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Effect of a contralateral lesion on neurological recovery from stroke in rats

Fen Sun^{a,b}, Lin Xie^b, XiaoOu Mao^b, Justin Hill^b, David A. Greenberg^b, and Kunlin Jin^{a,b,*}

^aDepartment of Pharmacology and Neuroscience, University of North Texas, TX, USA

^bBuck Institute for Research on Aging, CA, USA

Abstract

Purpose—Clinical studies suggest a correlation between changes in activity of the contralesional cerebral cortex and spontaneous recovery from stroke, but whether this is a causal relationship is uncertain.

Methods—Young adult Sprague-Dawley male rats underwent unilateral or bilateral permanent distal middle cerebral artery occlusion (dMCAO). Infarct volume was determined by 2,3,5-triphenyltetrazolium chloride (TTC) staining 24 hr after dMCAO, and functional outcome was assessed 1–28 days after dMCAO using the ladder rung walking and limb placing tests.

Results—Infarct volume was unchanged, but functional neurological deficits were reduced 1 day after bilateral compared to unilateral dMCAO.

Conclusions—Activity in the contralesional cerebral cortex may inhibit functional motor recovery acutely after experimental stroke.

Keywords

Stroke; ischemia; recovery; rat

1. Introduction

Several lines of evidence suggest that the contralesional cerebral cortex plays a role in functional recovery from stroke (Cramer, 2008). Interhemispheric inhibition arising in the contralesional motor cortex is increased in patients with stroke and correlates with poor functional outcome (Murase et al., 2004), whereas reducing such activity by cutaneous anesthesia of the unaffected hand (Floel et al., 2008) or by transcranial direct current stimulation (Hummel and Cohen, 2006) enhances motor function in stroke patients. Functional imaging studies also show an inverse correlation between motor task-associated recruitment of contralesional cortex and stroke outcome (Ward et al., 2003). Conversely, following stroke induced by middle cerebral artery occlusion in rats, injection of lidocaine into the contralesional cerebral cortex (Biernaskie et al., 2005) or disconnection of the hemispheres by corpus callosotomy (Jin et al., 2011) exacerbates deficits.

If contralesional cortex influences stroke outcome, the nature of this influence might be clarified by producing a second lesion contralateral to the stroke, and evaluating its effect on

associated neurological deficits. Accordingly, we compared functional recovery after unilateral versus bilateral cerebral cortical infarcts induced by distal middle cerebral artery occlusion (dMCAO) in rats. Although infarct volume did not differ between the two groups, neurobehavioral deficits in the limbs contralateral to dMCAO were improved at 1 day by an additional, contralateral dMCAO. This suggests that activity in the contralateral cortical hemisphere tends to limit acute recovery from cortical stroke in rats.

2. Materials and methods

2.1. Stroke model

All animal procedures were conducted in accordance with National Institutes of Health guidelines and with the approval of the Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats (250–300 g) were anesthetized with 3% isoflurane in 70% N₂O/30% O₂ using a mask. Permanent distal middle cerebral artery occlusion (dMCAO) was performed as described previously (Nawashiro et al., 1997; Won et al., 2006). This model was chosen to produce a pure cortical lesion, because we were interested in assessing cortico-cortical interactions, and because of concern that a more extensive stroke, produced bilaterally, might be difficult for the animals to survive. A 2-cm incision was made under the surgical microscope between the left orbit and tragus, the temporal muscle was retracted laterally, and a 3-mm diameter craniotomy was made just rostral to the foramen ovale. The dura was incised with a 26-gauge needle and the left MCA was exposed and occluded by electrocoagulation without damaging the brain surface. For bilateral dMCAO, both left and right MCA were occluded in the same operative session. The temporal muscle was repositioned and the skin was closed. Rectal temperature was maintained at $37 \pm 0.2^{\circ}\text{C}$ throughout with a heating blanket. In sham-operated controls the MCA was visualized but not occluded.

2.2. Infarct volume

Some rats were deeply anesthetized with isoflurane 1 day after MCAO. Brains were then removed and sliced into 2-mm sections with the aid of brain matrices. Sections were placed into 2% TTC (2,3,5-triphenyltetrazolium chloride) in PBS and incubated at 37°C for 30 min. TTC solution was replaced with 4% paraformaldehyde for overnight fixation at 4°C . Stained sections (6 per rat, 5 rats per condition) were scanned on a desktop scanner and infarct area was measured using NIH Image software. Infarct volume was expressed as a percentage of normal hemisphere volume.

2.3. Neurobehavioral testing

Unilateral MCAO ($N=10$), bilateral MCAO ($N=13$), and sham-operated ($N=6$) rats underwent neurobehavioral tests to assess functional outcome. Animals were trained prior to MCAO and deficits were assessed 1, 3, 7, 14, and 28 days thereafter. The investigator performing the tests was blinded to the experimental condition.

The ladder rung walking test is sensitive for quantifying skilled locomotion. The degree of motor dysfunction after MCAO was measured by counting the number of foot-faults of the impaired limbs per round. The apparatus was modified to produce a 122-cm-long horizontal plastic-rung runway with 3-mm diameter rungs and 7–14 mm between rungs (Metz and Whishaw, 2002). Baseline and post-operative testing sessions consisted of three traverses across the ladder. An error was scored for any foot slip or misstep (i.e., missing or slipping from a rung). The number of errors and steps of the affected forelimb and hindlimb in each trial was counted. The mean number of errors in three traverses was calculated.

The limb placing test was used to measure lateralized postischemic sensorimotor dysfunction (De Ryck et al., 1989). Rats were evaluated for the ability to place the limbs on a tabletop surface in response to visual, tactile, or proprioceptive input. Each of seven tasks was scored by a blinded observer as: 0, no placing; 1, incomplete or delayed placing; or 2, complete, immediate placing. Forelimb and hindlimb scores were averaged for each animal (normal score, 14).

2.4. Statistical analysis

Results were expressed as mean values $\pm$ SEM. Infarct volume was analyzed by Student's *t*-test. Non-parametric (neurobehavioral) data were analyzed using the Mann-Whitney U test. *P* values <0.05 were considered significant.

3. Results

First we asked if there was a difference in infarct volume between rats who received unilateral and bilateral dMCAO. As shown in Fig. 1, infarct volume in rats with bilateral dMCAO was unchanged compared to that in rats with unilateral MCAO.

Next we asked if bilateral MCAO affected the severity of neurological deficits. Rats with bilateral dMCAO had a significant reduction in the number of missteps by the right (affected) forelimb (Fig. 2A), but not hindlimb (Fig. 2B), and a less severe deficit in the limb-placing test (Fig. 2C) when compared to the unilateral dMCAO group at 1 day, but the severity of deficits converged thereafter.

4. Discussion

The main finding of this study is that infarct volume was unchanged, but motor function was improved transiently, in rats with bilateral compared to those with unilateral dMCAO. These findings are consistent with other evidence that the contralesional hemisphere influences functional motor recovery after stroke. For example, functional magnetic resonance imaging showed that stimulation of the affected limbs 3 days after MCAO activated cortical regions of the contralesional hemisphere (Dijkhuizen et al., 2001).

The ladder rung walking and limb placing tests were used to evaluate sensorimotor integration elicited by tactile and proprioceptive stimulation of the forelimb and hindlimb (De Ryck et al., 1989). Because these tests assess unilateral performance rather than performance asymmetry, we believe that the improvement observed after bilateral dMCAO is genuine, and not the artifactual result of comparison with an impaired, rather than an unimpaired, "control" side. However, the observed improvement was transient, being evident 1 day after dMCAO but not beyond. Thus, bilateral dMCAO hastened behavioral recovery rather than enhancing the maximal extent of recovery.

Our results are consistent with several previous experimental (Allred et al., 2010; Rha et al., 2011; Zhao et al., 2009) and clinical (Boggio et al., 2007; Bradnam et al., 2011; Kang et al., 2011; Nair et al., 2011) studies, in which attenuating the effect of the contralesional hemisphere improved functional motor recovery after brain infarction. However, other experimental (Jin et al., 2011; Napieralski et al., 1998; Shanina et al., 2006) and clinical (Takeuchi et al., 2012) stroke studies have yielded opposite conclusions. Methodological differences such as lesion type, size, severity, and location, and the behavioral tests used, might account for these differences. We noted previously that corpus callosotomy, which like contralesional dMCAO interrupts interhemispheric connections, exacerbated some of the same postischemic neurobehavioral deficits that were improved in the present study (Jin et al., 2011). This discrepancy may relate to the longer interval between disconnection

surgery and MCAO, larger infarct size (proximal rather than distal MCAO), or inclusion of a reperfusion phase (temporary rather than permanent MCAO) in the earlier study. In addition, the deleterious effects of callosotomy depended on the extent of callosal lesions (Jin et al., 2011), and the extent and anatomical basis of interhemispheric disconnection differs between callosotomy and contralesional dMCAO. After MCAO, for example, interhemispheric communication through noncallosal pathways—which in the rat include the anterior, posterior, tectal, and supraoptic commissures, and the massa intermedia (Mohn and Russell, 1981)—could also be affected.

The basis for improved function in the bilateral compared to the unilateral dMCAO group in our study is uncertain. One possible mechanism is loss of interhemispheric inhibition (Ferbert et al., 1992), which has been implicated in effects of the contralesional hemisphere on ischemic deficits (Madhavan et al., 2010). Interhemispheric inhibition is exerted largely through the corpus callosum (Meyer et al., 1998), and appears to involve GABAergic transmission mediated through GABA_B receptors (Palmer et al., 2012). Better understanding of interhemispheric interactions following stroke may suggest new therapeutic targets. To this end, future studies might focus on pharmacologic strategies that can mimic the effect of surgical disconnection approaches in hastening or otherwise improving recovery from stroke.

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Fig. 1. Infarct volume after unilateral left or bilateral dMCAO. (A) Rats were euthanized 24 hr after unilateral (left) or bilateral (right) dMCAO and brain slices were stained with TTC to delineate infarcts (white). (B) Left hemisphere infarct volume was expressed as a percentage of normal hemisphere volume; there was no significant difference between the unilateral and bilateral dMCAO groups ($N=5$ per group, Student's t -test).

Fig. 2.

Neurobehavioral function measured by the ladder rung walking test (A, B) and limb placing test (C) after unilateral or bilateral dMCAO. Tests were performed 1, 3, 7, 14, and 28 days after unilateral dMCAO (black, $N=10$), bilateral dMCAO (red, $N=13$) or sham surgery (green, $N=6$). *Means = 6.5 and 3.0, $U=142.5$, $P<0.05$ compared to unilateral dMCAO; **means = 7.0 and 10.5, $U=158$, $P<0.03$ compared to unilateral dMCAO (Mann-Whitney U test).