysis of the data from the second phase of
the experiment also revealed an age-related performance of the animals at P40 and P60, $F_{3,39} = 3.299$, $p<0.05$. The post test indicated a superiority of the LR rats at P40 to those at P60 ($p<0.03$). Figure 6 compares the LR and DR performances across age.

**DISCUSSION**

Learning and memory phenomena are found upon the basis of consolidating information entering the brain. The information can be stored in many parts of the brain including the neocortex and the hippocampus. Particularly, the hippocampus is critical for spatial learning and memory. It is also well known that the visual system is a main source of sensory input into the nervous system. There are numerous reports addressing how the lack of visual experience prominently affects the candidate mechanisms of learning and memory, i.e. long-term potentiation and long-term depression (Berry et al., 1993; Fathollahi and Salami, 2001; Katz and Shatz, 1996; Kirkwood et al., 1996; Salami et al., 2000; Sermasi et al., 1999). From the behavioural point of view, however, evidence is scarce (Tees et al., 1981; Prusky et al., 2000). On the other hand, despite extensive investigation on age dependency of spatial memory in old animals, little is known about young animals. Here, the present results discuss an interaction of the sensory experience and age on the spatial working and reference memory tasks.

The comparison of the working and reference memory task performances made by the P40 rats suggests a substantial difference between the LRs and DRs. It appears that the DR rats have a greater capability to solve the working memory tasks than the LR rats. In addition, compared to the LR group, the DR group seems to perform better in the reference memory tasks. An important point in the behavior of the P40 rats is that the DR rats began with a higher performance than the P40 LR rats. On the other hand, as illustrated in Fig. 3 and 5, the LRs displayed a better improvement through the proceeding trials resulting in a similar performance of the LR and DR rats in the second half of the working and reference memory experiments. From these results, it is concluded that early visual experience effectively influences spatial representations. Inconsistent results on LR and DR performance are reported by Tees et al. (1981). They found that visually deprived animals make significantly more errors than normally reared animals. In another study, Tees et al. (1990) also reported that visually experienced rats proved to be more competent than their DR counterparts in water maze task. Jarrard (1983) reported that a high performance in the radial

**amaze is dependent on the integrity of the hippocampal formation.** The hippocampus, in turn, receives sensory inputs from related cortices directly (Yukie, 2000) and indirectly, via entorhinal cortex (Lavenex and Amaral, 2000). Therefore, it can be concluded that the early exposure to visual deprivation influences the cognitive function of the hippocampus. Here, a role can be contributed to the pineal gland and its hormone, melatonin. This gland in mammals is connected to the network of nerves that activates or inhibits the enzyme system responsible for the synthesis of melatonin. Darkness stimulates melatonin release while light inhibits it (Presl, 1993). Some evidence suggests that melatonin may modulate cognitive plasticity. The potential mechanisms by which melatonin may modulate cognitive plasticity are examined within hippocampal long-term potentiation frameworks (Yun et al., 2004). Melatonin that increases glutathione peroxidase activity in the rat hippocampus has a positive effect on water maze performances (Gonene et al., 2005). In addition, melatonin treatment has reversed cognitive deficits in aged and ethanol intoxicated mice (Raghavendra and Kulkarni, 2001). Consequently, if darkness leads to an increase in melatonin synthesis, it would be reasonable to contribute a substantial role to melatonin in modulating spatial representations in visually deprived animals. Inconsistent results, however, are reported where application of melatonin in rats significantly inhibited spatial learning and memory (Feng et al., 2002).

The experiments on the P60, in contrast to P40, reveal that the performances in the LR group resemble those in the DR group. This means that, regardless of rearing condition, only 3 weeks of elapsed time is sufficient to mask the difference between normal and visually deprived rat performances. The P60 LR rats showed considerable differences on the working as well as reference memory tasks in respect to the P40 rats. In relation to the working memory, the older animals outperformed the younger animals. In the reference memory experiments, however, the P40 animals showed superiority to their P60 counterparts. Although the rats in DR groups also followed a similar pattern of behaviour; the variations were less pronounced compared to the LR animals. It seems that the age prominently affects performance of the LRs rather than the DRs. Hence, the present results also confirm an age dependency in spatial memory performances; however, it differently underlies the working and reference memory. Again, a role is can be attributed to melatonin where it reserved cognitive deficits associated with age in mice (Raghavendra and Kulkarni, 2001). Altogether, the present results
demonstrate that the early exposure to light deprivation leads to a better performance in these spatial tasks. Time significantly affects this ability so that it makes the difference across only three weeks. Thus, it can be concluded that the sensory experience dependency of spatial memory declines with age.

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