Dark and light rearing during early postnatal life impairs spatial learning of rats in Morris water maze

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ABSTRACT

In early postnatal life, sensory-driven processes deeply affect structure and function of sensory cortices. Because some visual signals pass from visual cortex to the hippocampal formation, the aim of this study was to investigate the effects of change in visual experience on rat’s spatial learning and memory. This experimental study was carried out on 30 Wistar male rats (45 days old) which were randomly distributed into 3 groups; the CO (Control group) was in 12 light/12 dark cycle through birth to the end of the study, the LR (Light Reared) group was in complete lightness and the DR (Dark Reared) group was in complete darkness (n=10 for each). Using MWM (Morris Water Maze), the animals learned to find a hidden platform for 4 trials per day during 5 days. After removing the platform, spatial memory was tested at day 5 in one trial (probe trial). Our results indicated that in the learning stage, the CO rats spent less time and distance to find the hidden platform than the other groups. There was no difference between all groups in probe trial. Change in visual experience impairs spatial learning of rats in Morris water maze and their spatial memory formation is not influenced.

Keywords: Visual Experience; Critical Period; Spatial Learning and Memory; Morris Water Maze; Rat

INTRODUCTION

Critical periods are specific windows during development when both genetic driven and environmental processes interact to establish functional characteristics [1]. On the other hand, critical period is a time window wherein the growing brain is most malleable and shows heightened responsiveness to external environmental influences [2]. Neuronal circuits in the brain are shaped by experience during ‘critical periods’ linearly postnatal life [3]. It is now understood that many regions of the brain have critical periods that occur at different times and are activated and regulated by distinct mechanisms [3]. With maturation of the sense organs, the developing brain relies less on spontaneous activity and increasingly on sensory experience [4]. Environmental experiences and training are known to induce changes in cerebral cortex including neurochemical, altered cortical thickness, size of synaptic contacts and dendritic structure as well as improving performance on learning tests. For example, it has been revealed that, espousing central nervous system to environmental enrichment in critical period induces robust neuronal plasticity in both intact and injured central nervous system, including improved spatial learning and memory function [5-7]. Vision is critical for the functional and structural maturation of connections in the mammalian visual system. Visual experience, however, is a subset of a more general requirement for neural activity in transforming immature circuits into the organized connections that sub-serve adult brain function [4].The critical period for susceptibility to environmental influences is one of the central concepts to emerge from studies of visual system development [8]. It is also indicated that changes in the external visual environment can alter preexisting neuronal connections and this phenomenon has been well studied in the visual cortex [2, 9]. Consistent with this idea, dark rearing has been shown to delay onset of the critical period [10,11].

The visual cortex provides a crucial sensory input to the hippocampus, and is a key component for the creation of spatial memories [12]. Spatial memory is the ability to encode, store and retrieve information about spatial layouts in the environment, thus enabling the learning of a path between two points or remembering the location of objects
The hippocampus receives sensory inputs directly [14], from the neocortex, as well as indirectly, converging on the Entorhinal cortex [15]. The sensory cortices, the hippocampus undergoes a period of postnatal development [16]. Much of hippocampal synaptogenesis occurs postnatally and hippocampal synapses exhibit plasticity before the emergence of mature behavioral function [17]. It is the maturation of these anatomical and physiological properties that is thought to underlie the emergence of adult-like behavior [18]. Therefore, the postnatal development of the hippocampus seems similar to that of the primary sensory cortices; it undergoes a period of synaptic plasticity before the emergence of mature behavioral function. These developmental similarities suggest that the hippocampus may also undergo a postnatal sensitive period, during which neuronal activity shows increased responsiveness to environmental stimulation [16].

An understanding of how visual cortical neurons respond with synaptic plasticity to visual experience, and whether these responses influence the induction of hippocampal plasticity, is fundamental to our understanding of the neuronal mechanisms and functional consequences of visuo-spatial information processing [12]. Thus, the aim of this study was searching about effects of change in visual experience during early postnatal life on spatial learning and memory of rats.

MATERIALS AND METHODS

Thirty male Wistar rats were randomly assigned to 3 experimental groups: One group was normally reared on a 12 light/dark cycle (Control, CO). The two other groups were maintained either on a total lightness (Light reared, LR) or total darkness (Dark reared, DR) from birth through experiment. Food and water were continuously available in the home cages ad libitum. The temperature (22±2°C) and humidity (55±5%) were kept constant. All procedures were performed in strict accordance with the Deputy of Research, Kashan University of Medical Sciences.

There were no significant changes in animal weight all over the experiments. Testing was conducted in the Morris water maze (a tank made of galvanized metal, 160 cm diameter, 80 cm depth) that was filled with water (22°C) up to 20 cm below the rim. The pool was divided into four quadrants of equal area arbitrarily called northeast, southeast, southwest and northwest. A removable, circular platform (10 cm diameter) was submerged 1.5 cm below the water surface and located in the center of one quadrant of the pool, remained in the same position throughout the experiment. The testing room contained a number of extra-maze visual cues (including some geometric shapes, posters, objects, and equipment) remained in fixed situations over the experiment. The latency and swim paths of the rats were monitored by a closed-circuit video camera and a computerized tracking system. Behavioral variables were quantified with the aid of a customized-software (Radiab 7, I. R. Iran).

Behavioral testing

On each day, subjects were moved to the testing room 30 min prior to the experiment. Each subject was placed by the tail into the water, immediately facing the perimeter, at one of four start points (north, south, east or west). Upon release into the water the rat was allowed to swim for a maximal time of 90 s. In the behavioral training, the animals were required to locate a hidden platform that remained in the same position in relation to external visual cues. Once the maximum time had elapsed and the subject failed to find the platform (which usually happened during beginning trials), it was gently set on it at the end of the trial where it remained for 15 s. After a period of 15 s on the platform, the rat was immediately re-placed in the pool. The starting location was varied in each trial, and the sequence of start locations was randomly determined by the software. Following the completion of the trial, the rats were dried and returned to their home cage. The training procedure took 5 consecutive days, each subject having four training trials per day, with a 15 min inter-trial interval.

Spatial memory retrieval test (Probe trial)

On the day 5 and after the last run of training in each experiment finished, rats were given a probe test to evaluate the animals’ retrieval performance of spatial memory under full-cue conditions. The platform was removed from the pool and the animals were released from the quadrant opposite to where the platform was and allowed to swim freely for 90 s to assess their memory for the platform location. Spatial retention in probe trials was measured by the percentage of total time spent in the platform (target) quadrant.
The time schedule for the experiments was constant for all groups over 5 days and each numbered animal had faced the maze the same way as the order of the other days.

Analysis
The total time required to find the platform (s) and the distance swum by the rat from the point of release of the animal until it finds the platform (cm) were considered as indicators of learning in the training experiments. The mean speed (cm/s) of animals in the maze was considered as an index for locomotion activity, also. The time during which the animal remained in the proper quadrant of the water maze and the distance traveled by the rats in this area were measured for the probe trials. For the training experiments, the data are presented as a mean±SEM for each group in each trial. However, for more illustration, trials were collapsed in blocks of 4 trials. For the probe trials, the mean±SEM for each group is reported. A repeated measures ANOVA was performed for learning trials using the SPSS software (version 17.0). The Bonferroni Post hoc analysis was applied if statistically significant was observed for interactions. Probe trials data were compared by the ANOVA test. P<0.05 was considered statistically significant.

RESULTS

A- Learning trials
a- Time spent to find the platform:
Concerning the time spent in the water maze, the repeated measures ANOVA test indicates significant difference between all groups ($F_{2,117}=9.162; P<0.001$). As illustrated in figure 1, spatial navigation of the control, LR and DR animals in the water maze indicate that, the three groups improved their behavior over 5 days of training. However, the control rats displayed a better function compared with both LR and DR’s ($P=0.012$ and $P<0.001$, respectively). The CO animals preserved their superiority through experiment by the last day of testing. Although, the LR rats generally exhibit a higher behavior in locating the platform as did the DR ones, however, no significant difference was observed between the two groups ($P=0.679$).

![Figure 1](image1)

*b- Distance spent to find the platform:
Statistical analysis also, indicates that, the subjects in the different groups vary in the distance traveled in the Morris Water Maze ($F_{2,117}=15.459; P=0.001$). Figure 2 shows that the path traveled by the rats in CO group was significantly shorter than that traveled in LR and DR animals ($P=0.005$ and $P=0.001$, respectively), but no significant differences were observed between DR and LR groups ($P=0.941$).
Figure 2. The distance traveled in the maze to find the platform by the control (CO, open triangles), dark reared (DR, solid squares) and light reared groups (LR, open squares) during 5 days of water maze training. Each data point indicates mean±SEM summed results of four daily trials. The statistical analysis showed that there was a significant difference between CO and LR (P=0.005), moreover, CO and DR (P=0.001) rats.

c- Mean speed of animals in the maze:
Considering the mean speed of animals in the maze during 5 days of training (figure 3), the statistical analysis indicates that there was no significant difference between all groups ($F_{2,117}= 8.273; P=0.769$).

Figure 3. The mean speed of different groups in the maze during 5 days of training each data point indicates mean±SEM summed results of four daily trials. The statistical analysis showed that there was significant difference between all of groups.

B- Retrieval trial
a- Time spent in correct quadrant:
Animals of all groups were introduced to the probe trials on day 5 after finishing the acquisition stage of experiments. As indicated in figure 4, in missing platform trials, there was no significant difference between all groups in time ($F_{2,25}= 2.191; P=0.131$) spent in the platform-containing quadrant. Figure 5, is the schematic illustration of pass traveled in this stage by rats in different groups.
Figure 4. Spatial memory was evaluated in a probe trial on days 5. The histograms represent the mean time spent (seconds) in the correct zone (training quadrant) of the Morris Water Maze on 90 s probe trial. There was no significant difference between all of groups.

Figure 5. The schemas show the pool with the platform location (small circle) and the pass traveled by the CO, LR and DR animals.

b- Distance spent in correct quadrant:
Concerning the traveled distance passed in the correct quarter of the maze, there is no significant difference between all of groups ($F_{2,29} = 1.381; P=0.269$). Figure 6 shows the distance traveled by the 3 groups in the correct zone (training quadrant) of the Morris Water Maze on 90 s of probe trial.
DISCUSSION

Our results indicate that animals with change in visual experience (reduce or enhance light in normal light cycle; LR and DR groups) unlike animals with normal light cycle (12h light/12h dark; CO group) had a significant deficit in spatial learning. This change had no significant effect on locomotion activity of rats, also. Light- and dark-reared animals, however, did not differ in their ability to locate a flagged platform following spatial memory stage. These data indicate that change in visual experience in rats can influence measurement of their spatial learning.

It is revealed that, sensory experience during the postnatal critical period is essential for the fine-tuning of circuits in sensory cortices [19] and normal maturation of visual cortical circuits and function [8]. Lack of a sensory input not only alters the cortical circuitry sub serving the deprived sense, but also produces compensatory changes in the functionality of other sensory modalities [20]. It is generally accepted that the developing mammalian visual system progresses through “sensitive” or “critical” periods early in life, in which mature vision is facilitated through adaptive feedback from the visual environment, wherein visual deprivation leads to loss of visual function [21]. Thus, visual deprivation is effective only during an early ‘sensitive’ period, which is lengthened by dark rearing. The primary visual cortex of DR animals is not fully plastic, indicating a role for light stimulation in inducing visual cortical plasticity [22]. Visual deprivation during the critical period has catastrophic effects on visual function, including loss of visual responsiveness to the deprived eye, reduced visual acuity, and loss of tuning to many stimulus characteristics. These changes occur faster than the remodeling of thalamocortical axons, but the intracortical plasticity mechanisms that underlie them are incompletely understood [23]. Visual deprivation such as dark rearing (DR) prolongs the critical period for ocular dominance plasticity and retards the maturation of gamma-aminobutyric acid GABAergic inhibition in visual cortex [24].

Undoubtedly, information traffic between sensory cortices and the hippocampal formation play a key role in data processing for learning and memory. Evidence both in human [25] and animal models [26] indicates that the early stages of memory processing take place in the hippocampus and that later they rely on cortical–cortical connections [25] or cortical–hippocampal networks [27]. The hippocampus-dependent spatial learning and memory are correlated closely with hippocampal LTP, impairment of which often leads to memory deficits [28].

Environmental enrichment –an example of change in sensory experience- potentiates neural plasticity, enhancing acquisition and consolidation of memory traces. In the sensory cortices, after cortical circuit maturation and sensory function acquisition are completed, neural plasticity declines and the critical period ‘closes’. In the visual cortex, this process can be prevented by dark-rearing, and
it is showed that environmental enrichment can promote physiological maturation and consolidation of visual cortical connections in dark-reared rats, leading to critical period closure [29]. In rats, environmental enrichment induces structural and biochemical changes in the brain that correlate with better learning and memory, and these effects are commonly attributed to the interaction of complex social and inanimate stimulation [30]. Enrichment-induced enhancement of learning and memory might relate to cellular effects on synaptic plasticity and hippocampal neurogenesis, although a recent study suggests that increased hippocampal cell proliferation is not necessary for improved spatial memory performance [31]. Melatonin secrets in the dark phase of circadian cycle and it is suggested that the level of the hormone would be the highest in the DR and the lowest in the LR rats [32]. Evidences propose that melatonin could be a regulator in the processes contributing to memory formation and synaptic plasticity in different regions of brain including the hippocampus [33-37]. Electrophysiological studies have also revealed that melatonin may regulate the electrical activity of hippocampal neurons [37, 38] and alter synaptic transmission between hippocampal neurons [39-42]. A significant negative correlation is reported between blood melatonin levels and spatial memory performance in both male and female adolescent rats, suggesting an association between melatonin production and neurodevelopment [43]. Soto-Moyano and his colleagues also found that melatonin administered rats have a greater number of the errors committed and the time spent to solve the visuo-spatial task in the radial maze [44]. Thus, it can be said that the increased melatonin in the DR animals weaken the spatial task learning. However, Gonenc, et al reported that melatonin positively affected water maze performance of ethanol treated rats [45]. The role of melatonin in improvement of learning and memory performances impaired by ethanol [46] or hyperhomocysteinemia in rats [47] is also reported.

There are some reports about attenuating the effect of exogenous melatonin on psychomotor attention [48] or nocturnal activity [49], however, change in circadian rhythm of our rats could not influence their locomotion activity in maze. Immaturity of brain might be another possibility of poor function of the DR animals in learning the maze task. At least a main effective factor in developing brain is environmental inputs to the brain [50] and sensory deprivation delays maturity of the brain function [51,52]. Evidence supports susceptibility of different areas of brain such as visual cortex [53] and hippocampus [6,54] to sensory experience. Hence, decrease or lack of visual experience may lead to weakened cortical processing relating in the spatial task learning.

The time of testing could be the reason of low performance of the LR rats compared to the CO ones. The LR animals used in the present study were reared in a constant light throughout life. This condition influences circadian rhythm of the animal activity so that the light adapted rats as nocturnal animals were failed to have normal maze navigation as controls. Here again the effort of light-keeping animals in searching the water maze could be optional in locating the hidden platform. Ma, et al showed that three weeks exposure to constant light caused the animals to show a shorter spent time during initial phase of spatial learning, but impaired hippocampus-dependent spatial memory in Morris Water Maze [55].

Overlay, since memory related activation in the hippocampus and parahippocampus are affected by time of day and melatonin, and circadian clock and melatonin implicate in human memory processing during night [35], it can be concluded that the circadian rhythm modification-dependent changes may account for the poor performance of the LR and DR animals.

Our results illustrated that change in visual experience in early postnatal life of rats impairs spatial learning and their spatial memory formation isn't influenced.

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