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Research report

Social isolation after stroke leads to depressive-like behavior and decreased BDNF levels in mice

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HIGHLIGHTS

- Isolated mice have exacerbated stroke-induced histological injury compared to pair housed animals.
- Locomotion across all stroke mice is equivalent by day 3 in the Open Field Test (OFT).
- Isolation leads to increased immobility in the Forced Swim Test (FST) at 13 days post-stroke.
- Pair-housed animals have increased BDNF levels in stroke tissue at 49 days post-stroke compared to isolated cohorts.

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ABSTRACT

Social isolation prior to stroke leads to poorer outcomes after an ischemic injury in both animal and human studies. However, the impact of social isolation following stroke, which may be more clinically relevant as a target for therapeutic intervention, has yet to be examined. In this study, we investigated both the sub-acute (2 weeks) and chronic (7 weeks) effects of social isolation on post-stroke functional and histological outcome. Worsened histological damage from ischemic injury and an increase in depressive-like behavior was observed in isolated mice as compared to pair-housed mice. Mice isolated immediately after stroke showed a decrease in the levels of brain-derived neurotrophic factor (BDNF). These changes, both histological and behavioral, suggest an overall negative effect of social isolation on stroke outcome, potentially contributing to post-stroke depression and anxiety. Therefore, it is important to identify patients who have perceived isolation post-stroke to hopefully prevent this exacerbation of histological damage and subsequent depression.

1. Introduction

Stroke is the fourth leading cause of death and the primary cause of long-term adult disability in the United States (American Stroke Association, 2012). Stroke affects physical, cognitive, and social functioning. Perceived social isolation contributes to mortality and morbidity in patients with cerebrovascular disease, but the underlying mechanisms for this are unknown [1–3]. Pre-stroke social isolation leads to poor functional and cognitive recovery in humans, and has been linked to an increased risk of post-stroke depression (PSD) and post-stroke anxiety (PSA) [4–7]. PSA occurs with PSD in approximately 25% of isolated stroke patients [8,9]. Stroke patients report higher perceived social isolation after stroke than age-matched healthy individuals [10], which is thought to contribute to the higher recurrent stroke rates seen in isolated individuals [4].

Importantly, the detrimental effects of social isolation can be modeled in animals. Isolation prior to an induced stroke exacerbates histological ischemic damage in rodents [11–13], an effect mediated in part by an increased pro-inflammatory signaling via NF-κB [13]. However, since many "isolated" patients are not identified until they come to medical attention after a stroke has occurred, assessing the effects of social isolation after stroke on outcome is critical to translational efforts targeting social factors in stroke recovery.

Across 51 clinical studies, approximately one-third of stroke survivors are diagnosed with post-stroke depression (PSD) [14].

Abbreviations: MCAO, middle cerebral artery occlusion; ST, stroke surgery; SH, sham surgery; BDNF, Brain Derived Neurotrophic Factor; SI or ISO, social isolation; PH, pair-housed; PSD, post-stroke depression; PSA, post-stroke anxiety.

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Depression is associated with higher morbidity and mortality [15], greater disability, and poorer recovery after stroke [16–18]. Depression correlates with markers of inflammation such as interleukin-6 (IL-6) and C reactive protein (CRP) in humans [19]. The detrimental effects of pre-stroke isolation in animal models has been linked to an enhanced neuroinflammatory response to injury and elevations in IL-6, but the link between social behavior, inflammation, and depression remains unclear [13,20,21]. Much less is known regarding the contribution and involvement of these pro-inflammatory signaling pathways in isolation that occurs after stroke.

Neurotrophins play an important role in depression and are emerging as a possible role in depression following stroke [22–25]. Hippocampal levels of brain-derived neurotrophic factor (BDNF) clinically correlate with mood elevation after pharmacological treatment for depression in humans [26] and in rodents [27,28]. BDNF is a known regulator of neuronal plasticity and neurogenesis [29]. In addition, BDNF regulates behavioral and cellular metabolic responses to environmental stimuli [30] and environmental enrichment [31], and is higher in pair housed than in isolated rodents [21,32]. BDNF is also known to be neuroprotective after experimental brain injury [30] and enhances synaptic plasticity and functional outcome after MCAO [33]. Therefore, BDNF may play a role in both PSD and repair after ischemic injury. While the effects of pair-housing mice prior to an induced stroke leads to a positive impact on stroke recovery and infarct size in the stroke animal, the nature of the behavioral interactions induced by the partner have not yet been explored [13]. Living with an unwell individual as opposed to a healthy caretaker may affect stroke recovery and mood in both the patient and the caretaker. We investigated whether there was a benefit to housing with a healthy partner in our post-stroke isolation design.

This study investigates the interaction of post-stroke social isolation on histological outcomes, functional recovery, and depressive phenotypes. BDNF levels in brain, serum inflammatory markers, motor function, and depression-like and anxiety-like behavior in mice isolated after experimental stroke were examined.

2. Methods

2.1. Experimental animals

Eight-week-old male C57BL/6 mice (23–27 g) were obtained from Harlan Laboratories, Inc. (IN). Mice were housed in a temperature controlled room (74 ± 2 °F) with *ad-libitium* access to food and water and maintained under a 12-h light/dark cycle (lights on at 7:00 AM). Experimental procedures were conducted during the light phase. After arrival and acclimation for one week, mice were group-housed for one week and then pair-housed. Depending on the experimental group, mice were randomly assigned to remain pair-housed (PH) or were housed individually (socially isolated: "ISO") after surgery (Fig. 1). All experimental procedures were conducted in accordance with protocols approved by the Animal Care and Use Committee of the University of Connecticut Health Center. All behavioral testing was performed by an investigator blinded to housing condition.

2.2. Middle cerebral artery occlusion model

Focal transient cerebral ischemia was induced by 60 min of reversible right middle cerebral artery occlusion (MCAO) under isofluorane anesthesia followed by reperfusion as described previously [34,35]. Sham animals underwent the same procedure but the suture was not advanced into the MCA. During surgery and ischemia, rectal temperature was monitored with a Monotherm system (V WR LabShop, Batavia, IL, USA) and maintained at approximately 37 °C with an automated temperature control feedback system. Cerebral blood flow reduction of >80% of baseline after suture insertion was confirmed in all stroke animals by Laser Doppler Flowmetry (Moor Instruments). All animals, both stroke and sham, were administered 0.2 mL saline subcutaneously day 0–5 post-stroke to ensure survival and had free access to wet mashed food.

2.3. Experiment 1

In order to assess the impact of stroke and social isolation on anxiety- and depression-like behaviors, mice were randomized and subjected to a transient stroke (ST) or a sham surgery (SH). Immediately after surgery, mice were assigned one of six groups using 2 × 3 experimental design with stroke condition, sham (SH) or stroke (ST) as the first between-subjects factor and post-stroke housing condition, housed with sham (SH), housed with stroked (ST) for the healthy partner studies, or housed in isolation (ISO) as the second between-subjects factor. Thus, the six groups were: SH/SH ($n = 10$), SH/ST ($n = 10$), SH/ISO ($n = 8$), ST/SH ($n = 10$), ST/ST ($n = 10$), ST/ISO ($n = 10$). Mice remained in these housing conditions throughout the experiment.

The mice were then subject to behavioral testing by a blinded investigator. The Open Field Test (OFT) was administered on days 1, 3, 5, 7, and 10 post-stroke. On day 11, the Elevated Zero Maze (EZM) test was conducted, followed by the Forced Swim Test (FST) on day 13. Mice were sacrificed on day 13, 2 h after the FST (Fig. 1A).

2.4. Experiment 2

Experiment 2 was designed to assess long-term depression- and anxiety-like behavior after stroke. The same experimental procedures used in Experiment 1 were used. Behavior on the EZM was assessed on day 11 post-stroke and the FST was conducted on day 33 post-stroke. Animals were sacrificed on day 49. The experimental design was identical to that in experiment 1, and the experimental groups were as follows: SH/SH ($n = 8$), SH/ST ($n = 9$), SH/ISO ($n = 10$), ST/ST ($n = 9$), ST/SH ($n = 8$), ST/ISO ($n = 8$) (Fig. 1B). Mice remained in these housing conditions throughout the experiment.

2.5. Neurological scoring

Neurological deficit scores (NDS) were obtained on days 0–5 post MCAO surgery and after behavioral tests using a five point scoring scale. The scoring used was as follows: 0, no deficit; 1, forelimb weakness and torso turning to the ipsilateral side when held by tail; 2, circling to affected side; 3, unable to bear weight on affected side; and 4, no spontaneous locomotor activity or barrel rolling as described previously [36].

2.6. Behavioral testing

Mice were acclimated to the testing room in their home cages 1 h before behavioral testing. All apparatuses were wiped with 70% ethanol between animals. All testing was performed at the same time of day to avoid circadian variations in activity.

2.6.1. Open Field Test

General locomotor activity and anxiety-like behavior was assessed with the OFT Mice were placed in the front right corner of a clear acrylic box (16″ × 16″) and allowed to explore the box for 20 min. Locomotor activity was quantified as the total number of beam breaks by a computer operated PAS Open Field system (San Diego Instruments, San Diego, CA). The percentage of beam breaks in the center zone (13″ × 13″) was used as a measure of