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Research report

Structural and functional effects of social isolation on the hippocampus of rats with traumatic brain injury

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HIGHLIGHTS

- Social isolation (SI) enhances morbidity and mortality from neurological disorders.
- In this study the effect of SI on traumatic brain injury (TBI) was evaluated.
- SI enhanced cell injury and neuronal apoptosis in the hippocampus of rats with TBI.
- SI enhanced behavioral deficits in rats with TBI.
- Prevention of SI may improve outcomes for patients recovering from TBI.

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ABSTRACT

Social isolation has significant long-term psychological and physiological consequences. Both social isolation and traumatic brain injury (TBI) alter normal brain function and structure. However, the influence of social isolation on recovery from TBI is unclear. This study aims to evaluate if social isolation exacerbates the anatomical and functional deficits after TBI in young rats. Juvenile male rats were divided into four groups; sham operated control with social contacts, sham control with social isolation, TBI with social contacts, and TBI with social isolation. During four weeks after brain injury in juvenile rats, we evaluated the animal behaviors by *T*-maze and open-field tests, recorded brain activity with electrocorticograms and assessed structural changes by histological procedures in the hippocampal dentate gyrus, CA1, and CA3 areas. Our findings revealed significant memory impairments and hyperactivity conditions in rats with TBI and social isolation compared to the other groups. Histological assessments showed an increase of the mean number of dark neurons, apoptotic cells, and caspase-3 positive cells in all tested areas of the hippocampus in TBI rats with and without social isolation compared to sham rats. Furthermore, social isolation significantly increased the number of dark cells, apoptotic neurons, and caspase-3 positive cells in the hippocampal CA3 region in rats with TBI. This study indicates the harmful effect of social isolation on anatomical and functional deficits induced by TBI in juvenile rats. Prevention of social isolation may improve the outcome of TBI.

1. Introduction

Traumatic brain injury (TBI) is one of the most common causes of death and disability throughout the world. The groups at the highest risk of a TBI are males, teenagers and young adults [1]. A variety of morphological, cellular, molecular, bioelectrical and behavioral changes of human TBI, including alterations in ionic homeostasis, generation of free radicals, eliciting inflammatory responses,

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releases of excitatory amino acids, initiation of DC negative shifts and changes in neurotransmitter systems have been characterized by various experimental TBI models [2,3]. TBI causes structural changes in the brain and has important long-term consequences with respect to physical, cognitive, emotional, or behavioral functions [4,5]. Long-term consequences of TBI depend on the severity of injuries and the stage of brain development [6].

Social interaction is central to human well-being and involved in the maintenance of both mental and physical health. Social isolation (SI), an objective reflection of reduced social network size or lack of social contact, is associated to a higher risk of depression, anxiety, cognitive deterioration [7,8], cardiovascular disease [9] and infectious illnesses [10]. SI increases morbidity and mortality from cerebrovascular and cardiovascular diseases [11,12], and is associated with increased inflammatory and metabolic responses to stress, and modifies transcriptional pathways linked with glucocorticoid and inflammatory processes [13]. SI modulates the brain activity by changes in neurotransmitter release, including glutamate, dopamine, and serotonin [14]. SI induces behavioral deficits accompanied by a reduction of prefrontal cortex volume and a modulation of hippocampal synaptic plasticity [15]. Despite growing evidence implicating SI as a risk factor for cerebrovascular diseases, little is known regarding the possible influence of SI on TBI and its pathogenesis. The aim of the present study was to examine the influence of SI on behavioral deficits and neuropathological changes produced by TBI. Brain bioelectrical activity, memory retrieval performance as well as cell injury and neuronal apoptosis were compared in socially housed and isolated juvenile rats with TBI.

2. Materials and methods

Forty four juvenile male Wistar rats (25–35 days old; 45–90 g) were randomly housed in a 12-h light/dark cycle (lights on at 07:00 h, off at 19:00 h) and maintained at a temperature of $22 \pm 2\,^{\circ}C$. Food and drinking water was available ad libitum. Prior to experiments, all animals were housed together. Animals were randomly divided into four groups: (1) Sham + socially housed (SH): animals underwent all of the surgical procedures but did not undergo brain injury and kept in group during experimental procedures. (2) Sham + SI: animals underwent all of the surgical procedures but did not undergo brain injury and kept under isolated condition. (3) TBI: brain lesions were induced by a Hamilton syringe. Animals were housed socially during the study. (4) TBI + SI: brain lesion was produced by Hamilton syringe and animals were housed individually after leisoning. Animals under isolated condition were kept in a separate room, beyond smelling and hearing distance from the other rats [16]. The study was conducted in accordance with the National Institutes of Health guidelines for the care and use of animals and under protocols approved by the Ethics committee of Shefa Neuroscience Center, Tehran, Iran.

2.1. Surgery

Rats were anesthetized with ketamine (150 mg/kg; Sigma, USA). The head of each rat was placed in a stereotaxical instrument (Stoelting Instruments, USA). The scalp was incised and retracted, a guide cannula was placed in the skull at the following coordinates [17]: anterior-posterior: +1.8 mm anterior to the bregma; medial-lateral: −3.1 mm lateral to the sagital suture; dorsal-ventral: 0.7 mm down from the skull surface. A stylet was placed into the guide cannula to maintain patency. Silver electrodes were implanted (without injuring the dura mater) over the somatosensory cortex of all tested animals and reference electrodes were placed on the nasal bulb (Fig. 1A). Electrodes and cannula were fixed with dental acrylic cement. The scalp was sutured and animals were returned to their home cages. Before the beginning of the experimental procedures, all rats were housed socially for 1 week in well-ventilated boxes in an air-conditioned room to recover from surgery. Induction of TBI (by intracerebral penetration of a microneedle and injection of a small quantity of isotonic solution) has been previously described in detail [18,19]. Briefly, a sterilized microneedle (200 μm diameter) was inserted into the cannula. Using a Hamilton 100 μl syringe, 10 μl isotonic solution was injected over a period of 30 min. Brain activity was evaluated by electrocorticogram (ECoG) 10 min before and 60 min after TBI (injection of the solution). In addition, ECoG recordings were obtained from each rat 7, 14, 21 and 28 days after induction of TBI (1 h duration). Behavioral tests were performed in all rats for four consecutive weeks (7, 14, 21, and 28 days after TBI, once per week) before animals were sacrified for histopathological investigations.

2.2. T-maze test

Using a *T*-maze test, memory retrieval performance was assessed for four consecutive weeks after TBI. The apparatus is comprised of two arms, allowing left and right hand shifts. The floor of the maze is 10 cm wide and made of black glass; the walls are 17 cm high and made of clear glass. The stem is 40 cm long, with a guillotine door located 25 cm from the end of the stem, thereby creating a starting area. The cross piece was 140 cm long, and at each end, there was a food well (2 cm diameter and 0.75 cm deep). Animals were trained to the *T*-maze in normal light for 10 min to retrieve food from the right arm. Then rats underwent a food deprivation schedule for 24 h. During the behavioral test, red light was used in the right arm. Twenty minutes prior to the experiment, the cages were transferred to the observation room. Rats were monitored by a camera.

2.3. Open-field test

To evaluate the effect of SI on motor impairment after TBI, all groups (sham + SH, sham + SI, TBI, and TBI + SI) were assessed by open-field test. Rats were placed in an open-field box and locomotion was tracked over a 5-min period. Movements inside the 20 cm × 20 cm region were used to evaluate motor function by measuring the total distance moved (cm) and average movement velocity (cm/s). Behavioral evaluations were made at 7, 14, 21, and 28 days post-injury, once per week.

2.4. ECoG recordings

ECoG was recorded with silver electrodes connected to an amplifier (EXT-02 F, NPI, Germany; with band-pass filters at 0.5–30 kHz, sampling rate 10 kHz) and signals were digitized using an analog-digital converter (Digidata 1440A; Axon instrument, USA). The characteristic features of ECoG waves were visualized and analyzed by the software Axoscope (Version 10.2).

2.5. Histological assessment

Four weeks after induction of TBI, rats were anesthetized using a high dose of chloral hydrate (350 mg/kg; Sigma–Aldrich, Germany) and perfused transcardially via the ascending aorta with 200 ml of saline followed by 300 ml of 1% paraformaldehyde (PFA) solution. The brains were stored in a 1% PFA solution for at least a week. After that and the solution was replaced by 30% sucrose-formalin. For histological assessment, coronal uniform random sections were cut through the hemisphere ipsilateral to the TBI-site. Twelve successive sections were selected by random systematic sampling from each animal. These sections were stained by toluidine blue and