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Research report

Pair housing reverses post-stroke depressive behavior in mice

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HIGHLIGHTS

- Abnormal social behavior is seen after stroke.
- Pair housing after stroke enhances recovery from stroke-induced behavioral deficits.
- Animals isolated after stroke had a progressive deficit in sociability that worsened over time.
- The protective effects of pair housing may be mediated by M2 activation of microglia/macrophages.

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ABSTRACT

Social isolation (SI) has been linked epidemiologically to high rates of morbidity and mortality following stroke. In contrast, strong social support enhances recovery and lowers stroke recurrence. However, the mechanism by which social support influences stroke recovery has not been adequately explored. The goal of this study was to examine the effect of post-stroke pair housing and SI on behavioral phenotypes and chronic functional recovery in mice. Young male mice were paired for 14 days before a 60 min transient middle cerebral artery occlusion (MCAO) or sham surgery and assigned to various housing environments immediately after stroke. Post-stroke mice paired with either a sham or stroke partner showed significantly higher ($P < 0.05$) sociability after MCAO than isolated littermates. Sociability deficits worsened over time in isolated animals. Pair-housed mice showed restored sucrose consumption ($P < 0.05$) and reduced immobility in the tail suspension test compared to isolated cohorts. Pair-housed stroked mice demonstrated significantly reduced cerebral atrophy after 6 weeks ($17.5 \pm 1.5\%$ in PH versus $40.8 \pm 1.3\%$ in SI; $P < 0.001$). Surprisingly, total brain arginase-1, a marker of a M2 "alternatively activated" myeloid cells was higher in isolated mice. However, a more detailed assessment of cellular expression showed a significant increase in the number of microglia that co-labeled with arginase-1 in the peri-infarct region in PH stroke mice compared to SI mice. Pair housing enhances sociability and reduces avolitional and anhedonic behavior. Pair housing reduced serum IL-6 and enhanced peri-infarct microglia arginase-1 expression. Social interaction reduces post-stroke depression and improves functional recovery.

1. Introduction

Social support is one of the most recognized psychological factors influencing recovery from disease [1,2] and has the potential to alleviate maladaptive psychological responses to stress [3]. In contrast, social isolation (SI) exacerbates disease and slows recovery. Absence of social interaction has been linked epidemiologically with high mortality rates following various pathological stressors including stroke [4]. Social isolation and feelings of loneliness not only increase the risk of death but also delay recovery [5]. Social interaction has well -documented health benefits in humans and can be easily assessed in the clinic by tools such as the UCLA loneliness scale [6]. However, discovering the biological mechanisms underlying the benefits of social interaction requires the development of pre-clinical animal studies. Although reproducing social behavior in mice has limitations, there are many advantages [7].

As not all strokes can be prevented, evaluating the influence of social and emotional factors on stroke recovery is an important area of research. Depression and anxiety are common clinical findings after stroke, with up to 40% of stroke survivors affected [8]. These behavioral deficits have been associated with higher levels of disability and lower levels of social support [9,10].

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Post-stroke depression (PSD) leads to increased cognitive deficits, sexual dysfunction, anhedonia, feelings of despair and social withdrawal [11–15]. These disturbances not only affect the overall well-being of patients but also confound the recovery process by leading to further isolation. SI is predicted by depressive symptomatology using the geriatric depression scale (GDS) in clinical populations [16,17]. However, no such scale or validated behavioral paradigms to measure complex behavior changes after stroke exists for rodent stroke models. Historically, the lack of appropriate behavioral quantification and analysis has been a major limiting factor in drug development for stroke [18].

Housing conditions prior to an induced stroke are a strong determinant of long-term survival in mice. Mice pair housed prior to injury had a 100% survival rate after 7 days in contrast to a 40% survival rate among isolated animals [19]. The detrimental effects of social isolation on infarct volume and behavioral recovery were evident in both sexes [20]. However, many studies have examined isolation prior to stroke, and focused on motor recovery and survival at acute endpoints. Therefore in this study we analyzed and validated behavioral changes after stroke in long term survival cohorts with a focus on depressive behavior.

Emerging evidence indicates a relationship between SI and systemic inflammation [19]. Clinical studies have shown that plasma levels of inflammatory cytokines were increased after stroke [21]. Immune cells, most notably resident microglia or peripheral macrophages that migrate to the area of ischemic injury also participate in pathological changes during stroke by releasing various pro-inflammatory cytokines and chemokines [22–24]. Microglia/macrophages are highly plastic cells that can assume diverse phenotypes in response to specific micro-environmental signals [23–25]. These macrophages and/or resident microglia may either contribute to damage or enhance repair in the brain [25]. These divergent effects in the brain are mediated by distinct macrophage subsets, i.e., "classically activated" proinflammatory (M1) or "alternatively activated" anti-inflammatory (M2) cells. Their phenotype is dependent on the cytokine environment present during macrophage activation [23]. Myeloid cells are divided into these two distinct phenotypes based in part upon L-arginine metabolism. M1 (or classically activated) cells express high levels of iNOS and low levels of arginase-1 and participate in the clearance of intracellular pathogens. Conversely, M2 (alternatively activated) myeloid cells express the reverse pattern, and are involved in repair [26]. In this study, chronic behavioral changes induced by pair housing, and the potential contribution of microglia/macrophages to post-stroke depressive phenotypes were examined.

2. Materials and methods

2.1. Experimental animals

All animal protocols were approved by the University's Institutional Animal Care and Use Committee at the University of Connecticut Health Center, Farmington, CT and were performed in accordance with National Institutes of Health guidelines. Six-week-old C57Bl/6 male mice were purchased from Harlan laboratories, USA. All mice were maintained in an ambient temperature and humidity controlled vivarium with *ad libitum* access to food and water. After arrival, the mice were acclimatized in the animal care facility for two weeks in their original groups. Thereafter, all mice were randomly housed in groups of 2 mice per cage for an additional two weeks. During pair housing all the mice were examined daily for compatibility (observed for fighting, or the failure to gain weight in either partner). After two weeks of pair housing all mice were subjected to stroke or sham surgery. Immediately after surgery, mice were randomly assigned to one of six groups using a two way

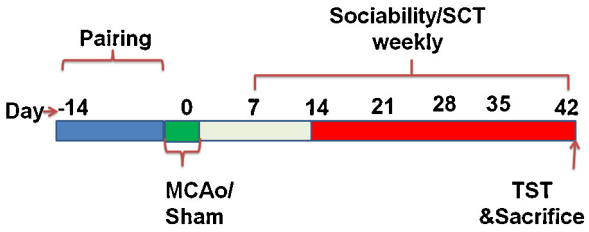

Fig. 1. Schematic of experimental design and behavioral testing (Cohort 2).

factorial design. Surgical condition (sham (SH) or stroke (ST)), was the first between-subjects factor and housing condition housed with sham (SH), housed with stroke (ST) or housed in isolation (ISO), was the second between-subjects factor [27]. Thus, the six groups were: SHcSH ($n = 6$), SHcST ($n = 8$), SH ISO ($n = 7$), STcSH ($n = 8$), STcST ($n = 8$), and ST ISO ($n = 9$). Mice remained in these housing conditions throughout the experiment (Fig. 1). If any mice died during the experiments, all of the subjects housed in the same cage were excluded from the study. The experiments were conducted in two separate cohorts. In the first cohort behavioral deficits (Sociability, SCT and TST tasks) were assessed at one time point, 6 weeks after stroke. In the second cohort the mice were tested (Sociability, SCT) weekly through 6 weeks with testing initiated at day 7 after MCAO [number of animals/group; ST-PH ($n = 10$), SH-ISO ($n = 6$), SH-PH ($n = 8$), and ST ISO ($n = 7$); detail for groups is described in Section 3.1].

2.2. Stroke model

In all stroke groups, transient focal cerebral ischemia was induced in mice (20–25 g) by 60 min of transient right middle cerebral artery occlusion (MCAO) under isoflurane anesthesia followed by reperfusion as described previously [28,29]. Briefly, a midline ventral neck incision was made, and unilateral right MCAO was performed by advancing a 6-0 silicone-coated nylon monofilament (Doccol Corporation, CA) into the internal carotid artery 6 mm from the internal carotid artery bifurcation via an external carotid artery stump. Rectal temperatures were monitored with a temperature control system (Fine Science Tools, Canada) and temperature was maintained with an automatic heating pad at ~37 °C during surgery. Cerebral blood flow measurements by Laser Doppler Flowmetry (DRT 4/Moor Instruments Ltd, Devon, UK) confirmed ischemic occlusion (reduction to 15% of baseline) during MCAO and restoration of blood flow during reperfusion. In sham mice, an identical surgery was performed except the suture was not advanced into the internal carotid artery.

2.3. Assessment of social interaction/sociability

The three-chamber paradigm, established by Crawley and colleagues [30] has been used to examine mouse sociability in models of autism and other psychiatric disorders. We used this paradigm to study PSD in PH versus ISO mice with minor modifications. In the first cohort sociability was assessed at 6 weeks after MCAO. In the second cohort, social behavior was tested at several time points after stroke, each at 7 day intervals through 6 weeks with testing initiated at day 7 after stroke.

The social testing apparatus was comprised of a rectangular, three-chambered Plexiglas box (22 in. $L \times 16$ in. $W \times 9$ in. H). Dividing walls had a single rectangular opening $2W \times 2L$ inches in diameter allowing access into each chamber (Supp Fig. 1). Initially, the test mouse was habituated to the test chamber for 5 min. In this habituation phase the mouse had full access to both sides of the chamber each containing an empty round wire cage. After this habituation period, the test mouse was returned to its home