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Full-length Article

Early enriched physical environment reverses impairments of the hippocampus, but not medial prefrontal cortex, of socially-isolated mice

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ABSTRACT

Early social isolation (SI) produces a variety of emotional, behavioral and cognitive abnormalities. Conversely, environmental enrichment (EE), a complicated social and physical construct, offers beneficial effects on brain plasticity and development. However, whether or not exclusive physical EE is sufficient to reverse the adverse consequences of early SI remains unclear. Here we reported that 1 month-old solitary mice housed in the EE for 8 weeks corrected spatial cognitive dysfunction, but did not ameliorate social interaction deficits and increased anxiety-like behavior. Pathological analyses revealed that the enriched environment decreased cellular apoptosis, synaptic protein loss, myelination defect and microglial activation in the hippocampus, but not medial prefrontal cortex (mPFC) of mice housed singly. Moreover, increased nuclear factor-kappaB and interleukin-1β levels, and downregulation of brain-derived neurotrophic factor signaling pathway were normalized in the hippocampus rather than mPFC of these animals. Our results revealed a brain region-specific effectiveness of physical EE in remediating brain impairment of adolescent SI mice, with a complete reversal of hippocampus-dependent cognitive dysfunctions, but without mitigation of mPFC associated anxiety and social interaction defects. This finding emphasizes the irreplaceable role of social life for the early brain development.

1. Introduction

A social life with useful communication is critical for humans as social creatures (Holt-Lunstad et al., 2010; Yang et al., 2016). Conversely, long-term social isolation (SI), defined as a lack of participation in social relationships and/or a complete or near-complete lack of interaction with others, is deleterious to both the physical and mental health of individuals (Grippo et al., 2007; Friedler et al., 2015; Tzanoulinou and Sandi, 2017). The deleterious effect on the central nervous system is particularly obvious during early development, because of the high rates of neurogenesis and synaptogenesis (Blakemore, 2008; Fuhrmann et al., 2015). Moreover, accumulating epidemiological evidence show that adults who suffer from SI early in life have a higher incidence of neuropsychiatric disorders, such as depression, schizophrenia, mania, and ethanol or drug abuse (Heinrich and Gullone, 2006; Blakemore, 2008; Khandaker et al., 2014; Yang et al., 2016). Therefore, it is worth examining potential interventions to reduce or even eliminate SI-induced neurodevelopmental impairments.

In contrast to the detrimental effects of SI, individuals experiencing a childhood enjoying environmental enrichment (EE), such as receiving a wealth of verbal communication, free exploration and affectional relationship, often have better intelligence levels and social interaction abilities, and less incidence of depression and anxiety-relevant behaviors (Christensen et al., 2014; Tost et al., 2015). In rodent studies, EE not only includes enriched physical environment, a large housing room is filled with novelties and toys that are routinely changed during the experimental period, but also denotes complex social interactions, where 4–6 littermates live together (van Praag et al., 2000). Growing evidences reveal that the beneficial effect of EE on animal cognitive functions is associated with improving dendritic branching and length, synapse plasticity and hippocampal neurogenesis (Nithianantharajah and Hannan, 2006; Wolf et al., 2006; Lonetti et al., 2010; Garthe et al., 2016). However, whether enriched physical environment referring to increased housing space equipped

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with novel items is exclusively sufficient to ameliorate or even eliminate SI-induced abnormal neuropsychiatric behaviors remains to be determined.

In the present study, we assessed therapeutic effects of EE on learning and memory, social interaction and anxiety-like behavior of mice that, 1 month since their birth, were housed singly for 8 weeks. Our results reveal that there is a brain region-specific effectiveness of enriched physical environment in remediating brain impairment of young adult SI mice, with a complete reversal of hippocampus-dependent cognitive dysfunctions, and without mitigation of medial prefrontal cortex (mPFC)-associated social interaction defects and anxiety.

2. Materials and methods

2.1. Animals

The experimental design was shown in Fig. 1A. Following weaning at postnatal day 30, male CD1 mice from each litter were randomly assigned to four groups (n = 16 in each group): group housing (GR), social isolation (SI), group housing in an enriched environment (GR + EE), and social isolation housing in an enriched environment (SI + EE). Mice in the GR + EE and SI + EE groups were housed in large plastic cages, measuring 47 cm × 30 cm × 23 cm. Cages contained a variety of objects, i.e., toys, ropes, hanging platforms, wooden houses, tunnels, ladders, running wheels and nesting materials to enhance sensory, cognitive and motor stimulation (Nithianantharajah and Hannan, 2006). The contents of each cage were renewed every 4 days for ensuring novelty of the items to the animals. The relative positions of the objects were also rearranged for spatial variability. Animals in the GR and SI groups were housed in standard plastic cages (31 cm × 22 cm × 15 cm) without any exposure to stimulation. Littermate pups were housed in GR or GR + EE group of four mice per cage, while only one mouse was housed in each cage in SI or SI + EE group (Fig. 1B). All animals were kept in their respective experimental conditions until behavioral experiments were carried out two months later.

Mice were maintained at a constant room temperature (18–22 °C), with a relative humidity of 30–50%, controlled illumination (12:12 h light/dark cycle), and food and water were available ad libitum. All experiments were conducted in accordance with international standards on animal welfare and the guidelines of the Institute for Laboratory Animal Research of Nanjing Medical University.

2.2. Behavioral tasks

2.2.1. Y-maze test

The Y maze consisted of 3 arms, named as novel arm (NA), starting arm (SA), and other arm (OA). The test contains two 5-min stages, including training stage and testing stage, with an interval of 2 h. The NA was blocked by a black baffle during the first stage, but was opened during the second stage. The time spent in the NA and the numbers of entries into the NA were calculated (Huang et al., 2016).

2.2.2. Elevated plus maze test

The elevated plus maze consisted of four arms (50 cm × 10 cm) and a central square (10 cm × 10 cm) connecting these arms, which was elevated 100 cm above the floor. Two opposite arms were open, while the remaining opposite arms were closed with 40 cm high walls. Each mouse was placed into the maze center facing an open arm, and left to freely explore the apparatus for 5 min. Arm entry was defined as entering an arm with all four paws. The open arm duration and entries were calculated (Huang et al., 2016).

2.2.3. Social interaction test

The social interaction testing apparatus included three plastic compartments (40 cm × 40 cm × 30 cm), separated by two doors

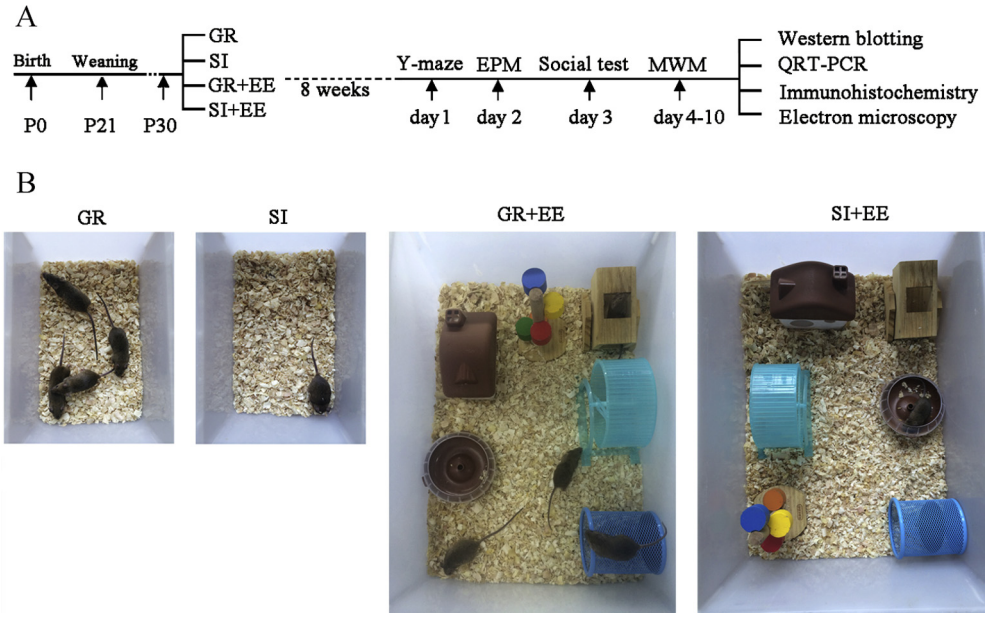

Fig. 1. (A) Schematic representation of the experimental design. Animals were randomly assigned to four groups (n = 16 in each group): group housing (GR), social isolation (SI), group housing in an enriched environment (GR + EE), and social isolation housing in an enriched environment (SI + EE) at postnatal day 30. The four groups animals lived in respective housing conditions for 8 weeks and then were tested by the Y-maze on day 1, the elevated plus maze (EPM) on day 2, the social interaction test (social test) on day 3 and the Morris water maze (MWM) on day 4–10. Animals were sacrificed (S) 1 h after MWM. Western blotting, quantitative real-time PCR, immunohistochemistry and electron microscopy were performed on 4 mice per group, respectively. (B) Living conditions of different experimental groups. Mice in the GR and SI groups were housed in standard plastic cages (31 cm × 22 cm × 15 cm). Mice in the GR + EE and SI + EE groups were housed in large plastic cages (47 cm × 30 cm × 23 cm) consisting of social interaction (4 mice in the cage), stimulation of exploratory behavior with objects, and a running wheel for exercise.