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Research report

Regional vulnerability of the hippocampus to repeated motor activity deprivation

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HIGHLIGHTS

- Rearing-deprived rats indicated levels of emotional disturbances after rearing deprivation.
- HPA system was significantly affected by chronic rearing deprivation.
- Spatial search strategies were considerably influenced by the deprivation protocol.
- Hippocampal CA2-specific vulnerability was observed in relation to rearing deprivation.

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ABSTRACT

Spontaneous vertical and horizontal exploratory movements are integral components of rodent behavior. Little is known, however, about the structural and functional consequences of restricted spontaneous exploration. Here, we report two experiments to probe whether restriction in vertical activity (rearing) in rats could induce neuro-hormonal and behavioral disturbances. Rearing movements in rats were deprived for 3 h/day for 30 consecutive days by placing the animal into a circular tunnel task. Rats temporarily deprived of rearing behavior showed elevated plasma corticosterone levels but no detectable psychological distress and/or anxiety-related behavior within an elevated plus maze. However, rats emitted a greater number of 22-kHz ultrasonic vocalizations and spent significantly more time vocalizing than controls when deprived of their rearing behavior. Despite intact spatial performance within wet- and dry-land spatial tasks, rearing-deprived rats also exhibited a significant alteration in search strategies within both spatial tasks along with reduced volume and neuron number in the hippocampal subregion CA2. These data suggest a new approach to test the importance of free exploratory behavior in endocrine and structural manifestations. The results support a central role of the CA2 in spontaneous exploratory behavior and vulnerability to psychological stress.

1. Introduction

From an evolutionary viewpoint, animals' exploratory behavior including horizontal and vertical movements [36] serves the purpose to determine benefits (e.g., food and partners) and recognize potential dangers (e.g., predators) in a novel environment [2,23]. Specifically in rats, this behavior not only represents an organized sequence of trips and progressions with variable direction and speed [67,70] but also includes an unpredictable number of stops each of which is mostly linked to vertical or rearing behavior. Rearing in rats during exploration can be observed when the animal intermittently discontinues its horizontal activity and rears up by lifting its forelimbs from the ground. In this position, the animal

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usually seems stationary and appears to be visually inspecting the environment [3]. Thus, in conjunction with horizontal movements, rearing plays a key role in the integrity of exploratory behavior.

Vertical activity along with its potential cognitive importance in exploration also is a manifestation of emotional state. Rearing during open-field exploration was previously regarded as reflecting emotional components of behavior [27]. If this is the case, it seems justified to hypothesize that rearing deprivation could impair emotional behavior along with the integrity of free and goal-directed exploration.

Importantly, the neuroanatomical correlates of vertical activity indicate a close correlation between the hippocampal morphology and the occurrence of rearing in rats and mice [13,54,12,29,62,1]. For example, cholinergic responses in the hippocampus seem stronger in animals which show more frequent rearing behaviors during open-field exploration [61]. Also, animals with a higher rate of rears show larger intra- and infra-pyramidal mossy fiber projections in hippocampus compared to their less frequently-rearing counterparts [31]. However, the hippocampal regional involvement in vertical activity has not yet been directly investigated.

The present experiments were designed to examine the neurohormonal, emotional and behavioral consequences of reduced exploratory movements, with particular focus on vertical activity (rearing). Rats were deprived of rearing for a limited time each day by placing animals into a circular tunnel task that resembled features of a rat's natural environment. The tunnel segment in a rat burrow allows only one rat to pass at a time without space for rearing. The burrow usually ends in a cup-shaped nest or dead end where the rat establishes a colony, spends extended periods of time and, more importantly, is free to show vertical activity. Here, we examined whether restricted rearing influences the activity of the hypothalamic-pituitary-adrenal (HPA) axis, a system that is intimately linked to psychological distress and hippocampal function [55]. The experiments investigated established endocrine (corticosterone and glucose), affective (anxiety-like behavior and ultrasonic vocalizations) and cognitive (spatial learning and memory) manifestations of altered HPA axis activity [19,23]. Furthermore, we asked whether rearing deprivation induces regional alterations in the hippocampus based on evidence that changes in rearing frequency are reflected in altered hippocampal morphology and function [1]. Results of the present study indicate neuro-hormonal disturbances and regional hippocampal vulnerability to restricted exploratory activity. The results support a central role of the CA2 in exploratory hippocampus-dependent behaviors and vulnerability to psychological stress.

2. Materials and methods

This study involved 29 male Wistar rats (8–9 weeks old). The animals were housed in pairs under a 12:12 h light/dark cycle with light starting at 07:30 h. Animals were provided with water and food ad libitum. The room temperature was set at 22 °C, and experimental procedures were conducted during the light phase of the cycle at the same time of day. All procedures were approved by the Avicenna Institute of Neuroscience (AIN) Animal Care Committee and were carried out in accordance with NIH guidelines.

3. Experimental procedures

3.1. Experiment 1 Vertical activity deprivation and spatial performance in wet- and dry-land tasks

Fourteen rats (control, $N = 6$; deprived, $N = 8$) in this experiment were trained and tested before and after vertical activity deprivation for spatial performance in two tasks, the Morris water task (MWT; [59,49] and the ziggurat task (ZT; [20]. All behavioral analyses were performed by an experimenter blind to the group identities.

3.1.1. Assessment of vertical activity

Preliminary assessment (pre-test) of vertical activity (number of rears) during free navigation was performed before rearing deprivation using a square black open-field arena (70 × 70 × 35 cm) under dim illumination. Rats were individually placed in the middle of the field, and the number of rears was counted for 10 min regardless of whether rears occurred on or off the walls [61]. The same procedure for counting the number of rears was used for the post-test session. Vertical activity in this experiment was scored when rats reared on their hind limbs with their forelimbs unsupported. During a rearing movement, the rat appears stationary with slow or absent whisker movement [3].

3.1.2. Morris water task (MWT)

A hidden platform version of the MWT was employed to assess spatial performance [19]. Briefly, animals were taught to escape from the water (22 ± 1 °C) by climbing onto the hidden platform. Each trial began with the rat being placed in the pool at one of the four cardinal compass positions around the perimeter of the pool according to a pseudo-random sequence. The location of the hidden platform remained constant from trial to trial. Thus, we were able to assess trial-independent spatial learning. The maximum duration of each swim trial was 60 s. Animals in this experiment were tested in 12 trials for one day before (pre-test) and after (post-test) vertical activity deprivation. Latency and path speed were recorded and analyzed by an image-computerized tracking system (HVS Image, UK). A no-platform probe trial was also performed two hours after the completion of the single session hidden platform testing as an additional measure for reference memory. The platform was removed from the pool and the rats were allowed to swim freely for 30 s. Because in one of our pilot studies both aged ($N = 7$) and young ($N = 5$) rearing-deprived rats showed (data not presented) more thigmotaxis (wall hugging behavior) and circling (swimming in tight circles; [7] within the MWT, the number of circling during spatial navigation in the task was manually calculated later via the path graphics for each animal generated by the tracking system.

3.1.3. Ziggurat task (ZT)

The procedures for ZT testing were previously reported [21]. Briefly, animals were food-restricted one week prior to habituation sessions and spatial testing in the ZT, and maintained at about 90–95% of their initial body weight throughout the experiment. To maintain body weight, rats were given an additional amount of food in their home cage at least 2–3 h after completion of the behavioral training and testing. Animals were weighed daily throughout the experiment in order to monitor food consumption.

Rats were habituated to the ZT environment for four days. After habituation, the testing sessions were conducted over 12 trials per day within the standard version of the ZT, and began the day immediately following the last session of habituation. Two sets of ziggurats were defined in the arena. First, "start" ziggurats, located in each corner, and second, the rest of ziggurats or "goal" ziggurats [21]. On the testing day, rats were released from each starting point and allowed to explore the environment. One central goal ziggurat was baited with spaghetti for each trial. During each testing trial, rats started from one of four different starting points in a randomized sequence. Across trials, the starting location varied among the four corners of the apparatus, and on each trial, animals navigated in the environment for 80 s or until they found the central goal ziggurat. It should be pointed out that the location of the goal ziggurat remained constant from trial to trial.