

## STROKE RECOVERY MODEL

<b>MODEL:</b>	<b>PERMANENT ELECTROCOAGULATION OF THE PROXIMAL MIDDLE CEREBRAL ARTERY (MCA)—MODIFIED TAMURA MODEL</b>
<b>TEST NUMBER:</b>	BTFX-R01
<b>CATEGORY:</b>	Permanent focal ischemia model
<b>SPECIES:</b>	Mature Sprague-Dawley rats
<b>APPLICATION:</b>	Testing recovery-promoting agents in a model of focal stroke (permanent occlusion)
<b>METHOD:</b>	<p>Focal cerebral infarcts are made by permanent occlusion of the proximal right middle cerebral artery using a modification of the method of Tamura <i>et al.</i> [1-4]: Male Sprague-Dawley rats (300-350 g) are anesthetized with 2-3% halothane or isoflourane in N<sub>2</sub>O/O<sub>2</sub> (2:1) and anesthesia is maintained with 1-1.5 % halothane or isoflourane. The proximal MCA is exposed through a subtemporal craniectomy without removing the zygomatic arch and orbital contents and without transecting the facial nerve (Figs. 1, 2). The artery is then occluded by microbipolar coagulation from just proximal to the olfactory tract to the inferior cerebral vein, and is then transected. Body temperature is maintained at 37.5 ± 0.5° C during the anesthesia using a heating blanket connected to a temperature controller.</p> <p>Following stroke surgery, putative recovery-promoting drugs are given systemically (intraperitoneally, intravenously) or intracerebrally (intraventricularly or intracisternally, see Fig. 3). These drugs are typically given by single or multiple injection starting 1-3 days after stroke.</p> <p>Behavioral assays of sensorimotor recovery are done at regular intervals during the first month after stroke:</p> <ul style="list-style-type: none"><li>Limb placing tests:<ul style="list-style-type: none"><li>Forelimb placing tests</li><li>Hindlimb placing tests</li></ul></li><li>Body swing test</li></ul> <p>The tests are done before MCAO, 1 day after MCAO (or before the administration of drug treatment), 3 and 7 days after MCAO, then once per week (e.g. 14, 21 and 28 days after MCAO). For a full description of these tests, see below.)</p>

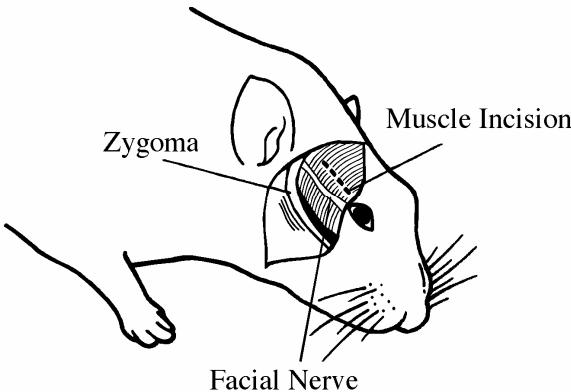
One month following stroke, animals are anesthetized deeply with chloral hydrate and perfused transcardially with heparinized saline followed by 10% formalin. Brains are removed and processed for paraffin embedding, sectioned (5  $\mu$ m), and stained with hematoxylin and eosin (H&E). The area of cerebral infarcts is determined on seven slices (+4.7, +2.7, +0.7, -1.3, -3.3, -5.3, and -7.3 compared to bregma respectively), using a computer-interfaced imaging system. Infarct area on each slice is calculated using the "indirect method" as (the area of the intact contralateral hemisphere - the remaining uninfarcted area of the ipsilateral hemisphere) to correct for brain shrinkage during processing. Infarct areas are then summed among slices and multiplied by slice thickness to give the total infarct volume, which is expressed as a percentage of the intact contralateral hemispheric volume. Infarct volumes are also determined separately for the cerebral cortex and striatum using similar methods. H&E-stained brain sections are also examined for gross histological abnormalities, including tumor formation, hydrocephalus, etc.

Animals are weighed on the days of behavioral assessment. Behavioral scores and body weight are analyzed by two-way repeated measures analysis of variance (ANOVA; treatment X time). Infarct volume is analyzed by one-way ANOVA.

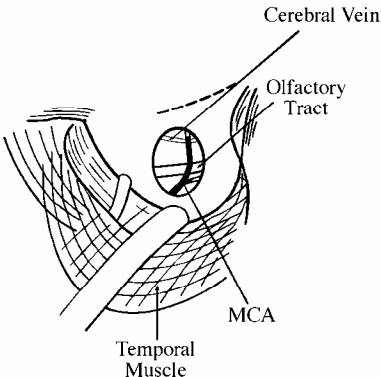
**ENDPOINTS:** Long-term sensorimotor behavioral recovery; infarct volume

**DESCRIPTION OF INFARCTION:**

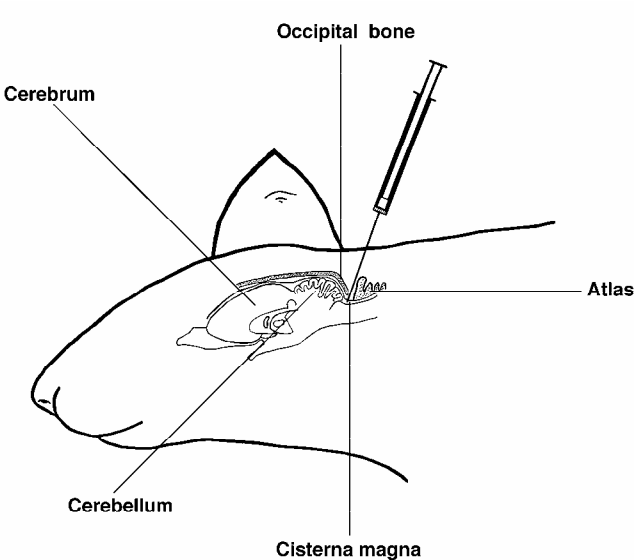
This method produces an infarct in the dorsolateral cerebral cortex and underlying striatum (Figs. 4, 5). It involves cortical regions important in sensorimotor function of the contralateral forelimb and hindlimb (including FL and HL regions, respectively). The infarct is smaller than that produced by intra-arterial suture occlusion and is thus compatible with long-term animal survival. Biotrofix investigators have used this model to test stroke recovery promoting drugs, i.e., drugs that *do not* necessarily reduce infarct volume but *do* enhance functional recovery.



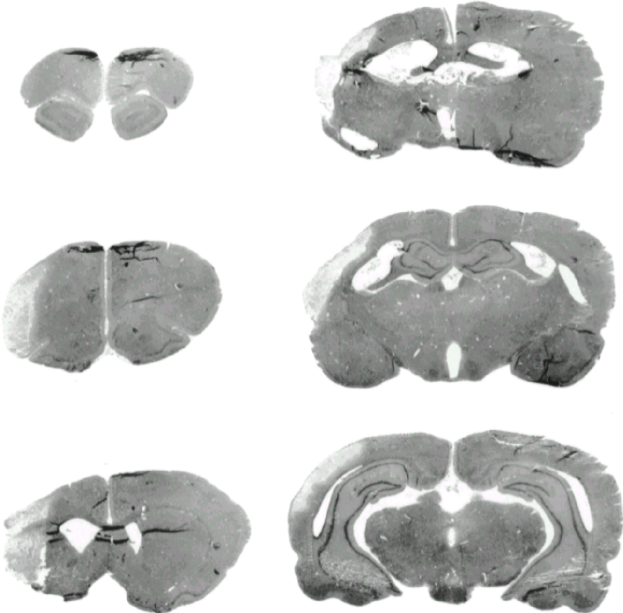
**Figure 1.** Modified Tamura Model. Exposure of craniectomy site.



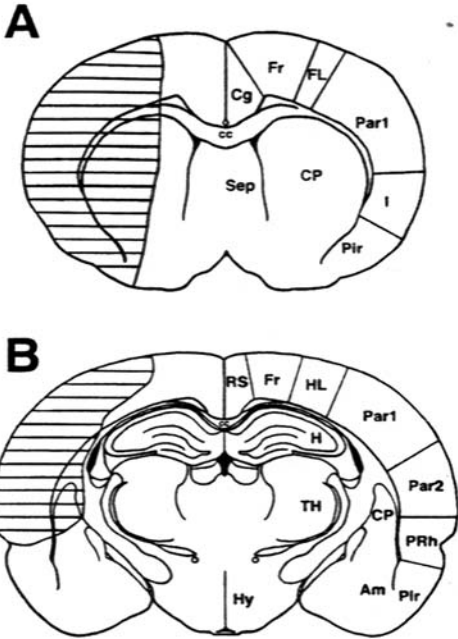
**Figure 2.** Modified Tamura Model. Exposure of the proximal middle cerebral artery (MCA).



**Figure 3.** Technique of Percutaneous Intracisternal Injection.



**Figure 4.** Modified Tamura Model. Location of cerebral infarcts in six coronal brain slices (+4.7, +2.7, +0.7, -1.3, -3.3, and -5.3 mm compared to bregma; top left to bottom right, respectively.)

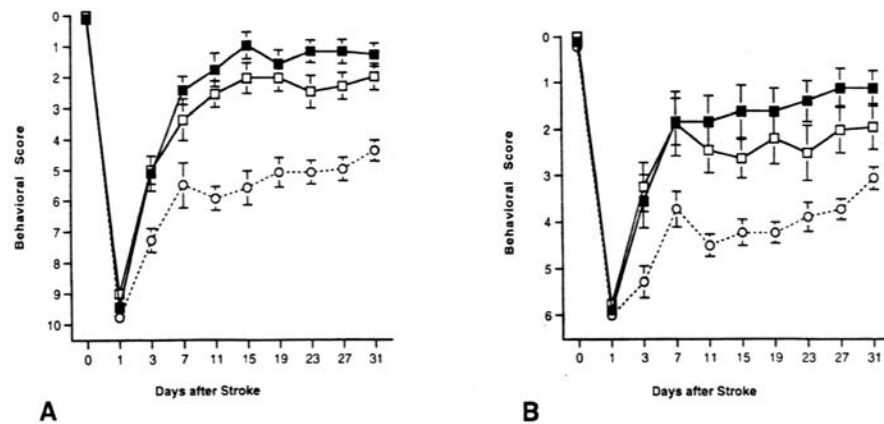


**Figure 5.** Modified Tamura Model. Location of cerebral infarcts (shaded regions) on slices (A) +0.7 mm, and (B) -3.3 mm compared to bregma. Infarcts involve the dorsolateral cerebral cortex and striatum (caudoputamen, CP), including cortex subserving sensorimotor function of the contralateral forelimb (FL) and hindlimb (HL).

**BEHAVIORAL RECOVERY TESTS**

**LIMB PLACING TESTS:**

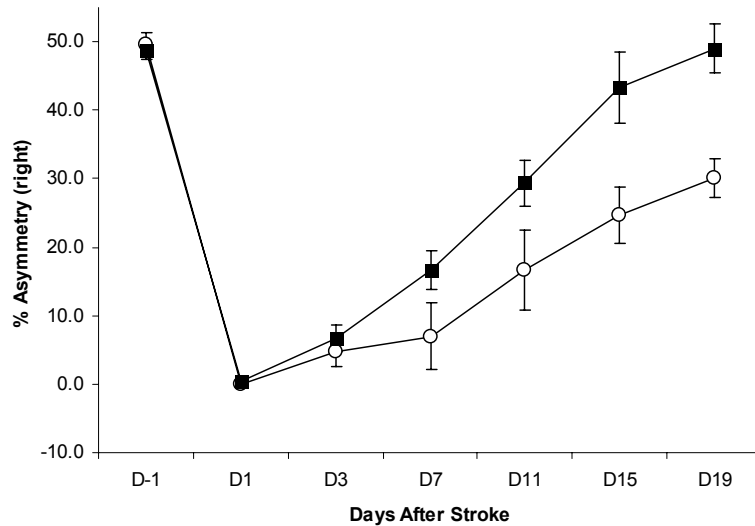
These tests have been used extensively by many investigators to examine sensorimotor recovery of the contralateral (impaired) limbs following focal stroke [2, 5, 6]. Limb placing tests have been used extensively by ViaCell Neuroscience investigators to examine the recovery-promoting effects of polypeptide growth factors after stroke [1-4]. For these tests, rats are handled for 10 min. each day for seven days before stroke surgery. For the *forelimb placing test*, the examiner holds the rat close to a table top and scores the rat's ability to place the forelimb on the table top in response to whisker, visual, tactile, or proprioceptive stimulation. Similarly, for the *hindlimb placing test*, the examiner assesses the rat's ability to place the hindlimb on the table top in response to tactile and proprioceptive stimulation. Separate subscores are obtained for each mode of sensory input and added to give total scores (for the forelimb placing test: 0 = normal, 12 = maximally impaired; for the hindlimb placing test: 0 = normal; 6 = maximally impaired). Animals are tested just before stroke surgery, on the first day following surgery, the third and seventh day following surgery, and then once a week thereafter for 21 - 28 days after stroke. Typically, there is a slow and steady recovery of limb placing behavior during the first month after stroke. Growth factors and other treatments enhance the rate and degree of recovery.



**Figure 6.** Enhancement of (A) forelimb placing and (B) hindlimb placing following intracisternal injection of osteogenic protein-1 (OP-1, BMP-7) administered at 1 and 4 days after stroke. Open circles = vehicle; open squares = 1 µg OP-1; closed squares = 10 µg OP-1. (see [3])

**BODY SWING TEST:**

This test, developed by Sanberg and colleagues, has been used to examine side preferences after stroke [7, 8]. The animal is held approximately 1 inch from the base of its tail. It is then elevated to an inch above a surface of a table. The animal is held in the vertical axis, defined as no more than 10° to either the left or the right side. A swing is recorded whenever the animal moves its head out of the vertical axis to either side. Before attempting another swing, the animal must return to the vertical position for the next swing to be counted. Thirty total swings are counted. A normal animal typically has an equal number of swings to either side. Following focal stroke, the animal tends to swing to the contralateral side. There is a slow spontaneous recovery of body swing during the first month after stroke. Recovery on this test can be enhanced by several agents.



**Figure 7.** Body Swing Test. The Y axis represents the body swing asymmetry score. The X axis is days after stroke. A sharp decline in asymmetry score followed by a gradual recovery is seen during the first month after stroke. In this experiment, enhancement of recovery was seen with test drug (solid squares) compared to vehicle (open circles).

## REFERENCES:

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