Corpus Callosum and Experimental Stroke

Studies in Callosotomized Rats and Acallosal Mice

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Background and Purpose—Interhemispheric inhibition via the corpus callosum has been proposed as an exacerbating factor in outcome from stroke.

Methods—We measured infarct volume and behavioral outcome after middle cerebral artery occlusion in callosotomized rats and acallosal mice.

Results—Neither callosotomy in rats nor callosal agenesis in mice improved infarct volume or behavioral outcome after middle cerebral artery occlusion.

Conclusions—These findings argue against a role for transcallosal projections in exacerbating focal cerebral ischemia. (Stroke. 2011;42:2584-2588.)

Key Words: callosal agenesis ■ callosotomy ■ corpus callosum ■ ischemia ■ stroke

Focal cerebral ischemia produces changes in electric activity, blood flow, and metabolism at distant brain sites, including the contralesional hemisphere;1 however, how these changes affect outcome from stroke is uncertain. In fact, clinical studies have provided evidence for both adaptive2–4 and maladaptive5–7 effects of contralesional inputs. Interhemispheric influences in stroke are likely to be mediated through the corpus callosum, which is the principal conduit between the hemispheres in all placental mammals, including rodents8 and humans.9 However, the net (excitatory or inhibitory) effect of transcallosal projections is uncertain and variable.10 For example, both interhemispheric facilitation11–13 and inhibition12,14 of human motor cortex have been reported.

The small number of studies that have used callosotomy to evaluate the effect of interhemispheric signaling on various unilateral cerebral lesions also have yielded conflicting results. Callosotomy had no effect on recovery from aspiration of rat motor cortex in a study,15 but resurrected deficits after frontal or parietal cortex aspiration in another study.16 In contrast, callosotomy reversed the detrimental effect of training the unaffected limb on recovery from endothelin-1–induced lesions of sensorimotor cortex.17 Accordingly, disagreement persists regarding whether input from the contralesional hemisphere after stroke is adaptive, maladaptive, or inconsequential for recovery.

We sought to test the hypothesis that interhemispheric inhibition via the corpus callosum14 adversely affects outcome from stroke by studying the effects of callosotomy in rats and callosal agenesis in mice on infarct size and behavioral outcome after middle cerebral artery occlusion (MCAO). The rationale for this approach was that if transcallosal signaling exacerbates ischemic brain injury, then interventions that interfere with this pathway might have therapeutic benefit, as observed for callosotomy in epilepsy18 and as suggested by studies on repetitive transcranial magnetic stimulation of the contralesional hemisphere in chronic stroke.19,20

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 280 to 310 grams were obtained from Charles River Laboratories (Hollister, CA). BTBR T1/J (BTBR;21), LP/J,22,23 and C57BL/6J mice weighing 20 to 27 grams were purchased from the Jackson Laboratory (Sacramento, CA). Animals were kept under controlled temperature and humidity conditions with standardized light and dark cycles and free access to food pellets and tap water. Experiments were approved by the Buck Institute’s Animal Care and Use Committee and were conducted according to National Institutes of Health guidelines.

Callosotomy

Rats were anesthetized with 4% isoflurane in 70% N2O and 30% O2 and placed in a stereotaxic apparatus (David Kopf Instruments). The skull was opened with a drill and an adjustable wire knife24 was positioned with a guide in the midline, 5.9 mm deep to bregma, and lifted to cut the corpus callosum from +2.0 to −2.0 mm (rostrocaudal callosotomy), +2.0 to 0.0 mm (rostral callosotomy), or 0.0 to −2.0 mm (caudal callosotomy) relative to bregma. The knife was then withdrawn, the guide was removed from the skull, and the skin was sutured. Sham callosotomy consisted of introducing the guide without the wire knife.

Focal Cerebral Ischemia

Transient focal cerebral ischemia was induced using the suture occlusion technique as described previously.25 Rats were anesthe-
tized with 4% and mice were anesthetized with 2% isoflurane in 30% O₂ and 70% N₂O using a vaporizer. A midline incision was made in the neck and the right external carotid artery was carefully exposed and dissected. A silicon-coated monofilament nylon suture (4-0) was inserted from the external into the right internal carotid artery to occlude the origin of right middle cerebral artery (MCA). After occlusion for 90 minutes (rats) or 60 minutes (mice), the suture was removed to allow reperfusion. The external carotid artery was ligated and the wound was closed. Sham-operated rats underwent identical surgery, except that the suture was not inserted. Regional cerebral blood flow was measured using a Moor VMS-LDF laser Doppler monitor (Moor Instruments). Arterial blood gases and glucose were measured with an I-STAT portable clinical analyzer (Heska). Rectal temperature was maintained at 37±0.5°C using a heating pad and a heating lamp. At various times after reperfusion, animals were anesthetized and perfused through the heart with 4% paraformaldehyde in phosphate-buffered saline (pH 7.4).

**Elevated Body Swing Test**

The elevated body swing test²⁵ was used to test asymmetrical motor behavior. Rats held by the base of the tail were raised ~10 cm above the testing surface. The initial direction of swing, defined as turning the upper body by >10 degrees to either side, was recorded in 3 sets of 10 trials, performed over 5 minutes. The number of turns in each (left or right) direction was recorded, and the percentage of turns made to the side contralateral to the ischemic hemisphere (percent left-biased swing) was calculated. Average scores were determined for each rat.

**Cylinder Test**

Forelimb use bias was analyzed by observing the rat’s movements over 3-minute intervals in a transparent, 18-cm-wide, 30-cm-high poly(methyl methacrylate) cylinder,²⁷ which was sufficiently large to permit movement but small enough to promote rearing and wall exploration. A mirror behind the cylinder made it possible to observe and record forelimb movements when the rat was facing away from the examiner. After an episode of rearing and wall exploration, a landing was scored for the first limb to contact the ground or for both limbs if they made simultaneous contact. Percent use scores were calculated for both the unimpaired and impaired limb, relative to the total number of movements. Percentage use of the impaired limb was subtracted from percentage use of the unimpaired limb to yield an overall limb bias score. Wall exploration and landing movements were analyzed separately. Average cylinder test scores were calculated for each animal and for each week of testing, which consisted of 2 sessions per week.

**Beam Walking Test**

A 1-m-long, 2.5-cm-wide wooden beam was suspended 23 cm above a bench top, which was covered with soft pads to protect the rat in case of a fall. Rats were pretrained for 2 consecutive days (5 trials per day) on the beam. Each rat was given 5 trials, which were videotaped, and the average number of slips per trial was used for statistical analysis. Slips were counted only while the rat was in forward motion. A fault was defined as any foot slip off the top surface of the beam or any limb use on the side of the beam.²⁸

**Infarct Volume**

Rats or mice were anesthetized and decapitated 24 hours after the onset of ischemia. Brains were removed and 1-mm coronal sections were immersed in 2% 2,3,5-triphenyltetrazolium chloride in saline for 20 minutes at 37°C and then fixed for 2 hours in 4% paraformaldehyde. Infarct areas were measured by a blinded observer, calculated as a percentage of the area of the contralateral hemisphere, and multiplied by the distance between sections to obtain the respective volumes.²⁹

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**Results**

**Callosotomy in Rats**

The temporal relation of callosotomy to MCAO and outcome measurement is shown in Figure 1A. Callosotomy was performed before MCAO to allow early and late postischemic effects to be detected, and MCAO was delayed for 1 day after callosotomy to give animals time to recover between surgeries. The 2,3,5-triphenyltetrazolium chloride staining 1 day after MCAO was used as an early measure of the effect of callosotomy on pathoanatomic outcome, whereas longer-term consequences of callosotomy were assessed by behavioral testing. The extent of callosotomy was varied and confirmed by postmortem sectioning (Figure 1B).

**Callosotomy and Infarct Volume**

MCAO with and without previous callostomy produced similar changes in cerebral blood flow (Figure 2) and was associated with similar arterial blood pH, \(P_{O_2}\), \(P_{CO_2}\), HCO³⁻, and glucose (\(P>0.05\) by \(t\) test). In rats not undergoing callosotomy, infarct volume measured by 2,3,5-triphenyltetrazolium chloride staining 1 day after MCAO was ~40% of hemispheric volume. Neither rostral nor caudal callosotomy affected this significantly, but a more extensive rostrocaudal lesion increased infarct volume by ~50%. Rostrocaudal callosotomy without MCAO caused no defect in 2,3,5-triphenyltetrazolium chloride staining (not shown).

**Callosotomy and Behavior**

We used a battery of sensorimotor behavioral tests to assess functional outcome from MCAO in control and callosoto-
mized rats consisting of the elevated body swing test,26 cylinder test,27 and beam walking test.28 Rats with callosotomy but no MCAO performed normally on these tests, showing no laterality on the elevated body swing test or cylinder test and few slip steps on beam walking (Figure 3). In contrast, MCAO without callosotomy produced defective performance on all tests, which tended toward normalization over the course of 2 weeks. Rats that underwent rostrocaudal callosotomy before MCAO performed somewhat worse, whereas rostral or caudal callosotomy was associated with improvement in the elevated body swing test at early (24–72 hours, but not 1 to 2 weeks) intervals after MCAO. Thus, callosotomy caused worsening or, at best, transient improvement in behavioral outcome measures.

**Figure 2.** Effect of callosotomy before middle cerebral artery occlusion (MCAO) on infarct volume in rats. A, Regional cerebral blood flow (CBF) in the distal middle cerebral artery (MCA) territory ipsilateral to MCAO without (□) and with (■) prior callosotomy. B, Infarct volume was measured by 2,3,5-triphenyltetrazolium chloride (TTC) staining (red) 1 day after MCAO. C, Infarct volume was increased in rats undergoing rostrocaudal callosotomy 1 day before MCAO compared to rats subjected to MCAO alone. *P<0.05 compared to no callosotomy (none).

**Callosal Agenesis in Mice**

To evaluate the effect of callosal agenesis on MCAO, we compared BTBR mice, which lack a corpus callosum, to two other mouse strains: LP/J, which is the strain most closely related to BTBR based on simple sequence length22 and single nucleotide polymorphism analysis and has been used...
but infarct volume was
somewhat more distantly related.22,23 Neither LP/J30
which is the principal conduit for
interhemispheric inhibition, would improve outcome after
MCAO in rodents. We found, however, that neither calloso-
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tomy in rats nor callosal agenesis improved infarct size or
behavioral outcome after MCAO, but behavioral effects of callosotomy have been
documented in other spheres. In studies of the visual system,
for example, posterior (but not anterior) callosotomy disrupts
interocular transfer of visual information36 and complete
callosotomy impairs visuomotor coordination37 but improves
left–right discrimination in a water maze task.38 Accordingly,
surgery alone or callosotomy in particular could have a net
deleterious effect on recovery from MCAO despite relieving
whatever impediment to recovery interhemispheric inhibition
presents. Additional factors that may contribute to the overall
impact of callosotomy include stimulation (or suppression) of
trophic factor expression,39 induction of Wallerian degener-
ation leading to death of transcortical neurons40 (although this
was not observed in retrograde tracer studies41), impaired
postischemic cell migration,42 and altered cerebral blood
flow.43

The effect of callosotomy on deficits associated with
cerebral cortical lesions in rats has been assessed in a limited
number of previous studies. In one case, a fairly extensive
callosotomy (1.2 to −3.6 mm relative to bregma) was
performed 20 days after aspiration of parasagittal cortex over
a similar rostrocaudal distance, and motor performance in an
elevated beam test was evaluated up to 15 days later.15
Callosotomy had no effect on performance in either control or
lesioned rats. In another study, a less extensive callosotomy
(0.5 to −1.0 mm relative to bregma) was performed 4 weeks
after aspiration of parietal, medial frontal, or motor cortex,
each of which produced a contralateral neglect syndrome that
subsequently resolved.16 Callosotomy transiently reestab-
lished neglect, although the specificity of this effect is
uncertain because a variety of superimposed insults are
known to be capable of reviving neurological deficits that are
no longer clinically apparent.44,45 In a third study, moderately
extensive callosotomy (1.0 to −1.5 mm relative to bregma)
performed at the time of endothelin-induced lesions of
sensorimotor cortex abolished the adverse effect of contralat-
eral limb training on pellet recovery by the affected limb.17
Therefore, callosal fibers appeared to mediate an inhibitory
effect of the contralateral hemisphere on outcome.

Discussion
The possibility that interhemispheric inhibition of injured by
contralateral cerebral cortex may adversely affect recovery from stroke5–7,32 led us to investigate whether interruption of the
corpus callosum, which is the principal conduit for
interhemispheric inhibition, would improve outcome after
MCAO in rodents. We found, however, that neither callosotomy
in rats nor congenital absence of the corpus callosum in mice was beneficial in this regard. Moreover, both extensive
(rostrocaudal) callosotomy and callosal agenesis were asso-
ciated with increased infarct size, and rostrocaudal callosotomy
also impaired behavioral outcome.

We chose coordinates for callosotomy based on the known
organization of rat motor and sensory cortex33 and the fact
that transcallosal projections in the rat are exclusively homo-
typical.5,34 In this way, we expected to be able to detect most
interhemispheric effects on motor and sensory functions assessed by our behavioral testing. We performed calloso-
tomy before MCAO in an effort to detect effects occurring
anytime during the first 2 weeks after ischemia, but different
timing of callosotomy in relation to MCAO might produce
different results. GABAergic inhibition is increased in peri-
infarct cerebral cortex for at least 2 weeks after photothrom-
botic stroke in mice, and infarct size is decreased when
GABA-mediated transmission is reduced pharmacologically
starting 3 days after stroke but increased when treatment is
begun immediately.35 Thus, increased cortical inhibition in
the immediate aftermath of stroke may be advantageous for
recovery, and delaying callosotomy until after this period
might be more likely to provide benefit.

Even if transcallosal interhemispheric inhibition limits
recovery from stroke, callosotomy may have effects besides
disconnecting interhemispheric circuitry, which could affect
recovery independently. We did not observe defects in motor
or sensory function in rats subjected to callosotomy without
MCAO, but behavioral effects of callosotomy have been
documented in other spheres. In studies of the visual system,
for example, posterior (but not anterior) callosotomy disrupts
interocular transfer of visual information36 and complete
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Therefore, callosal fibers appeared to mediate an inhibitory
effect of the contralateral hemisphere on outcome.

Conclusion
The finding that neither callosotomy in rats nor callosal
agenesis in mice improved infarct volume or behavioral
outcome after MCAO argues against a role for transcallosal
projections in exacerbating focal cerebral ischemia.
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None.

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