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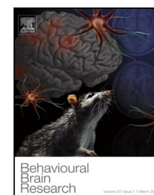

Research report

Chronic social isolation decreases glutamate and glutamine levels and induces oxidative stress in the rat hippocampus

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HIGHLIGHTS

- SI increased anxiety level but impaired social interaction and spatial working memory.
- SI decreased levels of glutamate, glutamine, and phosphocreatine in the hippocampus.
- SI increased levels of H_2O_2 in caudate putamen and hippocampus.
- SI decreased activities of some antioxidant enzymes.

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ABSTRACT

Social isolation (SI) rearing of rodents is a developmental manipulation, which is commonly compared with the psychological stressors in humans as it produces several behavioral outcomes similar to those observed in humans with early life stress. To explain the SI-induced behavioral outcomes, animal studies have been performed to examine the dopaminergic and glutamatergic systems in the brain. In this study, we measured possible changes in levels of glutamate and glutamine of SI-rats using proton magnetic resonance spectroscopy. We also assessed the oxidative stress parameters in certain brain regions to see if glutamate and/or glutamine changes, if any, are associated with oxidative stress. SI rearing for 8 weeks decreased the activities of antioxidant enzymes catalase, glutathione peroxidase, superoxide dismutase, and the total antioxidant capacity, but increased levels of hydrogen peroxide, in certain brain regions, of which prefrontal cortex and hippocampus were most vulnerable. It also decreased levels of glutamate, glutamine, N-acetyl-L-aspartate (NAA), and phosphocreatine in the dorsal hippocampus, but not in the cerebral cortex. Decreased phosphocreatine and NAA indicate energy metabolism deficit in brain cells; the latter also suggests the neuronal viability was inhibited. Decreased glutamate and glutamine may suggest the neuron-glial integrity was implicated by chronic SI. These neurochemical and biochemical changes may contribute to the SI-induced behavioral abnormalities including a high level of anxiety, social interaction deficit, and impaired spatial working memory shown in this study.
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1. Introduction

Social isolation (SI) rearing of rodents is a developmental manipulation in which an animal is singly housed upon weaning. This manipulation is commonly compared with the psychological stressors in humans as it produces several behavioral outcomes similar to those observed in humans with early life stress. The SI-induced behavioral outcomes include anxiety/depression-like behaviors [2,4,6,9,51,54] and deficits in social interaction [33,34,52,55], prepulse inhibition (PPI) of the acoustic startle reflex [12,33], sensory gating and mismatch negativity (MMN)-like responses [50], and in cognition and memory [13,15,36]. Of the SI-induced behavioral outcomes, deficits in PPI, MMN deficits [43], and increased preservative behavior resemble some of the symptomatology of schizophrenia. Moreover, the behavioral effects of isolation rearing on rats were ameliorated by the antipsychotic clozapine [33,34].

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In quest of the neural substrate of the SI-induced behavioral deficits, previous animal studies compared some specific brain regions of isolation-reared animals with normal controls in terms of the indices relevant to the dopaminergic and glutamatergic systems, both of which are implicated in schizophrenia [7,42]. For example, isolation-reared rats showed higher levels of basal extracellular dopamine in the stratum [16]. Other studies tried to find glutamatergic alterations in isolation-reared animals. For example, an earlier in situ hybridization study noted that mRNA of the N-methyl-D-aspartate (NMDA) receptor subunit NR1A was decreased in the hippocampus of isolation-reared rats compared with their socially housed counterparts [17]. Also, SI was reported to up-regulate NR2A expression in the prefrontal cortex (PFC) of rats [47], increase NMDA receptor binding in frontal cortex [46], and stimulate the mRNA expression of NR2A and NR2B in the hippocampus [55]. In a more recent study, 'SI' increased the binding activity of the group II metabotropic glutamate receptor (mGluR2/3) in the PFC and hippocampus of mice [24]. These previous data suggest the existence of glutamatergic alterations in brains of isolation reared rodents. However, there is little information available regarding the brain metabolic profile measured in living animals using non-invasive neuroimaging methods.

The SI-induced behavioral outcomes were also associated with cellular oxidative stress. In mice, SI for 6–8 weeks induced oxidative damage in the brain by enhancing the production of nitric oxide and depleting brain glutathione content, an endogenous antioxidant [21]. In rats, chronic SI compromised the activities of both glutathione peroxidase (GPx) and catalase in the hippocampus [11]. According to the authors, the mechanism for the SI-induced oxidative imbalance in the hippocampus is likely due to poor systemic energy conditions set by this stressor.

The aims of this study were to detect possible changes in levels of glutamate and glutamine caused by SI using the proton magnetic resonance spectroscopy (^{1}H-MRS) and to see if glutamate and/or glutamine changes are associated with oxidative stress that has been reported in isolation reared animals as reviewed above. ^{1}H-MRS is a non-invasive approach with relatively high spatial resolution and able to quantitatively measure neurochemistry in brains of living subjects [39]. It has been widely used to measure *in vivo* neurochemical integrity in patients with schizophrenia [29,39] or other neuropsychiatric diseases, but few reports were available in living small animals.

2. Materials and methods

2.1. Animals and experimental procedures

Timed-pregnant Sprague Dawley rats (the Animal Center of Shantou University Medical College, Guangdong, China) were individually housed in clear polycarbonate cages (30 cm × 50 cm × 20 cm). The day of birth was designated as postnatal day 0 (PD0). On PD21 (day of weaning), twenty male rat pups from 4 litters were pooled and randomly assigned to two groups, each comprising 10 rats/group. All animal procedures described below (Fig. 1) were carried out in accordance with the guidelines laid down by the NIH in the US regarding the care and use of animals for experimental procedures and approved by the Ethical Committee of Experimental Animals of Shantou University Medical College, China.

The animals in the SI group were socially isolated (1 rat/cage) for 8 weeks, whereas those in the non-stressed controls remained in group housing (GH, 3–4 rats/cage) during the same period. The rats were reared under identical conditions: same size of cages with sawdust, temperature (22 ± 1.0 °C, humidity (50 ± 10%), 12 h light:12-h dark cycle and free access to food and water. SI and GH rats experienced minimal handling and no environmental enrichment. Sawdust was changed per 3–4 days. During PD77–79, the behaviors of rats in social interaction, elevated-plus maze (EPM), and Y-maze were measured in order. On PD80 and PD81, the ^{1}H-MRS procedure was performed for each animal to measure the brain metabolites. On PD82, rats were sacrificed under a deep anesthesia (with chloral hydrate). The rat brain was removed out of the cranium and the brain regions of caudate putamen (CPU), cerebellum, cerebral cortex (CTX), hippocampus, PFC, and thalamus were dissected out and stored at −80 °C until biochemical analysis for the measurement of oxidative stress parameters.

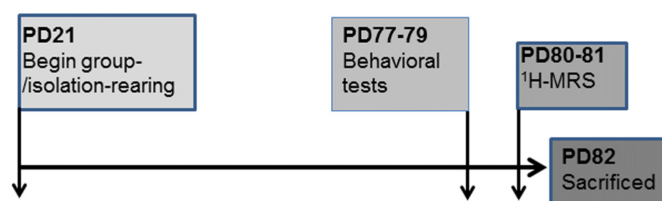

Fig. 1. The experimental procedures. Social isolation (1 rat/cage) started on postnatal day (PD) 21 and continued for 8 weeks. During PD77–79, the behavioral tests of social interaction, elevated-plus maze (EPM), and Y-maze were performed in order. On PD80 and PD81, the ^{1}H-MRS procedure was performed for each animal to measure the brain metabolites. On PD82, rats were sacrificed under a deep anesthesia. The rat brain was removed and the brain regions of caudate putamen (CPU), cerebellum, cerebral cortex (CTX), hippocampus, prefrontal cortex (PFC), and thalamus were dissected out and stored at minus 80 °C until biochemical analysis for the measurement of oxidative stress parameters.

2.2. Behavioral tests

A social interaction test adapted from a previous description was performed [3]. Individual rat was introduced into an open-field (100 cm × 100 cm) and its trajectory was tracked for two consecutive sessions of 150 sec. During the first session, an empty wire mesh cage (12 cm × 12 cm × 18 cm) was placed at one end of the open-field. During the second session, the conditions were identical except that a social target animal (an unfamiliar rat) had been introduced into the cage. Between the two test sessions, the tested rat was removed from the arena and placed back to its home cage for approximately 60 s. The video-tracking data from both the no target and target conditions were used to determine the time spent by the tested rat in the interaction zone (a 16 cm-wide corridor surrounding the cage) and the corners of the open-field opposite to the location of the cage using a video tracking program (Noldus Information Technology, Wageningen, Netherlands).

Anxiety-like behavior was assessed by performing the EPM test. The maze consisted of four radial arms (50 cm × 10 cm) elevated 60 cm above the floor. Two opposing arms were enclosed by black polypropylene walls (40 cm high), and the other two arms were open. Rat was placed at the central junction, facing an open arm, and the activity of him was recorded for 5 min. Decreases in open-arm time, and increases in closed arm time, were used as a measure of anxiety-like behavior.

The spatial working memory of rats was measured with the Y-maze as described previously [49]. Briefly, each rat was placed at the end of one arm of a symmetrical Y maze and allowed to move freely through the maze during an 8-min test period. The total number and series of arm entries were recorded. The number of overlapping entrance sequences (e.g., ABC, BCA) defines the number of spontaneous alternations.

2.3. MRI/S acquisition

Rat was initially anesthetized using a chamber with 5% isoflurane in oxygen and subsequently maintained with1.5–2% isoflurane

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