Social isolation alters neuroinflammatory response to stroke

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Edited by William T. Greenough, University of Illinois, Urbana, IL, and approved February 24, 2009 (received for review October 24, 2008)

Social isolation has dramatic long-term physiological and psychological consequences; however, the mechanisms by which social isolation influences disease outcome are largely unknown. The purpose of the present study was to investigate the effects of social isolation on neuronal damage, neuroinflammation, and functional outcome after focal cerebral ischemia. Male mice were socially isolated (housed individually) or pair housed with an ovariectomized female before induction of stroke, via transient intraluminal middle cerebral artery occlusion (MCAO), or SHAM surgery. In these experiments, peri-ischemic social isolation decreases poststroke survival rate and exacerbates infarct size and edema development. The social influence on ischemic damage is accompanied by an altered neuroinflammatory response; specifically, central interleukin-6 (IL-6) signaling is down-regulated, whereas peripheral IL-6 is up-regulated, in isolated relative to socially housed mice. In addition, intracerebroventricular injection of an IL-6 neutralizing antibody (10 ng) eliminates social housing differences in measures of ischemic outcome. Taken together, these data suggest that central IL-6 is an important mediator of social influences on stroke outcome.

focal cerebral ischemia | neuroinflammation

Social interaction is an important modulator of both mental and physical health. Social relationships perceived as being supportive are associated with improved health, whereas perceived social isolation and stressful social interactions can be detrimental to health. Within the clinical literature, low perceived social support and social isolation predict the onset of depression, as well as increased morbidity and mortality from cardiovascular and cerebrovascular disease (1–4). Despite growing evidence implicating limited or negative social interactions as risk factors for cerebrovascular disease, little is known regarding the mechanisms through which psychosocial factors influence stroke pathogenesis. The health benefits of social interaction in humans are typically attributed to improved health behaviors such as decreased smoking, decreased alcohol consumption, better nutrition, or better medical compliance, which in turn improve cerebrovascular health (5). However, both social isolation and perceived lack of social support are predictive of disease outcome independent of health behaviors (6, 7). Furthermore, the negative effects of social isolation on stroke and cardiac arrest outcome can be reproduced in mice, and the data suggest that socially isolated and socially housed mice mount a quantitatively different pathophysiological response to ischemic damage (8, 9).

Inflammatory processes have a fundamental role in the pathophysiology of ischemic injury. Indeed, chronic and acute infection, as well as low-grade systemic inflammation [i.e., elevated serum C-reactive protein (CRP)], are predictive of future strokes, as well as death from stroke and cardiac arrest (10–14). CRP is an acute phase protein that increases substantially in response to proinflammatory cytokine release and as such is used clinically as an index of chronic low-grade inflammation (15). Importantly, emerging evidence indicates a relationship between the social environment and systemic inflammation (16, 17), and in otherwise healthy humans, low social integration is associated with increased CRP concentrations (18, 19). Further, socially isolated mice exhibit increased intraischemic serum CRP concentrations relative to socially housed animals after experimental stroke (8). Although a direct causative role for CRP on the extent of ischemic injury has not been established, both the clinical (11, 16–18) and animal (8) data provide evidence of a strong correlation between social factors and the inflammatory response typically associated with ischemic injury.

The goal of the current study was to examine the influence of social housing on stroke outcome. Specifically, poststroke cytokine expression, edema formation, infarct development, and functional recovery were compared in socially housed and isolated mice.

Results

Social Isolation Influences Poststroke Survival and Ischemic Damage. Housing condition was a strong determinant of poststroke survival rate and ischemic damage. Following middle cerebral artery occlusion (MCAO) only 40% of socially isolated mice survived 7 days, compared with 100% of socially housed mice (U = 20.00, P < 0.05, r = 0.63), which limits interpreting the day 7 infarct and behavior data as being truly representative of the 2 experimental groups. However, it is interesting to note that the 4 surviving mice in the socially isolated group were similar in infarct size and behavior to the socially housed group on poststroke day 7 (P > 0.05; although we caution that this comparison suffers from the statistical limitation inherent in having a small sample size in one of the experimental groups).

To address the issue of differential long-term survival, all remaining measurements were made 24–72 h after initiation of reperfusion, when survival rates were not statistically different between groups (90% socially isolated and 100% socially housed on day 3; U = 45.00, P > 0.05, r = 0.22). Social isolation exacerbated infarct volume at 24 and 72 h (24 h, t20 = 1.738, P < 0.05; r2 = 0.12; 72 h, t20 = 2.568, P < 0.05, r2 = 0.11) (Fig. 1A). Social isolation also significantly exacerbated cerebral edema 48 h after MCAO; socially isolated animals experienced a 2-fold increase in edema relative to socially housed animals (t20 = 1.801, P = 0.05, r2 = 0.16) (Fig. 1B).

Within the open field, there were no effects of social housing on locomotor activity or exploratory behavior measured 24 h before surgery (all P > 0.05). A 2-factor ANOVA (factors were surgery and housing condition) revealed an effect of surgery on rearing behavior 72 h post-MCAO or SHAM surgery (F1,26 = 28.61, P < 0.05). After MCAO, mice reared significantly

Author contributions: K.K. and A.C.D. designed research; K.K., G.J.N., N.Z., J.S.M., and H.P. performed research; K.K. and A.C.D. analyzed data; and K.K. and A.C.D. wrote the paper.

The authors declare no conflict of interest.

www.pnas.org/cgi/doi/10.1073/pnas.0810737106

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0810737106/DCSupplemental.

PNAS | April 7, 2009 | vol. 106 | no. 14 | 5895–5900
less than SHAM; however, there were no effects of housing condition on total locomotor activity or exploratory behavior (all \( P > 0.05 \)) [supporting information (SI) Fig. S1 and Table S1]. Additionally, there were no social housing differences in open field central tendency (a measure of anxiety-like behavior) at the presurgical or postsurgical time points (\( P > 0.05 \)). Further, during rearing in a cylinder, there was no significant effect of housing condition on contralateral paw use pre- or postsurgery (all \( P > 0.05 \)).

There were no significant housing effects on body mass (\( F_{1,52} = 2.189, P = 0.05, \eta^2 = 0.04 \)), body temperature during surgery (\( F_{1,52} = 0.038, P > 0.05, \eta^2 = 0.0004 \)), or neuroscore (\( F_{1,52} = 2.901, P > 0.05, \eta^2 = 0.05 \)) across or within the experiments.

### Social Isolation Alters the Neuroinflammatory Response to Stroke.

Poststroke gene expression of macrophage antigen complex-1 (MAC-1), a pattern recognition complement receptor protein expressed on macrophage-lineage cells (\( F_{1,96} = 5.699, P < 0.05, \eta^2 = 0.05 \)), and glial fibrillary acidic protein (GFAP), an intermediate filament protein that is up-regulated in astrocytes following injury (\( F_{1,92} = 5.519, P < 0.05, \eta^2 = 0.05 \)), were significantly elevated in the ipsilateral (ischemic) relative to the contralateral (nonischemic) hemisphere across both time points after MCAO (Fig. S2 and Table S2). At the 12 h time point, there was a main effect of housing within the striatum on MAC-1 (\( F_{1,10} = 8.709, P < 0.05, \eta^2 = 0.46 \)) and GFAP (\( F_{1,15} = 6.63, P < 0.05, \eta^2 = 0.31 \)) gene expression, and a post hoc analysis revealed that both glial markers were significantly elevated in socially isolated animals relative to socially housed animals (\( P < 0.05 \)) (Fig. 2). Cortical gene expression of MAC-1 (\( F_{1,15} = 0.297, P > 0.05, \eta^2 = 0.02 \)) and GFAP (\( F_{1,15} = 2.67, P > 0.05, \eta^2 = 0.16 \)) did not vary significantly by housing conditions (\( P > 0.05 \)).

Overall, relative gene expression of proinflammatory cytokines interleukin-1 beta (IL-1\( \beta \)) (\( F_{1,146} = 11.429, P < 0.05, \eta^2 = 0.07 \)), tumor necrosis factor alpha (TNF-\( \alpha \)) (\( F_{1,136} = 30.876, P < 0.05, \eta^2 = 0.17 \)), and interleukin-6 (IL-6) (\( F_{1,12} = 15.180, P < 0.05, \eta^2 = 0.10 \)), as well as transforming growth factor beta (TGF-\( \beta \)) (\( F_{1,128} = 7.886, P < 0.05, \eta^2 = 0.22 \)) and cyclooxygenase-2 (COX-2) (\( F_{1,147} = 7.773, P < 0.05, \eta^2 = 0.05 \)) were significantly up-regulated in the ipsilateral (ischemic) hemisphere relative to the contralateral (nonischemic) hemisphere across both time points (Fig. S3). Post hoc analyses revealed that there were no effects of housing on IL-1\( \beta \), TNF-\( \alpha \), TGF-\( \beta \), or COX-2 expression (all \( P > 0.05 \)). However, IL-6 gene expression was significantly lower in socially isolated mice than socially housed mice at 12 h (striatum, \( F_{1,10} = 5.689, P < 0.05, \eta^2 = 0.36 \)). Further, brain IL-6 protein expression was significantly lower (cortex: \( F_{1,10} = 8.711, P < 0.05, \eta^2 = 0.49 \)), whereas serum IL-6 concentrations were significantly higher in socially isolated relative to socially housed mice (\( F_{1,15} = 9.297, P < 0.05, \eta^2 = 0.39 \)) (Fig. 3).

### IL-6 Antibody Infusion Eliminates the Influence of Social Interaction on Ischemic Outcome.

Treatment with an IL-6 neutralizing antibody significantly increased infarct volume. A 2-factor ANOVA revealed main effects of treatment (\( F_{1,24} = 16.081, P < 0.05 \)), housing (\( F_{1,24} = 5.057, P < 0.05 \)), and a treatment by housing interaction (\( F_{1,24} = 7.315, P < 0.05 \)) on infarct volume (\( \eta^2 = 0.32 \)). Among vehicle artificial cerebrospinal fluid (aCSF) treated mice, a Tukey post hoc analysis revealed that infarct volume was significantly larger in socially isolated than socially housed mice (\( P < 0.05 \)) but the infarct size was equivalent between animals in both housing conditions that received IL-6 antibody treatment.

Further, across both treatment conditions, a 2-factor ANOVA revealed a main effect of housing on serum concentration of IL-6 protein (\( F_{1,21} = 7.984, P < 0.05; \eta^2 = 0.28 \)). A Tukey post hoc revealed that socially isolated mice had significantly higher concentrations of circulating IL-6 protein compared with socially housed mice in the vehicle treated group (\( P < 0.05 \)). However, central administration of the IL-6 neutralizing antibody eliminated the difference in circulating IL-6 between socially housed and isolated mice. Thus, central IL-6 immunoneutralization in turn eliminated social influences on both postischemic infarct volume and peripheral IL-6 concentration (Fig. 4).

### Poststroke Serum Corticosterone (CORT) Concentrations.

A 2-factor ANOVA revealed a main effect of reperfusion time on CORT concentration (\( F_{1,47} = 10.975, P < 0.05; \eta^2 = 0.18 \).
presented as a ratio of ischemic to nonischemic hemisphere concentrations. Socially isolated mice. Gene and protein expression data in the CNS are compared to socially housed mice. Serum IL-6 measured via ELISA is up-regulated in socially isolated relative to socially housed mice (B). Serum IL-6 is up-regulated in vehicle-treated socially isolated mice (A) and cortical protein concentration measured via ELISA (C) are significantly down-regulated in the ischemic hemisphere of socially isolated relative to socially housed mice. (C) Serum IL-6 measured via ELISA is up-regulated in socially isolated mice. Gene and protein expression data in the CNS are presented as a ratio of ischemic to nonischemic hemisphere concentrations. * significantly different from socially housed mice, \( P < 0.05 \).

**Discussion**

Social environment influences immune function and disease outcome (17, 19). However, the mechanisms underlying the interaction of psychosocial factors and pathophysiology in ischemic injury require clarification. Data from the current study indicate that social housing condition is a strong determinant of the pathophysiology and long-term survival after experimental stroke. The survival rate to 7 days after experimental stroke was 100% for socially housed mice, compared with only 40% of socially isolated mice. The biased distribution in survival may reflect increased damage in socially isolated animals that consequently did not survive to day 7. Indeed, infarct and edema analyses at earlier time points indicate significantly greater ischemic damage in socially isolated mice than socially housed mice (Fig. 1). These data confirm and extend previous reports that social isolation potentiates the pathophysiological response to ischemia (8, 9) and suggest that social isolation contributes to early differences in the trajectory of ischemic injury development.

A separate cohort of animals was used to determine whether the increase in infarct size among socially isolated mice was associated with a difference in the neuroinflammatory response to MCAO. The neuroinflammatory response is triggered by activated microglia and astrocytes (i.e., reactive gliosis), as well as an up-regulation of proinflammatory cytokine release in response to neuronal damage (20–22). As expected, there was increased gene expression of MAC-1 and GFAP in the ipsilateral relative to the contralateral hemisphere after MCAO (Fig. S2). Importantly, within the ipsilateral hemisphere, gene expression of both MAC-1 and GFAP was increased in socially isolated mice relative to socially housed mice (Fig. 2). These data complement a recent report on social isolation-induced potentiation of neuroinflammatory responses in a model of global cerebral ischemia (9). The functional role of glia in ischemic injury is unclear; studies report both neuroprotective and damaging effects of glial products after an ischemic event (23–27). Although the current study does not indicate a causal relationship between the up-regulated glial markers and infarct volume, there is evidence that inhibition of microglial activation (via administration of minocycline) reduces stroke damage (28). Thus, taken together with increased infarct volume in socially isolated animals, it is possible that the secondary processes triggered by increased glial activation exacerbate neuronal damage.

We further conducted mRNA gene expression profiles on several genes that are central to the neuroinflammatory response in cerebral ischemia. Key among these genes are the cytokines IL-1β, TNFα, IL-6, TGF-β and the COX-2 enzyme. These inflammatory mediators are produced and secreted by activated glia within hours of ischemic injury and thus contribute significantly to the extent of neuronal damage after MCAO (21,
Our data indicate that gene expression of IL-1β, TNF-α, TGF-β, and COX-2 is significantly up-regulated in the ipsilateral relative to the contralateral hemisphere (Fig. S3), but, contrary to our initial hypothesis, these inflammatory markers do not appear to be influenced by social housing conditions. In contrast, IL-6 signaling is significantly altered by housing conditions; gene expression of striatal IL-6 is decreased in socially isolated relative to socially housed mice (Fig. 3A). These data were confirmed through protein analysis, which also indicated a decrease in central IL-6 protein expression in socially isolated mice (Fig. 3B).

Despite conflicting data on the functional role of IL-6 (29–32), studies demonstrate that central expression of this cytokine plays a critical neuroprotective role during an ischemic event (30, 31). Intracerebroventricular (ICV) administration of IL-6 reduces infarct size, possibly through a mechanism involving suppressed excitotoxicity (30, 31). Likewise, blockade of IL-6 signaling results in increased apoptotic cell death and infarct size, as well as poor neurological outcome (32). To address a role for central IL-6 as a mediator of the social housing effects on stroke outcome, mice were treated with an IL-6 neutralizing antibody or vehicle aCSF before MCAO. Treatment with the IL-6 antibody increased infarct volume in the socially housed group and eliminated the effect of social housing condition on infarct size (Fig. 4A). In contrast to reported effects of IL-6 on infarct volume (31), antibody treatment in our study did not affect infarct volume of socially isolated mice. One possible explanation for the absence of an effect among socially isolated mice is that poststroke central gene expression and protein concentrations of IL-6 in isolated mice were similar (or even lower) within the ischemic compared with the nonischemic hemisphere in our study; however, IL-6 was significantly elevated in the ischemic hemisphere of socially housed mice. Thus, the use of neutralizing antibody may reveal a “floor effect,” whereby IL-6 levels in the socially isolated mice cannot be further reduced. On the other hand, preventing the increase in IL-6 signaling via the neutralizing antibody potentiated infarct development in socially housed mice.

In addition to measuring central IL-6 protein levels, we assessed circulating concentrations of IL-6. Our data indicate that although central IL-6 is down-regulated (Fig. 3B), peripheral levels of IL-6 protein are up-regulated (Fig. 3C) in isolated relative to socially housed mice. This association between elevated levels of IL-6 and increased infarct size is consistent with the clinical literature on serum IL-6 concentration and stroke outcome. Within the clinical literature, elevated peripheral IL-6 is a reliable predictor of stroke occurrence, severity, and mortality (33, 34). The relationship between peripheral IL-6 and stroke outcome is indicative of an increased proinflammatory state, largely because of IL-6 mediated signaling of acute phase protein induction (i.e., CRP) after stroke (15, 35). Thus, contrary to its central actions, peripheral IL-6 is proinflammatory and is therefore a target of ongoing clinical trials for stroke patients (36). Data from the current study indicate that social housing condition influences both the neuroinflammatory and systemic inflammatory response to stroke. Importantly, both the central and peripheral IL-6 protein expression assays were performed in the same cohort of animals. Taken together, an up-regulation of peripheral IL-6, along with low central IL-6 expression, is consistent with an altered inflammatory state that contributes to poorer ischemic outcome in the socially isolated mice. Further, the increase in serum IL-6 among socially isolated mice is consistent with a previous report of increased intraischemic serum CRP concentrations in isolated relative to socially housed mice (8). Additionally, ICV treatment with the IL-6 antibody eliminated this group difference in serum IL-6 concentrations (Fig. 4B). An increase in serum IL-6 likely reflects an increase in the systemic inflammatory response to the substantial increase in infarct volume that occurred after treatment with the IL-6 antibody. In the current study, serum IL-6 concentrations are related to infarct size and do not appear to be independently modulated by social interaction in the postischemic period.

Another physiological system known to contribute to the extent of ischemic injury is the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis functions in part to coordinate the body’s physiological response to stressors by regulating glucocorticoid release (37). CORT plays an important modulatory role in ischemic cell death (38–40). After restraint stress, elevated postischemic serum CORT concentrations influence infarct size and functional outcome (40); in humans, poststroke cortisol concentration predicts mortality (41). Because social isolation is a stressor among several species, including Mus poschiavinus (42, 43), and is often associated with altered HPA-axis responsiveness (44), circulating CORT was measured in the current study at 12 h and 24 h after experimental stroke. CORT concentrations were similar between socially housed and socially isolated mice at both of these time points, despite a housing difference in infarct size in the 24 h cohort (Table S3). Although the data from the current study do not support a role for CORT underlying housing effects on infarct size, it remains possible that there may have been group differences in CORT concentration at earlier time points or that the stress of social isolation may be influencing IL-6 and infarct through a CORT-independent mechanism. Additional research is necessary to identify the upstream mechanisms underlying the effects of social isolation on ischemic outcomes.

In summary, socially isolated mice were less likely to survive a stroke and had increased infarct volumes and edema compared with socially housed mice. The increase in ischemic damage among socially isolated mice was accompanied by an altered neuroinflammatory response that was consistent with a neurocompromising influence of social isolation. Poststroke IL-6 signaling was down-regulated in the CNS and up-regulated in the periphery among socially isolated mice. Further, treatment with the IL-6 neutralizing antibody eliminated the effect of social isolation on serum IL-6 concentrations (i.e., CRP) after stroke (8). However, the behavioral assessments in the previous study were conducted at a later time point, suggesting that over time, socially housed mice may be better able to recover from functional deficits than socially isolated mice.

Measures of perceived social isolation or social support are as powerful, and in some cases more powerful, predictors of outcome than measures of actual social isolation or support in clinical studies examining health and well-being (45–47). It is not possible to differentiate between actual and perceived social isolation in mice, nor is there a measure in mice that would be comparable with social support in humans; however, the current study provides evidence that the presence or absence of a cohabitating conspecific is sufficient to alter stroke pathogenesis and outcome. Furthermore, the current study identifies differential expression of IL-6 as one factor contributing to the difference in infarct size between socially housed and isolated mice. Both social isolation and elevated serum IL-6 concentrations are associated with poor outcome in human stroke patients (1, 33, 34), but whether there is a causal link between these 2 factors in humans, as there appears to be in mice, will need to be empirically tested. Additional studies comparing the effects of social interaction on IL-6 expression in human stroke patients would also be informative.

In summary, socially isolated mice were less likely to survive a stroke and had increased infarct volumes and edema compared with socially housed mice. The increase in ischemic damage among socially isolated mice was accompanied by an altered neuroinflammatory response that was consistent with a neurocompromising influence of social isolation. Poststroke IL-6 signaling was down-regulated in the CNS and up-regulated in the periphery among socially isolated mice. Further, treatment with the IL-6 neutralizing antibody eliminated the effect of social isolation on serum IL-6 concentrations (i.e., CRP) after stroke (8). However, the behavioral assessments in the previous study were conducted at a later time point, suggesting that over time, socially housed mice may be better able to recover from functional deficits than socially isolated mice.
housing on infant size. Although numerous reports exist on neuroinflammatory measures in ischemia, they rarely describe housing conditions of the experimental mice, making it difficult to interpret those data independent of social/environmental influences. To our knowledge, the current study is the first to investigate the modulation of neuroinflammatory responses by social housing after experimental stroke. Taken together, these data support a causal role for IL-6 underlying the increase in ischemic injury associated with social isolation and provide evidence that social modulation of immune function can significantly influence stroke outcome.

Materials and Methods

Animals. Adult male C57/BL6 mice (23–30 g) (Charles River) were maintained in a 14:10 light/dark cycle in a temperature- and humidity-controlled vivarium. All animals were allowed ad libitum access to food and water. Experimental animals were housed either individually (socially isolated) or with an ovariectomized female (socio housed) for a period of 2 weeks before surgery and throughout the reperfusion period. The study was conducted in accordance with National Institutes of Health guidelines for the care and use of animals and under protocols approved by the institutional animal care and use committee.

Experimental Procedures

The influence of social housing on measures of stroke outcome was assessed in separate cohorts of mice at 5 different reperfusion periods. In experiment 1, mice were assessed for poststroke behavior, blood CORT concentration, and infarct size at 24 h (pair-MCAO, n = 10; single-MCAO, n = 10), 72 h (pair-MCAO, n = 13; single-MCAO, n = 11; pair-SHAM, n = 6; single-SHAM, n = 6), or 7 days of reperfusion (pair-MCAO, n = 8; pair-SHAM, n = 10; single-MCAO, n = 4 (6 died before sampling); single-SHAM, n = 10). Edema was determined at 48 h, the earliest time point at which secondary damage is observed after MCAO (pair-MCAO, n = 6; single-MCAO, n = 6).

In experiment 2, gene expression of inflammatory markers was measured in the cortex and striatum after stroke. Tissue was collected from separate cohorts of animals at 12 and 24 h of reperfusion (pair-MCAO, n = 6 per time point; single-MCAO, n = 6 per time point).

Experiment 3 was designed to test the role of central and peripheral levels of IL-6 in mediating the effects of social interaction on stroke outcome. In experiment 3a, blood and tissue were collected at 24 h of reperfusion (pair-MCAO, n = 6; single-MCAO, n = 6) for protein assay. In experiment 3b, mice were treated with IL-6 neutralizing antibody (10 ng) or vehicle (aCSF) 1 h before MCAO. Blood and brain tissue were harvested at 24 h of reperfusion and assessed for infarct volume and circulating IL-6 protein concentration (pair-MCAO-IL6 antibody, n = 7; pair-MCAO-aCSF, n = 6; single-MCAO-IL6 antibody, n = 7; single-MCAO-aCSF, n = 7). The ELISA for IL-6 requires a large amount of blood; among socially housed mice, 2 samples from the IL-6 antibody group and 2 samples from socially isolated mice were not sufficiently large to allow the assay. For determination of blood CORT and protein IL-6 concentrations, see SI Materials and Methods.

Surgery. Transient focal cerebral ischemia was induced by MCAO. The mice were anesthetized with 1.5% isofluorane in oxygen-enriched air provided through a face mask. Body temperature was maintained at 37 ± 0.5 °C through the use of a homeothermic blanket system. Briefly, unilateral right MCAO was achieved by insertion of a 6–0 nylon monofilament into the internal carotid artery to a point 6 mm beyond the internal carotid-ptygopalatine artery bifurcation. After 60 min of occlusion, the animal was reanesthetized and reperfusion was initiated by removal of the filament. For a detailed description of the MCAO procedure and determination of stroke volume and edema, see SI Materials and Methods.

Behavioral Testing. Animals in Experiment 1 underwent paw preference and open field behavioral testing. Both tests were conducted under similar environmental conditions (i.e., lighting, temperature, level of background noise, and time of day) at 24 h before MCAO and again at 72 h of reperfusion. However, it is important to note that the mouse’s familiarity with the testing environment was different at baseline testing (their first exposure to the testing chamber) and postsurgical testing (their second exposure to the testing chamber), which complicates comparison of behavior across these 2 time points. Thus, emphasis was placed on comparing experimental groups independently at each time point. Behavioral testing was conducted during the light phase and scored by an individual who was not aware of group assignment. The apparatuses were thoroughly cleaned between animals using a 70% alcohol solution (see SI Materials and Methods).

Real-Time PCR. RT-PCR was conducted at 12 and 24 h of reperfusion after MCAO. Bilateral samples were dissected from the cortex and striatum, and total RNA was extracted by using a homogenizer (Ultra-Turrax T8, IKA Works) and an RNeasy Mini Kit (Qiagen) according to manufacturer’s protocol. Extracted RNA was suspended in 30 μL of RNase-free water, and RNA concentration was determined by a spectrophotometer (NanoDrop ND-1000). The following inventoried primers and probes (Applied Biosystems) were used: GFAP, MAC-1, interleukins IL-6 and IL-1β, TNFα, COX-2, and TGF-β. A TaqMan 18S rRNA primer and probe set, labeled with VIC dye (Applied Biosystems), were used as a control gene for relative quantification. Amplification was performed on an ABI 7000 Sequence Detection System by using Taqman Universal PCR master mix. The universal 2-step RT-PCR cycling conditions used were: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min.

Intracerebroventricular Cannulation and IL-6 Neutralizing Antibody Injection. A guide cannula, targeting the left lateral ventricle, was implanted 1 week before experimental stroke surgery. The mice were anesthetized with 1%–1.5% isofluorane in oxygen-enriched air and were placed in a stereotaxic apparatus (David Kopf Instruments). A craniotomy was performed along the midline to locate bregma. The cannula, 2.00 mm below the pedestal (Plastics One), was positioned at +0.02 mm posterior and +0.95 mm lateral to bregma and secured with glue. Once the glue was dry, a dummy cannula was inserted into the guide cannula, and the mice were placed into their home cages for recovery. The neutralizing antibody to IL-6 (10 ng in 2 μL vehicle) (zcomR&D Systems) or vehicle, 2 μL aCSF, was infused 1 h before MCAO surgery. This dose has been used successfully to neutralize IL-6 signaling in mice (48). According to the manufacturer, this dose is within range of the 50% neutralization dose determined in the presence of 0.25 ng/mL mouse IL-6 (R&D Systems anti-mouse IL-6 Ab, AF-406-NA). The solutions were administered over 30 sec by using a 5 μL Hamilton syringe. Correct cannula placement was confirmed through cresyl violet staining.

Data Analysis. Results for surgical parameters, survival, infarct volume, edema, CORT concentrations, and serum IL-6 protein concentrations were analyzed via a 2-way ANOVA (factors were surgery and housing), a one-tailed t test where appropriate (edema), or by using nonparametric statistics (Mann–Whitney U). Gene expression and brain protein expression data were analyzed via 3-way ANOVA (factors were hemisphere, reperfusion period, and housing). Further, PCR data were also expressed as a ratio of ipsilateral to contralateral hemisphere (R/L) gene expression and were analyzed via 2-way ANOVA (factors were surgery and housing). Significant ANOVA results were followed by a Tukey HSD post hoc test. Behavior was analyzed by independent 2-way ANOVAs (factors were surgery and housing) at baseline and 72 h postsurgery because novelty of the testing environment may have differentially influenced behavior at these 2 time points; for the purpose of this study, across-group comparisons at the postsurgical time point are more informative than within group comparisons between baseline and the postsurgical time point. When the data did not meet assumptions of normality (ex. serum IL-6), a log transformation was conducted before analysis. Data were considered significant at P < 0.05, and effect sizes (r for nonparametric and eta squared, η² for parametric data) are reported for all relevant data.

Acknowledgments. We thank Zachary Weil and James Walton for technical support and assistance with data analysis and Zachary Weil for critiquing the manuscript. This work was supported by grants from the American Heart Association (Established Investigator Award to A.C.D. and predoctoral fellowship to J.K.K.), National Institute of Neurological Disorders and Stroke Behavioral Core Grant P30 NS045758 (to A.C.D.), National Institute of Neurological Disorders and Stroke Grant RO1NS04267–05 (to A.C.D.), and National Heart, Lung, and Blood Institute Grant RO1HL080249–01 (to A.C.D.).


Supporting Information

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SI Materials and Methods

Surgery. Transient focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO). The mice were anesthetized with 1.5% isoflurane in oxygen-enriched air provided through a face mask. Body temperature was maintained at 37 ± 0.5 °C through the use of a homeothermic blanket system. Briefly, unilateral right MCAO was achieved by insertion of a 6–0 nylon monofilament into the internal carotid artery to a point 6 mm beyond the internal carotid-pterygopalatine artery bifurcation. Once secured, the wound was sutured and the animal was allowed to awaken from anesthesia. After 60 min of occlusion, the animal was reanesthetized and reperfusion was initiated by removal of the filament. For SHAM surgery, the internal carotid artery was exposed, but not disturbed; all other aspects of the surgery remained the same. Sixty minutes after MCAO surgery, a neurological score was assigned to each animal as previously described (1).

Determination of Stroke Volume. Immediately after cervical dislocation and decapitation, fresh brains were removed, sectioned into five 2-mm-thick coronal sections, and incubated for 15 min with 2,3,5-triphenyltetrazolium at 37 °C, that stains live mitochondria. Slices were postfixed with 10% buffered formalin for 3–5 days before image analysis, at which point the slices were photographed, and infarct area throughout the cerebrum was analyzed by using Inquiry software (Loats Associates). Infarct size was determined as a percentage of the contralateral hemisphere after correcting for edema by using the following formula: [1-(total ipsilateral hemisphere - infarct)/total contralateral hemisphere] ×100.

Determination of Poststroke Edema. Brain tissue was collected at 48 h of reperfusion immediately after transcardial perfusion with 20 mL of 0.9% saline. The brain tissue was divided into ipsilateral (right) and contralateral (left) hemisphere, and an initial wet weight was obtained (W_R and W_L, respectively). The tissue was then dehydrated in an oven maintained at 70 °C, and dry weights of right and left hemispheres (D_R and D_L respectively) were obtained at 24-hour intervals until 2 consecutive weights yielded the same mass (2). An index of edema was calculated by using the formula: I = [(W_R/D_R – W_L/D_L)/(W_L/D_L)] ×100.

Behavioral Testing. Paw preference. Each animal was placed individually inside a clear plastic cylinder (8 cm internal diameter, 12 cm height) for 5 min and videotaped simultaneously from 4 angles. Paw preference was recorded as whether the left (contralateral) or right (ipsilateral) paw was first to contact the cylinder during rearing. Only the initial paw placement for each rear was recorded. If both paws were placed on the cylinder in such rapid succession that slow motion analysis could not determine which paw was placed first, then the placement was scored as being “simultaneous”. An index of contralateral paw preference was determined by using the following formula: [left/(left + right + simultaneous)] ×100.

Open field. Exploratory behavior was assessed in an open field apparatus by using Flex Field photobeam activity (San Diego Instruments). The apparatus was enclosed in a sound attenuating chamber equipped with a ventilating fan that provided masking noise. A clear Plexiglas insert (40 × 40 × 37.5 cm) was fitted inside a metal frame consisting of 16 equally spaced infrared photocell detectors. Interruptions in the infrared light sources by the experimental animal were recorded in the associated computer program. Animals were individually placed inside the apparatus for 60 min sessions, and data were analyzed to determine general locomotor activity and relative amount of activity occurring in the periphery versus the center of the apparatus.

Determination of blood corticosterone (CORT) concentrations. Trunk blood samples were collected immediately after rapid cerebral dislocation and decapitation. The samples were centrifuged at 6,000 rpm (model 5415R; Eppendorf, Hamburg, Germany) for 30 min at 4 °C; sera were collected and stored at -80 °C until assayed. CORT concentrations were determined by using an I125 CORT kit (MP Biomedical). The standard curve was run in triplicate, and samples were run in duplicate. All samples within an experiment were run in a single assay.

IL-6 ELISA. After MCAO, bilateral samples from the cortex and striatum, as well as blood serum, were collected for analysis of central and peripheral IL-6 protein expression. For protein extraction, brain tissue was homogenized in radioimmunoprecipitation assay (RIPA) buffer with protease inhibitors (Pierce). Brain tissue lysates and serum samples were diluted 1:5 and assayed by using a sandwich ELISA kit (BD Biosciences) according to manufacturer’s protocol.


Fig. S1. Exploratory behavior in the open field. (A) Total locomotor activity and (B) frequency of rearing measured 1 day before MCAO or SHAM (PRE) and at 72 h (POST), significantly decreases after surgery in both MCAO and SHAM mice, but does not vary by housing condition. *, Significantly different from SHAM; $P < 0.05$. 
Fig. S2. Relative gene expression of MAC-1 and GFAP in striatum is up-regulated in the ipsilateral ischemic (IPSILAT) relative to the contralateral non-ischemic (CONTRALAT) hemisphere. Data are collapsed across the 12- and 24-hour reperfusion timepoints. *, Significantly different from contralateral hemisphere; P < 0.05.
Fig. S3. Relative gene expression of inflammatory markers following MCAO measured via RT-PCR. mRNA gene expression of IL-1β, TNF-α, COX-2, IL-6, and TGF-β are significantly up-regulated in the ipsilateral relative to the contralateral hemisphere. *, Significantly different from contralateral hemisphere; *P < 0.05.
Table S1. Group means and standard deviations of functional outcome, infarct volume, and serum IL-6 concentrations

<table>
<thead>
<tr>
<th>Measure</th>
<th>Isolated, mean (± SD)</th>
<th>Paired, mean (± SD)</th>
<th>SHAM, mean (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total locomotor activity, beam breaks</td>
<td>PRE 6657392 (976.64)</td>
<td>POST 2425.67 (1316.00)</td>
<td>PRE 5767.38 (853.08)</td>
</tr>
<tr>
<td>Rearing, beam breaks</td>
<td>PRE 442.84 (104.10)</td>
<td>POST 55.31 (51.06)</td>
<td>PRE 397.67 (93.96)</td>
</tr>
<tr>
<td>Contralateral paw use, %</td>
<td>PRE 32.86 (14.69)</td>
<td>POST 10.17 (16.56)</td>
<td>PRE 34.48 (11.64)</td>
</tr>
<tr>
<td>Infarct volume, %</td>
<td>aCSF 32.64 (18.58)</td>
<td>aCSF 6.92 (5.41)</td>
<td>aCSF 6.92 (5.41)</td>
</tr>
<tr>
<td></td>
<td>IL-6 Ab 50.56 (6.13)</td>
<td>IL-6 Ab 47.78 (13.32)</td>
<td>–</td>
</tr>
<tr>
<td>Serum IL-6, log transformed</td>
<td>aCSF 3.53 (0.18)</td>
<td>aCSF 6.92 (5.41)</td>
<td>aCSF 6.92 (5.41)</td>
</tr>
<tr>
<td></td>
<td>IL-6 Ab 3.58 (0.15)</td>
<td>IL-6 Ab 47.78 (13.32)</td>
<td>–</td>
</tr>
</tbody>
</table>

Shown are summaries of measures of functional outcome at baseline (PRE) and postsurgery (POST), as well as infarct volume and serum IL-6 after artificial cerebrospinal fluid (aCSF) or IL-6 antibody (IL-6 Ab) treatment of socially housed and isolated mice.
## Table S2. Group means and standard deviations of relative gene expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Ipsilateral hemisphere, mean (±SD)</th>
<th>Contralateral hemisphere, mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CORTEX</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>0.99 (0.28)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>2.49 (2.31)</td>
</tr>
<tr>
<td></td>
<td>STRIATUM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>1.20 (0.72)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>1.09 (0.38)</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CORTEX</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>2.51 (2.01)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>2.62 (1.85)</td>
</tr>
<tr>
<td></td>
<td>STRIATUM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>3.40 (2.62)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>3.00 (3.18)</td>
</tr>
<tr>
<td></td>
<td>COX-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CORTEX</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>1.62 (1.10)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>2.96 (1.55)</td>
</tr>
<tr>
<td></td>
<td>STRIATUM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>1.14 (0.23)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>1.14 (0.51)</td>
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<tr>
<td></td>
<td>IL-6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CORTEX</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>1.51 (1.12)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>1.19 (0.58)</td>
</tr>
<tr>
<td></td>
<td>STRIATUM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>1.28 (0.87)</td>
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<tr>
<td></td>
<td>24 h</td>
<td>1.25 (0.91)</td>
</tr>
<tr>
<td></td>
<td>TGF-β</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CORTEX</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>0.92 (0.15)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>1.06 (0.24)</td>
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<tr>
<td></td>
<td>STRIATUM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>1.03 (0.12)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>1.26 (0.35)</td>
</tr>
</tbody>
</table>

Shown are summaries of relative gene expression data. Data are presented as gene expression relative to control gene (18S) expression. All data are collapsed across both housing conditions.
<table>
<thead>
<tr>
<th>Group</th>
<th>Isolated, ng/mL</th>
<th>Paired, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCAO-12 h</td>
<td>290.24 ± 84.44</td>
<td>269.70 ± 125.67</td>
</tr>
<tr>
<td>MCAO-24 h</td>
<td>163.63 ± 86.93</td>
<td>180.35 ± 106.77</td>
</tr>
</tbody>
</table>

Concentrations are mean ± SD.