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The Neuroprotective Effect of Xenon Administration during Transient Middle Cerebral Artery Occlusion in Mice

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Background: **Xenon has been shown to be neuroprotective in several models of *in vitro* and *in vivo* neuronal injury. However, its putative neuroprotective properties have not been evaluated in focal cerebral ischemia. The purpose of this study was to determine if xenon offers neuroprotection in a mouse model of middle cerebral artery occlusion.**

Methods: **C57BL/6 mice underwent 60 min of middle cerebral artery occlusion. The animals (n = 21 per group) were randomized to receive either 70% xenon + 30% O_2, 70% N_2O + 30% O_2, or 35% xenon + 35% N_2O + 30% O_2. After 24 h, functional neurologic outcome (on three independent scales: four-point, general, and focal deficit scales) and cerebral infarct size were evaluated.**

Results: **The 70% xenon + 30% O_2 group showed improved functional outcome (median [interquartile range], four-point scale: 2 [2], 70% xenon + 30% O_2 *versus* 3 [2], 70% N_2O + 30% O_2, $P = 0.0061$; general deficit scale: 9 [6], 70% xenon + 30% O_2 *versus* 10 [4], 70% N_2O + 30% O_2, $P = 0.0346$). Total cerebral infarct volumes were reduced in the 70% xenon + 30% O_2 group compared with the 70% N_2O + 30% O_2 group (45 ± 17 mm^3 *versus* 59 ± 11 mm^3, respectively; $P = 0.0009$).**

Conclusions: **In this model of transient focal cerebral ischemia, xenon administration improved both functional and histologic outcome.**

THE inert gas xenon has several properties that make it an ideal anesthetic gas, including a relatively safe cardiovascular profile (similar to or better than conventional anesthetics) and advantageous pharmacokinetic and pharmacodynamic properties compared with most other gaseous agents. A growing body of clinical and laboratory data characterize xenon as a gaseous anesthetic drug with analgesic effects, possessing a fast induction and recovery of anesthesia coupled with uncompromised hemodynamic properties.[1–3] In addition, its environmentally friendly characteristics make it an attractive option for inhalational anesthesia.[4] It does, however, have some disadvantages, particularly its higher cost, which has limited its development for clinical use.

Although the precise molecular mechanisms by which inhalational anesthetics produce general anesthesia are not known, it has been shown that hypnotic drugs work by activating inhibitory and/or blocking excitatory pathways.[5] Xenon has been shown to act *via* inhibiting the glutamatergic *N*-methyl-D-aspartate (NMDA) receptor.[6–8] It is also thought that through the inhibition of this excitatory pathway, xenon exerts neuroprotective effects. Previous *in vitro* and *in vivo* models have shown some putative neuroprotective effects,[9] but the effect of xenon on both functional and histologic outcome after reversible focal cerebral ischemia is unknown. We hypothesized that xenon would attenuate histologic injury and improve functional neurologic outcome after transient middle cerebral artery occlusion in the mouse.

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

Materials and Methods

The Duke University Animal Care and Use Committee approved the study, and all procedures met the National Institutes of Health's guidelines for animal care outlined in the *Guide for the Care and Use of Laboratory Animals* (Health and Human Services, NIH Publication No. 86-23, revised 1996).

Xenon-delivery System

To conserve xenon, we used a closed-loop ventilation system custom-designed for this protocol. This system consisted of a 5-l gas reservoir bag into which oxygen, xenon, nitrogen, and N_2O could be mixed. In addition to having in-line gas analyzers, the reservoir emptied into a pump that supplied the ventilator (Harvard® rodent ventilator model 683; Harvard Apparatus, Holliston, MA) with fresh gas flow. The exhaled gas was then recirculated through a carbon dioxide absorber (Sodasorb; W.R. Grace Co., Atlanta, GA). An additional parallel open circuit permitted a rapid change to allow for the use of conventional isoflurane anesthesia during the surgical preparation.

Surgical Procedures

Male C57BL/6 mice (8 weeks of age; body weight, 20–25 g; Jackson Laboratories, Bar Harbor, ME) were housed in a temperature-controlled environment with artificial light/dark cycle (12 h) and fasted overnight but allowed free access to water before experiments. The

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animals were anesthetized in a chamber with 3% isoflurane in 50% O_2 (balance nitrogen). The trachea was intubated (20G catheter) and the lungs were mechanically ventilated (tidal volume, 7 ml/kg; respiratory rate, 80 breaths/min). A needle thermistor was inserted below the left temporal muscle adjacent to the skull and temperature was servo-controlled to 37.0 ± 0.1°C using an infrared lamp and heating pad. *Via* a cervical neck incision, the left external jugular vein was cannulated allowing for a continuous infusion of fentanyl and intermittent infusion of vecuronium. Similarly, the right femoral artery was cannulated for continuous mean arterial pressure (MAP) monitoring.

The right carotid artery was surgically prepared for middle cerebral artery occlusion. Specifically, it was dissected and its branches (common carotid artery, internal and external carotid branches) were identified and isolated with suture. The external carotid artery was occluded and ligated remote from its origin and the proximal end was temporarily occluded with a microsurgical aneurysm temporary clip to allow subsequent intraluminal filament insertion. Isoflurane was then discontinued and a continuous fentanyl infusion was begun (bolus injection of 50 μg/kg and followed by 50 $\mu g \cdot kg^{-1} \cdot h^{-1}$). After establishing in unparalyzed pilot experiments that the animals did not exhibit escape behavior in response to noxious stimuli while using a similar anesthetic regimen, vecuronium was administered (0.1 mg/kg intravenous, repeated after 30 min) and the closed-loop gas delivery system was then initiated.

The animals were then randomized into three different anesthetic groups: 70% xenon + 30% O_2, 70% N_2O + 30% O_2, and 35% xenon + 35% N_2O + 30% O_2. After 15 min of equilibration, middle cerebral artery occlusion was initiated by insertion of a 6-0 nylon monofilament, blunted at the tip in a flame and then lightly coated with silicone, through the external carotid artery stump into the circle of Willis thereby occluding the origin of the middle cerebral artery.[10] An arterial blood sample was collected 30 min into the ischemic period for blood gas analysis. Because of the known confounding effect on cerebral blood flow of P_{CO_2} in this model, any animal that had an arterial blood gas that was out of range for P_{CO_2} (38–42 mmHg) was excluded *a priori* from any further analysis. After 60 min of middle cerebral artery occlusion, the filament was withdrawn and reperfusion began. Following topical administration of 1% lidocaine (0.1 ml), the catheters were removed, the vessels were ligated, and incisions were sutured closed. Anesthesia was then discontinued. On recovery of spontaneous ventilation, the tracheas were extubated and animals were recovered in individual cages enriched with 50% oxygen and a controlled environmental temperature (28.5–29.0°C).

Neurologic Evaluation

After 24 h, an observer blinded to group assignment evaluated the animals using three different neurologic assessment scales. The animal was observed for spontaneous activity and quantified by a four-point neurologic score (0 = normal behavior, 1 = deficit to extend the left forelimb, 2 = circling to the right, 3 = falling to the right, 4 = impossible to walk).[10] General and focal neurologic examinations were individually graded using 28-point scales, with a higher score corresponding to a greater severity of deficit.[11] The general neurologic deficit scale comprised observing the hair (0–2), ears (0–2), eyes (0–4), posture (0–4), spontaneous activity (0–4), and "epileptic" behavior (0–12) (see Appendix). The focal neurologic deficit scale was based on observations of: body symmetry (0–4), gait (0–4), circling behavior (0–4), climbing a 45° slope platform (0–4), forelimb symmetry (0–4), compulsory circling of forelimbs (0–4), and whisker response (0–4) (see Appendix).

Histology

After neurologic evaluation, the animals were reanesthetized with 5% isoflurane and euthanized *via* decapitation. The brain was immediately removed, immersed in methylbutane, frozen at −35°C, and sectioned. The infarct volume was measured using a previously published method.[10] Briefly, coronal sections (20-μm thick) were made and four sequential slices were collected and slide-mounted. The next 18 sections were discarded and a new four-slice cycle was initiated. The process began with the most rostral slice where the cerebral infarct was evident and continued throughout the extent of the infarct. The sections (eight or nine per animal) were stained with hematoxylin and eosin and digitized (1289 × 960 matrix of 210-μm^2 pixel units) using an image-analysis system (M2 Turnkey System; Imaging Research, Inc., St. Catharine's, Ontario, Canada). The infarct boundaries were separately delineated for the cortical and subcortical structures by an observer blinded to group assignment. Infarct volumes (mm^3) were computed as running sums of infarct area multiplied by the known interval (*e.g.*, 320 μm) between sections over the extent of the infarct expressed as an orthogonal projection.

Statistical Analysis

Statistical analysis was performed using Statview Software, version 5 (SAS Institute, Cary, NC). Results are expressed as mean ± SD or the median [interquartile range], as appropriate. Physiologic values and brain infarct volumes were compared among groups using analysis of variance followed by the Scheffé test for *post hoc* analysis when indicated by a significant F ratio. Neurologic scores were compared using the Kruskal-Wallis followed by the Mann-Whitney U tests, as appropriate. Statistical significance was considered when $P < 0.05$.

Table 1. Physiologic Values for Mice Subjected to 60-min Transient Focal Cerebral Ischemia

	Group		
	70% Xe	70% N_2O	35% Xe/35% N_2O
n	21	21	21
Body weight			
Preischemia (g)	23.8 ± 1.1	23.7 ± 1.7	23.0 ± 1.4
24 h postischemia (g)	22.4 ± 1.2	22.6 ± 1.6	22.0 ± 1.2
Preischemia			
MAP (mmHg)*	74 ± 2	73 ± 2	75 ± 1
Intraischemia			
MAP (mmHg)	81 ± 8	80 ± 8	78 ± 9
pHa	7.22 ± 0.05	7.22 ± 0.06	7.19 ± 0.09
$Paco_2$ (mmHg)	37 ± 7	38 ± 7	39 ± 9
Pao_2 (mmHg)	143 ± 19	134 ± 18	141 ± 17
Glucose (mg/dl)†	79 ± 13	66 ± 13	81 ± 11
Pericranial temperature (°C)	37.0 ± 0.1	37.0 ± 0.1	37.0 ± 0.1
Postischemia (reperfusion)			
MAP (mmHg)‡	76 ± 11	85 ± 11	75 ± 10

Values are mean ± SD.

* MAP before ischemia (70% N_2O *vs.* 35% Xe/35% N_2O: $P = 0.01$). † Glucose (70% Xe *vs.* 70% N_2O: $P = 0.008$; 70% Xe *vs.* 35% Xe/35% N_2O: NS; 70% N_2O *vs.* 35% Xe/35% N_2O: $P = 0.0015$). ‡ MAP after reperfusion (70% Xe *vs.* 70% N_2O: $P = 0.038$; 70% Xe *vs.* 35% Xe/35% N_2O: NS; 70% N_2O *vs.* 35% Xe/35% N_2O: $P = 0.029$).

MAP = mean arterial blood pressure; N_2O = nitrous oxide; pHa = arterial pH; Xe = xenon.

Results

After excluding three to five animals in each group because of unphysiologic arterial blood gas variables, 21 animals completed the experimental protocol in each of the three groups. Physiologic values are presented in table 1. Although modest MAP differences were present among groups before and after ischemia, there were no differences among groups during ischemia. There were no among-group differences in arterial pH, $Paco_2$, and Pao_2. Blood glucose concentrations were higher in both xenon groups compared with the 70% N_2O group, although values were similar for the two xenon groups (table 1).

Twenty-four hours after reperfusion, the functional performance on two of the three indices of neurologic scoring used was better in the animals anesthetized with 70% xenon compared with those in the 70% N_2O group, with the third nearly significantly different ($P = 0.0675$) (table 2). The 35% xenon + 35% N_2O group had an intermediate outcome (table 2). With respect to the histologic analyses, both xenon groups showed a significantly lower total cerebral infarct volume compared with the 70% N_2O group, which was largely attributable to a significantly smaller cortical infarct in the xenon groups (fig. 1).

Discussion

Xenon was first used as an anesthetic agent in the late 1940s.[12] Its unique properties, including a low blood-gas partition coefficient and inert characteristics with the absence of substantial side effects, make xenon an attractive anesthetic drug.[2] In recent years, *in vitro* evidence points to NMDA receptor antagonism as its principal mechanism for producing anesthesia.[8] This particular anesthetic mechanism also highlights a potential neuroprotective role for this drug whereby excessive activation of the glutamate receptor is central to mediating ischemia-related neuronal injury. Recently, both *in vitro* and *in vivo* experiments evidence showing neuroprotection from xenon have been published.[9] In addition, we have administered xenon to rats undergoing cardiopulmonary bypass (where cognitive dysfunction is common) and have similarly shown a neurologic benefit.[13] In the current study, 70% xenon decreased total infarct volume and improved neurologic outcome after transient focal cerebral ischemia in mice when compared with 70% N_2O, whereas 35% xenon had an intermediate effect.

Table 2. Results of Neurologic Evaluation 24 h after Reperfusion

	Neurologic Score		
Group	Four-point Scale	General Deficits	Focal Deficits
70% Xe	2 [2]	9 [6]	12 [14]
P value*	0.0061	0.0346	0.0627
70% N_2O	3 [2]	10 [4]	16 [22]
P value†	0.2224	0.0592	0.2272
35% Xe/35% N_2O	3 [2]	9 [6]	13 [16]

Values are median [IQR]; n = 21 in each group.

* *P* value, 70% Xe group *vs.* 70% N_2O group. † *P* value, 70% N_2O group *vs.* 35% Xe/35% N_2O.

Xe = xenon.

Filament occlusion of the middle cerebral artery in mice is a well-established experimental model of focal ischemia.[14–16] The assessment of brain injury after temporary middle cerebral artery occlusion has been previously validated using animal behavior and histology,[16] with a close association between functional neurologic outcome and the volume of brain infarction.[17] One of the strengths of our study was that three different scoring systems were used to define functional outcome. Two of the three scoring systems showed improved scores in the animals anesthetized with 70% xenon, with the third trending toward improvement ($P = 0.0627$). Although neurologic scores were not significantly improved in the 35% xenon group, numerically the values were intermediate between the 70% xenon and 70% N_2O groups, suggesting a dose-dependent effect of neuroprotective efficacy.

Xenon has a minimum alveolar concentration in rats of 160%. This is similar to the minimum alveolar concentration for N_2O in mice, which is estimated to be 150%.[3,4] In this protocol, xenon was administered at 70% in our experimental conditions and at 35% in combination with N_2O. Therefore, all three groups were

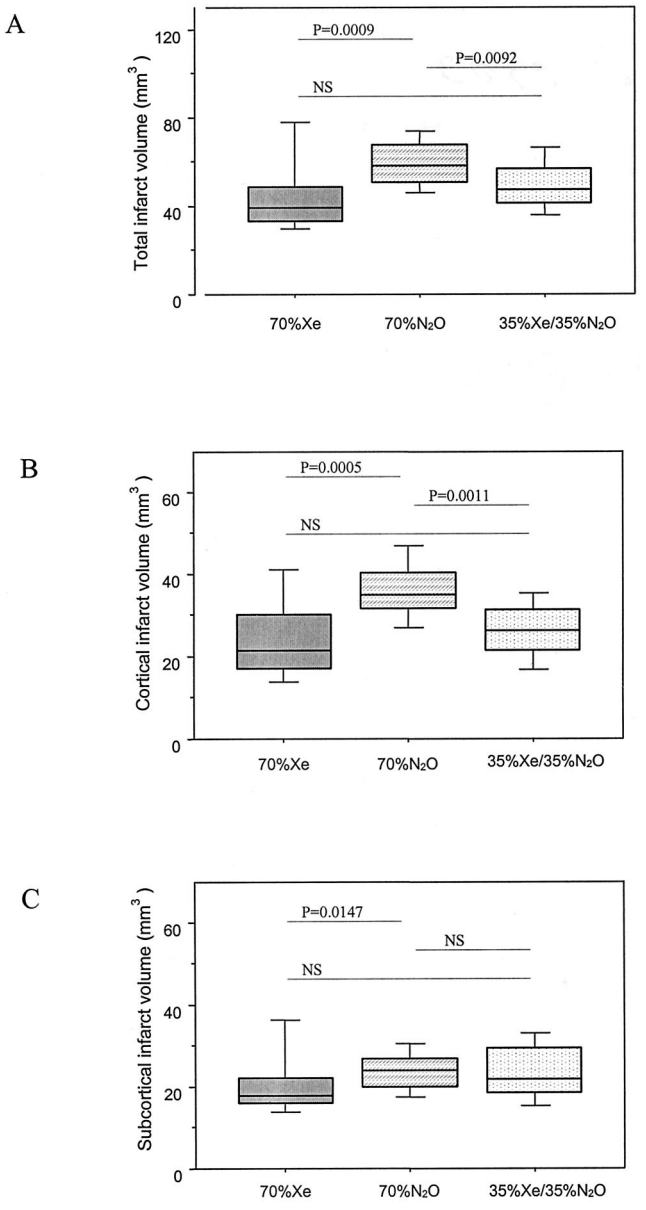

Fig. 1. Cerebral infarct volumes observed in each group after 24 h of reperfusion. (*A*) Total infarct volume (70% Xe, 45.2 ± 17.4 mm^3; 70% N_2O, 59.4 ± 11.5 mm^3; 35% Xe/35% N_2O, 49.8 ± 14.3 mm^3). (*B*) Cortical infarct volume (70% Xe, 24.1 ± 9.9 mm^3; 70% N_2O, 35.5 ± 8.6 mm^3; 35% Xe/35% N_2O, 26.6 ± 8.9 mm^3). (*C*) Subcortical infarct volume (70% Xe, 21.0 ± 8.2 mm^3; 70% N_2O, 23.9 ± 4.8 mm^3; 35% Xe/35% N_2O, 23.2 ± 6.0 mm^3). Xe = xenon.

maintained at similar levels of anesthetic depth. Differences in outcome, therefore, are unlikely to be attributable to variations in minimum alveolar concentration among groups.

Some physiologic differences were present among groups but, despite being statistically significant, these differences were unlikely to have influenced outcome. The 70% N_2O group had a slightly higher MAP after ischemia. Postreperfusion blood pressure is an important determinant of infarct volume, with a lower blood pressure usually being associated with worsened outcome.[18] A higher MAP may result in improved cerebral blood flow and consequently contribute to salvage of penumbral tissue. The fact that the animals anesthetized with xenon had a lower MAP may have obscured some fraction of its potential neuroprotective potency had blood pressure been held equal among groups using pharmacologic support. Intraischemic blood glucose concentrations were higher (albeit within an otherwise normal range) in the xenon-anesthetized animals. It is well established that increased serum glucose levels (usually above 180–200 mg/dl)[19,20] are associated with worsened neurologic outcome after brain injury. In our experiment, the xenon-anesthetized animals, despite having higher intraischemic serum glucose levels than the 70% N_2O group, had better neurologic scores and histologic outcomes. It is not clear why there were statistically significant, though arguably relatively minor, differences in blood pressure and glucose. One possible explanation is that xenon may have superior effects at blunting the stress response to surgery; however, this would only explain the lower blood pressure, not the slightly higher glucose levels. An alternative explanation concerning the higher MAP after ischemia in the 70% N_2O group is that the MAP may have been increased because the cerebral infarcts were larger, resulting in a more "stressed" animal with higher catecholamine levels.

There are some limitations to this study. From a physiologic control perspective, we were limited (owing to blood volume concerns of repeated sampling) to a single blood gas assessment. Potentially, alterations in blood gases before or after one sampling time may have affected results. In addition, the 24-h functional and histologic assessment interval may not have captured the final outcome differences between groups. Others have shown that apparent differences in outcome observed at 24 h dissipate with longer observation intervals. The 24-h assessment period, however, has been routinely used for mouse focal ischemia experiments, principally because maintaining survival for longer intervals is difficult.[10,14–16,21] The positive findings in the current study offer sufficient promise that long-term recovery studies in larger rodents or other species are warranted to test our findings. A final limitation is that we did not include a control, awake group of animals. It may be that the anesthetized state in all of our animals modulated outcome in a way that differences between groups may have been larger (or smaller) if a control nonanesthetized group had been included. The reason for the lack of an awake group related to the need for invasive pericranial temperature monitoring that would have not been tolerated in an awake animal. Including an appropriate temperature-controlled group (*i.e.,* pericranial) in mouse ischemia models is not feasible because of the lack of available technology to implant a thermistor into the brain of the animal, as is done in rat ischemia mod-

els,[22] thereby allowing a servo-controlled system to regulate brain temperature in the awake mouse.

Our experiment showed that xenon, alone or at a minimum alveolar concentration equivalent with N_2O, reduces brain injury after transient focal ischemia. It is plausible that this neuroprotective effect was mediated by the known NMDA receptor antagonism produced by xenon. NMDA receptor antagonists have been repeatedly shown to reduce focal ischemic outcome, when brain temperature is regulated, if treatment occurs during, but not after, the ischemic insult.[23] Curiously, N_2O has also been shown to provide NMDA receptor antagonism.[24] To our knowledge, the relative NMDA receptor antagonism potencies of xenon and N_2O have not been compared. If the mechanism of *in vivo* xenon neuroprotection is indeed *via* the NMDA receptor, it seems that xenon may be a more potent NMDA receptor antagonist of the two gases.

Principally, the neuroprotective effect was most prominent in the cerebral cortex, with little effect in the subcortex. Regional differences in the density of NMDA receptors may partially account for the lack of a neuroprotective effect in the subcortex *versus* the cortex. If indeed xenon protects by antagonizing NMDA receptors, the cortex, which generally has a higher density of NMDA receptors,[25,26] may be more susceptible to the effects of blocking these receptors. Another possible explanation for this differential cortical neuroprotection relates to differences in vascular distribution. The subcortex is supplied by an end artery (lenticulostriate) that when occluded by the filament results in a more dense ischemic period (*i.e.*, no blood flow for delivery of a blood-borne neuroprotective agent), whereas the cortex receives potential collateral blood flow from vessels across a watershed zone and results in a more well-defined penumbra, which may be more easily protected by a pharmacologic intervention.

NMDA receptor antagonists have not achieved acceptance as clinical neuroprotective agents principally for two reasons. Most clinical scenarios require treatment after stroke evolution is well under way (*e.g.*, patients admitted to the hospital with stroke), thereby exceeding the therapeutic window for this class of agents. However, because xenon could be administered intraoperatively, protection against intraoperative ischemic insults would be feasible. The second clinical limitation for NMDA receptor antagonists has been psychotomimetic adverse effects, which limit the doses administered to humans to levels that are subtherapeutic for neuroprotection.[27,28] Because xenon is rapidly eliminated, it is plausible that administration at neuroprotective concentrations during surgery may not be associated with psychotomimetic side effects after emergence.[29]

In summary, mice exposed to transient focal cerebral ischemia had reduced infarct size and improved neurologic outcome as a result of xenon anesthesia administered during the ischemic insult. Although these findings require considerable extension in other species and with longer-term recovery intervals to confirm the potential clinical relevance of our results, the current investigation, along with other recent encouraging results,[9,12] warrant further investigation into the clinical potential of xenon as a neuroprotective agent.

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Appendix: General and Focal Deficits (0–28)

Table 3 presents the general deficits, and table 4 shows the focal deficits.

Table 3. General Deficits (0–28)

	0	1	2	3	4	6	9	12
1. Hair	Normal	Localized disorder, especially around eyes and nose	Generalized disorder, ruffled and/or dirty fur	–	–	–	–	–
2. Ears	Normal position	Transient lope-eared	Steady lope-eared	–	–	–	–	–
3. Eyes	Normal	Rheumy	Dark discharge	Semi-closed	Closed	–	–	–
4. Posture	Normal	Hunched, unstable	Upright, head and/or body rests on ground	Lies on side, can assume prone position with strain	Passive, lies as placed	–	–	–
5. Spontaneous activity	Normal	Calm, quiet, explores slowly	Inert, somnolent, not exploring	Lethargic, stuporous, some movements in place	No spontaneous movements	–	–	–
6. Epileptic behavior	Not present	–	–	Hyperactive, timid and apprehensive	–	Aggressive appearance, hyperexcited	Extremely hyperexcited or focal seizures	Partially or fully developed grand mal seizures

General scoring system: For each of the six general deficits measured, animals can receive between 0–12 points depending on the severity. The scores on the six areas are then summed to provide a total general score ranging from 0–28. Reprinted from Clark WM, Lessov NS, Dixon MP, Eckenstein F: Monofilament intraluminal middle cerebral artery occlusion in the mouse. Neurol Res 1997; 19:641–8; used with permission.

Table 4. Focal Deficits (0–28)

	0	1	2	3	4
1. Body symmetry (open bench top)	Normal	Slight asymmetry	Moderate asymmetry	Prominent asymmetry	Extreme asymmetry
2. Gait (open bench top)	Normal	Stiff, inflexible	Limping	Trembling, drifting, falling	Does not walk
3. Climbing (gripping surface, 45° angle)	Normal	Climbs with strain, limb weakness present	Holds onto slope, does not slip or climb	Slides down slope, unsuccessful effort to prevent fall	Slides immediately, no effort to prevent fall
4. Circling behavior (open bench top)	Not present	Predominantly one-sided turns	Circles to one side (not constantly)	Circles constantly to one side	Pivoting, swaying, or no movement
5. Front limb symmetry (mouse suspended by its tail)	Normal	Light asymmetry	Marked asymmetry	Prominent asymmetry	Slight asymmetry, no body/limb movement
6. Compulsory circling (front limbs on bench, rear suspended by tail)	Not present	Tendency to turn to one side	Circles to one side	Pivots to one side sluggishly	Does not advance
7. Whisker response (light touch from behind)	Symmetrical response	Light asymmetry	Prominent asymmetry	Absent response ipsilaterally, diminished contralaterally	Absent proprioceptive response bilaterally

Focal scoring system: For each of the seven areas assessed, animals can be rated between 0–4 depending on the severity. The seven items are then summed to give a total focal score ranging between 0–28. Reprinted from Clark WM, Lessov NS, Dixon MP, Eckenstein F: Monofilament intraluminal middle cerebral artery occlusion in the mouse. Neurol Res 1997; 19:641–8; used with permission.