Social Interaction Improves Experimental Stroke Outcome

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Background and Purpose—Social interaction can have a profound effect on health. The purpose of the present study was to determine whether affiliative social interactions before and after stroke improve ischemic outcomes as assessed through histological analysis and behavioral assays.

Methods—Male and female C57BL/6 mice were housed individually or with an ovariectomized female. Behavioral assessments were made 24 hours before 60 or 90 minutes of transient intraluminal middle cerebral artery occlusion (MCAO) or SHAM surgery and after 7 days of reperfusion. Two hours after behavioral testing on day 7, infarct size was determined by 2,3,5-triphenyltetrazolium histology, and blood samples were collected for assessment of corticosterone and C-reactive protein (CRP) concentrations.

Results—Pair housing significantly decreased infarct size and improved contralateral paw use in 60-minute MCAO males and 90-minute MCAO females compared with socially isolated cohorts. Housing condition had no significant effect on infarct size in females that underwent 60 minutes of MCAO, but pair housing was associated with improved contralateral paw use relative to socially isolated mice. In a separate cohort of males, intraischemic CRP concentration was significantly reduced in pair-housed males relative to isolated males.

Conclusions—Affiliative interaction during the peri-ischemic period reduces intraischemic CRP concentration, decreases ischemic damage in male and female mice, and improves behavioral outcome. (Stroke. 2005;36:2006-2011.)

Key Words: behavior ■ ischemia ■ stroke

Social isolation predicts morbidity and mortality from a myriad of health conditions, including stroke and cerebrovascular disease.1–3 In contrast, patients with high levels of social support or large social networks exhibit more rapid and extensive functional recovery after stroke than socially isolated individuals.4,5 However, the mechanisms through which social support influence stroke outcome are not known.

Social influences on experimental stroke outcome have been studied most often in the context of environmental enrichment studies. The environmental enrichment typically includes a social component, a means of voluntary exercise (ie, running wheels), and novel stimulus objects. Housing rats in an enriched environment beginning several days after induction of stroke does not significantly alter infarct volume, but it does improve functional recovery,6–10 possibly through a mechanism that involves changes in dendritic structure in the contralateral hemisphere,11 altered gene expression,12 or increased neurogenesis.13

The mechanisms through which social environment can influence the histological and behavioral outcomes of stroke are largely unknown and are likely multifactorial. In the current study, we examined the effects of social isolation versus pair housing on stroke-induced infarct size and functional recovery in male and female mice. Further, we examined blood corticosterone and C-reactive protein (CRP) as possible mechanisms through which affiliative social interactions can influence ischemic outcomes.

Materials and Methods

Animals
Adult male and female C57BL/6 mice (Charles River; Wilmington, De) age 9 weeks of age were maintained in a temperature-controlled (≈70°F) vivarium on a 14/10 light/dark cycle, with ad libitum access to food and water. Experimental animals were housed either individually or with an ovariectomized female. The assigned housing condition was maintained for 2 weeks before the study and throughout the 7-day 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.

Surgery
Transient focal cerebral ischemia was induced in male and female mice by middle cerebral artery occlusion (MCAO). Mice were anesthetized with 1% to 1.5% halothane in oxygen-enriched air.
delivered through a facemask. Unilateral MCAO was achieved by inserting a 6-0 nylon monofilament into the internal carotid artery, via the external carotid artery, 6 mm beyond the internal carotid artery–pterygopalatine artery bifurcation. Once the filament was secured, the wound was sutured and the animal was allowed to emerge from the anesthesia. After 60 or 90 minutes of occlusion, a neurological score was assigned,24 then the animal was reanesthetized briefly and reperfusion initiated via withdrawal of the monofilament. For the SHAM surgery, the internal carotid artery was exposed but not disturbed. Throughout the surgery, rectal temperature was maintained at 37±0.5°C through the use of a homeothermic blanket system. The groups for experiment 1 (histology and behavior) included (1) socially isolated males, 60 minutes of MCAO (n=9); (2) pair-housed males, 60 minutes of MCAO (n=9); (3) socially isolated males, SHAM (n=8); (4) pair-housed males, SHAM (n=7); (5) socially isolated females, 60 minutes of MCAO (n=10); (6) pair-housed females, 60 minutes of MCAO (n=8); (7) socially isolated females, 60 minutes of SHAM (n=8); (8) pair-housed females, 60 minutes of SHAM (n=9); (9) socially isolated females, 90 minutes of MCAO (n=9); and (10) pair-housed females, 90 minutes of MCAO (n=9). Mice were recovered 7 days and assessed for behavioral performance. Then, 2 hours after behavioral testing and 1 hour before the onset of the dark cycle, tissue and blood were collected. Experiment 2 involved collection of intraschismic (60 minutes; pair n=12, single n=12) and postschismic (24 hours; pair n=13, single n=10) blood samples for assessment of corticosterone and CRP concentrations in male mice.

**Determination of Intraschismic Blood Flow and Corticosterone Concentrations**

Relative cerebral blood flow, blood gas and glucose concentrations, and blood corticosteroid concentrations were determined in a separate, nonsurviving, cohort of animals (socially isolated males n=6; pair-housed males n=6; socially isolated females n=6; pair-housed females n=6), as described previously. Blood flow was assessed using laser Doppler flowmetry (LDF) probe (DRT4; Moor Instruments, Ltd). LDF readings were taken at 15-minute intervals beginning 30 minutes before occlusion and continuing through 60 minutes of ischemia and 30 minutes of reperfusion. Blood samples were collected at baseline and after 30 minutes of ischemia for assessment of PaCO2, PaO2, and glucose (1-STAT Portable Clinical Analyzer with CG8+ cartridge; Heska). A separate blood sample (15 µL) was collected at 60 minutes of ischemia for assessment of corticosterone concentration.

**Determination of Blood Corticosterone Concentrations**

Blood samples were collected and assayed using an 125I corticosterone kit (ICN Biomedical) as described previously.14 Four intraschismic samples with values above the upper range of the assay were assigned a concentration of 1000 ng/mL (equivalent to the highest standard). Because of a technical malfunction, six 7-day reperfusion samples (distributed across 4 experimental groups) could not be assayed.

**Determination of CRP Concentrations**

For experiment 2, an intraschismic blood sample was collected after 60 minutes of MCAO in pair-housed (n=10) and socially isolated (n=10) male mice (females were not used in this experiment). A terminal blood sample also was collected in a separate cohort of mice at 24 hours after surgery (pair-housed n=13; socially isolated n=10). CRP concentration was determined using a mouse-specific high-sensitivity CRP ELISA kit (Kamiya Biomedical Company).

**Behavioral Testing**

Behavioral testing was conducted during the light phase by an individual unaware of experimental assignment. The apparatuses were thoroughly cleaned between animals using a 70% alcohol solution.

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Percent infarct relative to the contralateral hemisphere after 60 minutes of MCAO (mean±SEM). Pair housing decreased infarct size in male (isolated n=9; paired n=9), but not female (isolated n=10; paired n=8), mice. *P<0.05.

**Latency to Move**

Latency to initiate movement (LTM) was assessed as described previously.15 Data from one socially isolated male SHAM was excluded because its mean postsurgical LTM was >3 SD from the mean.

**Paw Preference**

The mouse was placed inside a clear plastic cylinder (8 cm internal diameter, 12 cm height) for 5 minutes and videotaped.16 The experimenter recorded each time the mouse placed its paw or paws on the side of the cylinder, noting whether the left (contralateral) or right paw touched first. Paw preference was determined using the formula \[ \text{Paw preference} = \frac{\text{left paw touches first}}{\text{total number of paw touches}} \times 100.\]

**General Locomotor Activity**

Locomotor activity was assessed during a 60-minute session in Flex Field photobeam activity systems (San Diego Instruments) as described previously.17

**Determination of Stroke Volume**

Immediately after cervical dislocation and blood sampling, brains were removed, sectioned into 5 2-mm-thick coronal sections, incubated for 15 minutes in 2,3,5-triphenyltetrazolium at 37°C, then fixated in 10% buffered formalin solution. The brain slices were photographed and measured using Inquiry software (Loats Associates Inc.) to determine infarct size as a percentage of the contralateral hemisphere after correcting for edema, as described previously.14

**Statistical Analyses**

The behavioral data from the animals that underwent 60 minutes of MCAO were analyzed via 3-way ANOVA for repeated measures (factors were sex, surgery, and housing). The behavioral data from animals that underwent 90 minutes of MCAO were analyzed via ANOVA for repeated measures. Corticosterone concentrations, blood gases, glucose, pH values, and infarct sizes were analyzed via ANOVA or t test. When the data did not meet the assumptions of ANOVA, a log10 transformation was conducted before analysis (ie, 24-hour CRP, intraschismic corticosterone).

**Results**

**Surgical Parameters and Infarct Size**

There was a significant effect of sex (F(1,32)=5.79; P<0.05) and housing (F(1,32)=9.09; P<0.05; Figure 1) on infarct size.
among animals subjected to 60 minutes of MCAO. Overall, males had larger infarcts than females, and socially isolated mice had larger infarcts than pair-housed mice. Within-sex analysis of housing revealed that pair housing significantly decreased infarct size relative to social isolation in males ($t_{16} = 2.35; P < 0.05$). Although a similar trend was observed in females, the difference in infarct size between the paired and socially isolated groups did not reach significance ($t_{16} = 1.98; P = 0.07$). Body mass at time of surgery was significantly lower in female than male mice ($F(1,32) = 15.06; P < 0.05$), but there was no effect of housing condition on body mass ($F(1,32) = 1.52, P > 0.05$). No differences were seen in body temperature, intraischemic LDF (reduced to $<13\%$ of baseline), or reperfusion LDF ($>95\%$ of baseline) between groups ($P > 0.05$).

To rule out a possible “floor effect” (the possibility that 60 minutes of occlusion produces too small of an infarct to effectively detect varying effects of housing conditions) in females, a longer duration of ischemia (90 minutes of MCAO) was added. In contrast to the results from the 60 minutes of MCAO experiment, the pair-housed females that had undergone 90 minutes of MCAO had significantly smaller infarcts than the socially isolated females ($t_{16} = 3.28; P < 0.01$; see Figure 3A). There were no significant group differences in body weight ($t_{16} = 1.54; P > 0.05$), body temperature during surgery ($t_{16} = 0.74; P > 0.05$), or neuroscore ($t_{16} = 0.97; P > 0.05$).

### Functional Outcome

Among animals that underwent 60 minutes of MCAO or SHAM, there was a significant overall effect of surgery ($F(1,60) = 4.59; P < 0.05$; higher locomotor activity in SHAM than MCAO) but no overall effect of sex or housing on general locomotor activity. However, there were significant differences between baseline and posturgical testing ($F(1,60) = 24.27; P < 0.01$; higher locomotor activity at baseline).

There also were significant effects of sex ($F(1,59) = 12.01; P < 0.01$) and housing ($F(1,59) = 4.23; P < 0.05$) but no effect of surgery ($P > 0.05$) on latency to move 1 body length. Overall, latency to move was significantly longer in male mice than female mice and longer in paired mice than isolated mice, regardless of surgical manipulation.

There also was a significant effect of surgery ($F(1,60) = 10.89; P < 0.01$) and an interaction between surgery and housing ($F(1,60) = 8.17; P < 0.01$) on contralateral paw use. After 60 minutes of MCAO, there was a significant decrease in contralateral paw use in isolated males ($t_{14} = 2.39; P \leq 0.05$) and isolated females ($t_{14} = 3.15; P \leq 0.05$) relative to baseline. In contrast, there was no effect of MCAO on contralateral paw use in pair-housed males ($t_{14} = 1.33; P > 0.05$) or females ($t_{14} = 0.41; P > 0.05$). SHAM surgery did not affect contralateral paw use in either males (pair $t_{14} = 0.25; P > 0.05$; isolated $t_{14} = 1.03, P > 0.05$) or females (pair $t_{14} = 1.61, P > 0.05$; isolated $t_{14} = 0.11, P > 0.05$; Figure 2).

Females exposed to 90 minutes of ischemia did not exhibit any alteration in general locomotor activity 7 days after surgery relative to baseline ($F(1,16) = 0.01; P > 0.05$).

However, overall, pair-housed females exhibited significantly less locomotor activity than socially isolated females ($F(1,16) = 5.45; P < 0.05$). There was no significant effect of surgery ($F(1,16) = 0.31; P > 0.05$) or housing condition ($F(1,16) = 0.11; P > 0.05$) on latency to move 1 body length. Overall, the ratio of contralateral (left) paw use decreased after MCAO relative to baseline ($F(1,16) = 5.52; P < 0.05$). Although there was not an overall effect of housing on contralateral paw placement ratio ($F(1,16) = 0.01; P > 0.05$), within-group analysis revealed a significant decline in contralateral paw use in the socially isolated group ($t_{16} = 2.64; P < 0.05$) but not in the pair-housed group ($t_{16} = 0.67; P > 0.05$; Figure 3B).

### Corticosterone

There was a main effect of sex on intraischemic corticosterone concentrations in blood collected at 60 minutes of occlusion ($F(1,23) = 1.86; P < 0.05$); corticosterone concentrations were lower in females than males. There was no effect of sex or housing on corticosterone collected at reperfusion day 7 in male or female mice subjected to 60 or 90 minutes of MCAO ($P > 0.05$; Table 1). Furthermore, within each housing condition, plasma corticosterone concentration was significantly greater in the MCAO cohorts than in the SHAM cohorts ($P < 0.05$).

### CRP Concentrations

Intraischemic CRP concentrations were significantly higher in socially isolated males than pair-housed males after 60 minutes of cerebral ischemia ($t_{22} = 2.24; P < 0.05$; Figure 4). By 24 hours of reperfusion, infarct size was significantly smaller in pair-housed than socially isolated animals ($t_{22} = 2.18; P < 0.05$), but blood CRP and corticosterone concentrations were similar between socially isolated and pair-housed animals ($P > 0.05$; Table 2).

### Discussion

Pair housing decreased infarct size and improved functional outcome after 60 minutes of MCAO in male mice and 90
minutes of MCAO in female mice (Figures 1 and 3A). The socially isolated 60-minute MCAO male mice and 90-minute MCAO female mice exhibited a significant decrease in contralateral paw use that was not observed in any of the pair-housed or SHAM-operated mice (Figures 2 and 3B). These data are in contrast to previous studies reporting that social housing improves behavioral recovery in the absence of an effect on infarct size.8,18 The most likely explanation for the difference in the effect of social housing on infarct size is that the rats in the 2 previous studies were housed in groups before and after ischemia but were socially isolated for 24 hours after stroke. Thus, it appears that immediate return to the social group is necessary to decrease stroke-induced neuronal death, but that there is a wider therapeutic window for the positive effects of social interaction on behavioral recovery. There are several other methodological differences between the studies that also could account for the discrepancy in results, including species (rats versus mice), method of inducing ischemia (proximal ligation versus intraluminal MCAO), and duration of occlusion (permanent versus transient). Importantly, in the present study, the experimental males were housed with 1 ovariectomized female rather than several other males, as in previous studies.8,18 Male rats that are housed together often establish a hierarchy and engage in different types of social interactions than male–female pairs,19 which could differentially affect their physiology and ultimately change their response to ischemia.

After 60 minutes of MCAO, female mice had significantly smaller infarcts than males, as expected.20 Social housing had no significant impact on infarct size (Figure 1) but improved behavioral outcome after 60 minutes of MCAO in females. Socially isolated females exhibited a significant decrease in contralateral paw use after MCAO (Figure 2). There was no significant change in contralateral paw use between pair-housed MCAO females or SHAM-operated females. In contrast, after 90 minutes of MCAO, infarct sizes were smaller and functional recovery was better in pair-housed females than socially isolated females (Figure 3). Thus, the lack of an effect of social interaction on infarct size in 60-minute MCAO females likely reflects a “floor effect” because the infarct sizes were already quite small (≈7% to 11% of the contralateral hemisphere). Whether the significant decrease in infarct size among pair-housed mice reflects permanently rescued neurons or merely delayed neuronal death remains to be determined in future experiments.

Cerebral ischemia activates the hypothalamic-pituitary-adrenal axis,21 which, in turn, can impact infarct size and functional recovery in rodents15,22 and predict morbidity and mortality in humans.23–25 Negative social interactions increase posts ischemic corticosteroid concentrations and

<p>| TABLE 1. Corticosterone Concentrations (ng/mL) at Reperfusion Day 7 |
|-------------------------|-------------------------|-------------------------|</p>
<table>
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<th>SHAM (Mean±SEM)</th>
<th>MCAO (Mean±SEM)</th>
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<td>Male</td>
<td>Isolated</td>
<td>119.41±56.87</td>
</tr>
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<td>Male</td>
<td>Paired</td>
<td>82.54±28.52</td>
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Figure 3. Infarct size and contralateral paw use after 90 minutes of MCAO (mean±SEM). A, Infarct volume is significantly decreased in pair-housed females (n=9) relative to socially isolated females (n=9). B, Isolated females exhibit a significant decrease in poststroke left paw use compared with baseline. *P≤0.05.

Figure 4. Intraischemic CRP concentrations (mean±SEM). Pair-housed males (n=13) have significantly lower CRP concentrations than socially isolated (n=10) males at 60 minutes of ischemia. *P<0.05.
are correlated with decreased bcl-2 expression and increased infarct size.\textsuperscript{15} In contrast, affiliative behaviors can suppress the hypothalamic-pituitary-adrenal axis and act as a buffer against stress.\textsuperscript{26} In the current study, housing did not have an effect on intraischemic or posts ischemic corticosterone concentration. These data suggest that corticosteroids are unlikely to mediate the effects of social pairing on ischemic outcome.

In contrast, social housing did influence CRP concentrations; pair-housed males had significantly lower blood CRP concentrations at 60 minutes of MCAO than socially isolated males (Figure 4). In apparently healthy humans, low social support is also associated with increased CRP concentrations.\textsuperscript{27} After inflammatory stimuli, CRP concentrations in blood increase so dramatically that CRP is often used clinically as an index of inflammation,\textsuperscript{28} which can be an important risk factor for stroke.\textsuperscript{29}

Indeed, high CRP is predictive of future stroke in men and women\textsuperscript{30,31} and is associated with higher Canadian Neurological Stroke Scores and Barthel Index Scores at 1 year.\textsuperscript{32} Whether elevated CRP influences ischemic outcome or is merely a marker of inflammation resulting from extensive neuronal damage has not been resolved,\textsuperscript{29,33} but studies in rats treated with human CRP suggest a causative role in exacerbating tissue damage during global and focal cerebral ischemia.\textsuperscript{34,35}

In summary, male and female mice that were socially isolated had larger MCAO-induced infarcts and greater functional deficits than pair-housed mice. The effects of pair housing on ischemic outcome do not appear to be related to intraischemic or posts ischemic corticosterone concentration because hormone concentration did not vary significantly as a function of housing. In contrast, intraischemic CRP concentration was significantly elevated in socially isolated mice relative to pair-housed mice. Future studies will be aimed at determining whether elevated CRP concentrations in socially isolated mice directly influence infarct size or whether they are a marker of an altered stroke-induced neuroinflammatory response.

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