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Impaired Functional Recovery After Stroke in the Stroke-Prone Spontaneously Hypertensive Rat

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Background and Purpose—To identify if the stroke-prone spontaneously hypertensive rat (SHRSP) exhibits impaired functional recovery after stroke compared with its normotensive reference strain, the Wistar Kyoto rat (WKY).

Methods—In study 1, a 2-mm distal middle cerebral artery occlusion (middle cerebral artery occlusion) was performed in both strains and recovery assessed using a 33-point neurological score. Because SHRSPs displayed much larger infarcts than WKYs, study 2 and study 3 involved extending the length of middle cerebral artery (MCA) occlusion in the WKY to increase the volume and distribution of infarction to comparable levels with SHRSP. Animals were assessed with the neurological score, tapered beam walk, and cylinder tests.

Results—In study 1, infarct volume (expressed as a percent of contralateral hemisphere) was WKY $13.1 \pm 3\%$ and SHRSP $19.8 \pm 1\%$. Initial neurological deficit was greater (WKY 25 ± 1 , SHRSP 22 ± 1 , out of a possible 33) and subsequent recovery was poorer in SHRSP. In studies 2 and 3, infarct volume and distribution (study 2, WKY $21.8 \pm 1.3\%$, SHRSP $22.9 \pm 3\%$; study 3, WKY $17.2 \pm 2\%$, SHRSP $16.5 \pm 3\%$) and initial neurological deficit at 2 hours after middle cerebral artery occlusion (study 2 WKY 23 ± 1 , SHRSP 22 ± 2 ; study 3 WKY 25 ± 1 and SHRSP 23 ± 1 ; mean \pm SEM) were comparable between strains. However, whereas WKY recovered toward normal scores, SHRSP scored significantly lower 2 weeks (study 2) and 4 weeks (study 3) after middle cerebral artery occlusion. Beam walk data revealed long-term impairment in SHRSP contralateral limb use, compared with WKY, at days 3, 7, and 28 ($P < 0.05$).

Conclusions—SHRSP exhibit impaired functional recovery after stroke compared with WKY. (*Stroke*. 2005;36:135-141.)

Key Words: brain injuries ■ middle cerebral artery occlusion ■ recovery of function ■ stroke

Cerebral ischemia caused by occlusion of the middle cerebral artery is reported to be the most commonly encountered type of stroke in humans,^{1,2,4} affecting an estimated 5.5 million people worldwide.² This can result in a variety of disabilities, including hemiparesis and dysphagia.⁵ It is now widely accepted that the brain has an inbuilt ability to recover after such an event;^{6,7} recently, research has focused on the study of long-term recovery after stroke.

To investigate potential mechanisms involved in recovery from stroke, the use of animal models of focal cerebral ischemia, in particular rodent models, is essential.¹⁰ A clinically relevant model of human stroke is the stroke-prone spontaneously hypertensive rat (SHRSP).^{3,8} This strain has been shown to have a genetic predisposition to cerebral ischemia and exhibits hypertension and an increased sensitivity to experimentally induced stroke.^{3,11} The Wistar Kyoto rat (WKY) is the normotensive strain from which the SHRSPs were derived and therefore represents a reference control strain. Although the SHRSPs have proved invaluable in assessing the relationship between hypertension, genetics, and stroke, their degree of sensorimotor impairment and

subsequent functional recovery after stroke has yet to be investigated. Understanding recovery in this clinically relevant model may improve our understanding of recovery after human stroke, which is an increasingly important aspect of stroke research.⁷

Because SHRSPs are hypertensive and display a greater sensitivity to experimental stroke, we tested the hypothesis that they would also exhibit a poorer outcome after middle cerebral artery occlusion compared with their reference strain, WKY.

Materials and Methods

Animals and Housing

All experiments were performed under license from the Home Office, United Kingdom and were subject to the Animals (Scientific Procedures) Act of 1986. SHRSP and WKY rats were obtained from inbred colonies within the Division of Cardiovascular and Medical Sciences at the University of Glasgow. Male rats aged between 3 and 6 months were individually housed in standard laboratory cages and food and water were available ad libitum. Animals were tamed by daily handling for up to 2 weeks before surgery and behavior testing.

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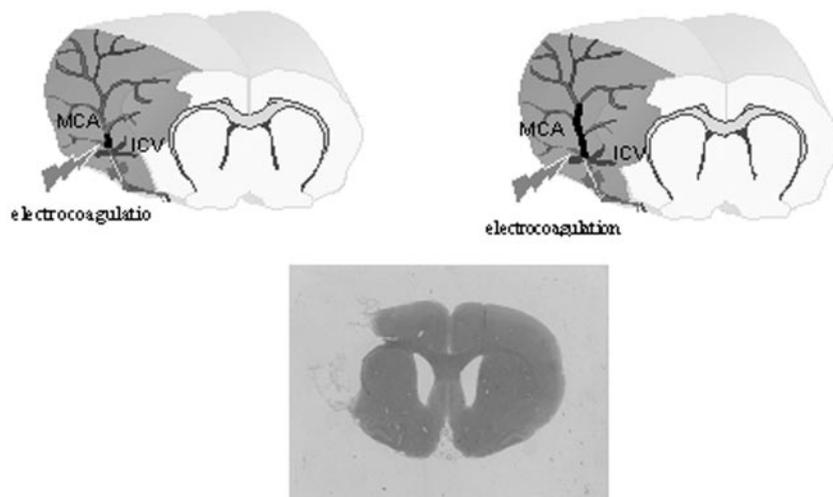


Figure 1. a, Two-mm distal middle cerebral artery occlusion with occlusion starting from a point just distal to the inferior cerebral vein (ICV) and extending 2 mm distally. b, Extended distal middle cerebral artery occlusion in WKY (studies 2 and 3) with occlusion starting just proximal to the ICV and extending distally to the first major branch of the middle cerebral artery (MCA). c, Representative hematoxylin and eosin-stained section showing tissue loss 4 weeks after middle cerebral artery occlusion in WKY.

Surgical Procedures: Focal Cerebral Ischemia

Anesthesia was induced with 5% halothane and maintained at 1% to 1.5% in oxygen–nitrous oxide (30:70) throughout the procedure by intubation of the animal. Body temperature was monitored via a rectal probe and maintained at 37°C using a heating lamp.

Study 1 included WKY (n=6) and SHRSP (n=5) (14-day survival) rats. Permanent focal cerebral ischemia was induced by electrocoagulation of the distal portion of the middle cerebral artery using a modification of the Tamura model, as previously described.⁹ In study 1, a 2-mm segment of the middle cerebral artery occlusion was electrocoagulated just distal to the inferior cerebral vein (Figure 1a). To ensure complete occlusion, the artery was cut at this position with microscissors.

Study 2 included 6 rats per strain (14-day survival), and study 3 included WKY (n=9) and SHRSP (n=11) (28-day survival) rats. A 2-mm distal middle cerebral artery occlusion was performed in SHRSPs. The occlusion began just proximal to the inferior cerebral vein. In WKYs, the occlusion was extended more distally, up to the first major branch of the MCA (Figure 1b).

Behavior Assessments: Neurological Score

A neurological assessment involving a 33-point score, modified from Hunter et al,¹² was used in all 3 studies. This comprised a battery of 11 tests that include assessment of paw placement, grip strength, ability to grip a horizontal bar, visual forepaw reaching, contralateral reflex, contralateral rotation, righting reflex, inclined plane, general condition, motility, and circling. Thirty-three is the maximum score the rat can achieve, and 31 to 33 would be considered normal scores. Scoring was performed before surgery and then after middle cerebral artery occlusion at 2 hours, 24 hours, 48 hours, 72 hours, 7 days, and 14 days/28 days.

Neurological scores were analyzed using a repeated measures 2-way analysis of variance (ANOVA) to determine the effect of time and strain on score. Individual time points were then assessed using an unpaired Student *t* test.

Tapered Beam Walk Test

This test, developed by Schallert et al,¹³ is a measure of forelimb and hind limb impairments after middle cerebral artery occlusion. Animals were trained to traverse a 130-cm-long tapered beam from a platform at one end to the animal's home cage at the other, and the number of foot slips onto an under-hanging ledge was recorded. Animals were pretrained for 5 to 7 days and tested preoperatively and then postoperatively at days 3, 7, 14, 21, and 28 in the light phase of the light/dark cycle.

The number of foot faults made was calculated as a percentage of the total number of steps taken.

Beam walk test data were analyzed using a repeated-measures ANOVA to determine the effect of time and strain on the number of

foot faults made. Data were log-transformed before analysis, and ipsilateral and contralateral faults were analyzed separately. A follow-up least significant difference test assessed differences in group means at individual time points.

Cylinder Test

The cylinder test, developed by Schallert et al,¹⁴ is a measure of forelimb asymmetry after middle cerebral artery occlusion. When placed in the cylinder, the animal will rear and explore the cylinder walls with its forepaws, allowing 3 categories of placements to be recorded: independent ipsilateral limb use, independent contralateral limb use, and both movements, when the animal uses both paws in unison or in quick succession. Animals were tested preoperatively and then postoperatively at days 3, 7, 14, 21, and 28, using a red lamp in the dark during the animals' light phase, to encourage exploratory behavior and rearing. The different forelimb preference, for placements made, was calculated as a percentage of the total number of placements made.

Cylinder test data were analyzed first with a mixed models repeated-measures ANOVA to determine effect of time, strain, and paw. A follow-up least significant difference test was used to analyze differences between the 2 strains for days and paws used.

Brain Processing: Perfusion–Fixation

At 2 (studies 1 and 2) and 4 (study 3) weeks after middle cerebral artery occlusion, animals were transcardially perfused with heparinized saline, followed by 4% paraformaldehyde. Brains were embedded in paraffin wax and 6- μ m sections were cut at 8 predetermined coronal levels throughout the middle cerebral artery territory.¹⁵

Assessment of Tissue Loss

Sections from each of the 8 coronal levels were stained with hematoxylin and eosin and assessed for tissue loss (Figure 1c). The area of intact tissue in the ipsilateral hemisphere and the area of the contralateral hemisphere were measured directly from sections at each of the 8 coronal levels using an MCID Image Analyser (Imaging Research). The volume of each hemisphere was calculated from the area under the curve of tissue area plotted against stereotaxic coronal levels. The endpoints were 12.5 mm anterior and 0.05 mm posterior to the interaural line. Swanson method was used to calculate the percent volume of tissue loss, as previously described.¹⁶ The area of tissue loss at each coronal level was also determined and compared in both strains.

The significance of differences in tissue loss was assessed using an unpaired Student *t* test.

Results

Study 1: Volume of Tissue Loss

A 2-mm distal middle cerebral artery occlusion resulted in mainly cortical damage (Figure 1c), which included the

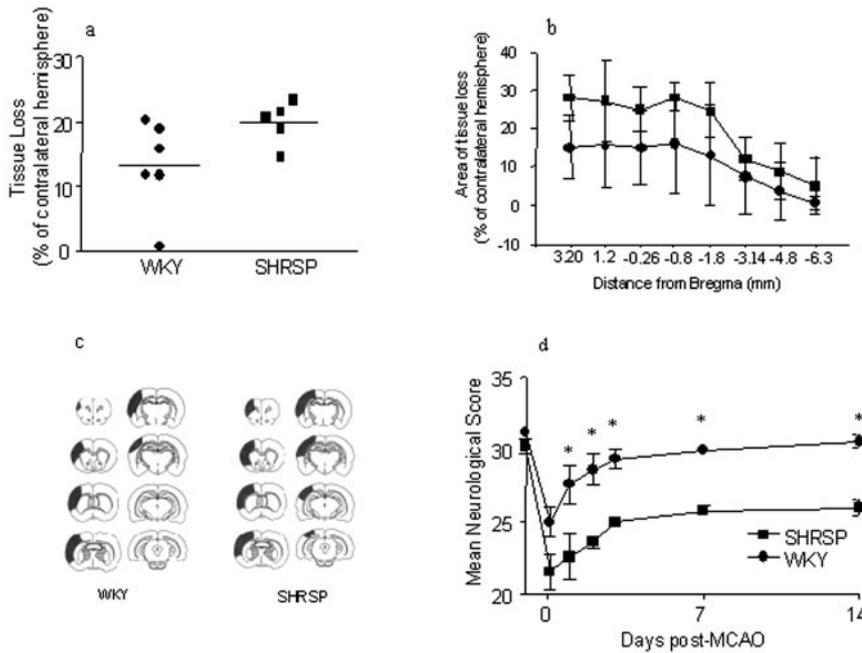


Figure 2. Study 1. Distribution and amount of tissue loss and neurological deficit in SHRSPs (n=5) and WKYs (n=6). a, Total tissue loss. b, Area of tissue loss at each coronal level. c, Distribution of tissue loss in the median animal from each strain. d, Neurological scores. WKY scored significantly higher than SHRSP from days 1 to 14 after middle cerebral artery occlusion ($P<0.005$).

sensorimotor cortex. The percent volume of tissue loss (Figure 2a) after a 2-mm distal middle cerebral artery occlusion was WKY $13.1\pm 3\%$ and SHRSP $19.8\pm 1\%$ (mean \pm SEM). Although the difference in the percent tissue loss was not statistically significant between the 2 strains, the distribution of tissue loss was less in WKYs than in SHRSPs at all 8 coronal levels examined (Figure 2b and 2c). The greatest difference was seen in dorsal regions of the motor cortex, which include the forelimb and hind limb regions. SHRSPs displayed substantially more damage in these areas than WKYs.¹⁷

Neurological Score

Mean neurological scores for both strains are presented in Figure 2d. Both SHRSPs and WKYs display a similar pattern of behavior: an initial deficit followed by some degree of recovery. At 2 hours after middle cerebral artery occlusion, SHRSPs exhibited a more severe deficit than WKYs (SHRSP 22 ± 1 versus WKY 25 ± 1 ; $P<0.005$). Repeated-measures ANOVA revealed a significant effect of time ($P<0.0001$) and strain ($P=0.0002$) on score, with WKYs scoring significantly higher than SHRSPs from 24 hours to 14 days after middle cerebral artery occlusion, ($P<0.005$). At 14 days after middle cerebral artery occlusion, SHRSPs continued to display deficits in paw placement, grip strength, horizontal bar, and general condition (SHRSP 26 ± 0.5 , WKY 31 ; $P<0.05$).

Study 2: Volume of Tissue Loss

After an extended distal middle cerebral artery occlusion, tissue loss in WKYs increased to comparable levels with SHRSP ($P=0.75$): WKY $21.8\pm 1.3\%$ and SHRSP $22.9\pm 3\%$ (mean \pm SEM) (Figure 3a). The overall distribution of damage in WKYs now incorporated substantially more of the medial motor cortex, including the forelimb and hind limb areas (Figure 3b and 3c).

Neurological Score

Mean neurological scores are presented in Figure 3d. The initial deficit in both strains is now similar, WKY 23 ± 1 and SHRSP 22 ± 2 (mean \pm SEM; $P<0.35$). However, repeated-measures ANOVA revealed a significant effect of time ($P<0.0001$) and strain ($P=0.0116$) on score, with WKYs scoring higher than SHRSPs from 48 hours to 14 days after middle cerebral artery occlusion ($P<0.05$). At 14 days after middle cerebral artery occlusion, SHRSPs continued to display deficits in paw placement, grip strength, horizontal bar, and general condition (SHRSP 26 ± 1 , WKY 30 ± 1 ; $P<0.05$).

Study 3: Volume of Tissue Loss

Figure 4a presents percent tissue loss in WKYs and SHRSPs at 28 days after middle cerebral artery occlusion. Both strains were comparable in terms of tissue loss (WKY $17.2\pm 2\%$, SHRSP $16.5\pm 3\%$; mean \pm SEM; $P=0.85$) and displayed a similar distribution of damage throughout the brain (Figure 4b and 4c).

Neurological Scores

Mean neurological scores are presented in Figure 4d. The initial deficit in both strains was similar, WKY 25 ± 1 and SHRSP 23 ± 1 (mean \pm SEM), although this small difference reached statistical significance ($P=0.02$). Repeated-measures ANOVA revealed a significant effect of time ($P<0.0001$) and strain ($P<0.0001$) on score, with WKYs scoring significantly higher from 2 hours postoperatively up to 28 days after middle cerebral artery occlusion ($P<0.05$).

Although WKYs recovered to normal scores by day 28, SHRSPs did not. SHRSPs showed long-term deficits in paw placement, grip strength, horizontal bar, and general condition (SHRSP 29 , WKY 32 ; $P<0.05$).

Beam Walk Test

Contralateral and ipsilateral foot faults for the beam walk test are presented in Figure 5a. At 3 days after middle cerebral

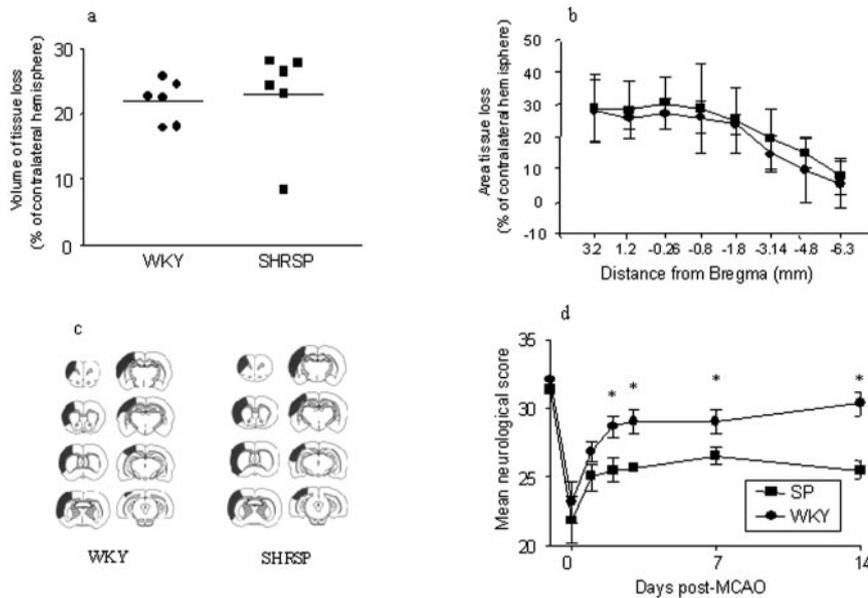


Figure 3. Study 2. Distribution and amount of tissue loss and neurological deficit in SHRSPs and WKYs (n=6/strain). a, Total tissue loss. b, Area of tissue loss at each coronal level. c, Distribution of tissue loss in the median animal from each strain. d, Neurological scores. WKYs scored significantly higher than SHRSPs from days 2 to 14 after middle cerebral artery occlusion ($P<0.05$).

artery occlusion, SHRSPs displayed a significantly greater number of contralateral limb foot faults than WKYs. Some evidence of recovery was apparent by day 7 in SHRSPs, but they continued to display a greater number of faults from days 7 to 28 compared with WKYs. There was a significant effect of time ($P=0.0001$) and strain ($P=0.016$) for the number of foot faults made with contralateral limbs. Further analysis revealed that this effect was significant at days 3, 7, and 28 ($P=0.004$, 0.017, and 0.010, respectively).

Analysis of ipsilateral limb faults revealed a significant effect of strain ($P=0.017$). This effect was significant at days 7 and 14 ($P=0.03$ and 0.02, respectively), with WKYs making more ipsilateral foot faults at these time points. The greatest number of faults was made with the hind limbs.

Figure 5b and 5c represent the number of contralateral faults made with the forelimb and hind limb, respectively. No additional statistical analysis was performed on these data, but it was evident that SHRSPs made more faults with both contralateral forelimb and hind limb from days 3 to 28, compared with WKYs.

Cylinder Test

The number of paw placements made with contralateral, ipsilateral, and both limbs are presented in Figure 6a, 6b, and 6c, respectively. Repeated-measures ANOVA on percent placement data revealed no significant effect of time ($P=1.0$) and strain ($P=0.9993$). Follow-up pair-wise comparisons revealed no significant difference between the 2 strains on

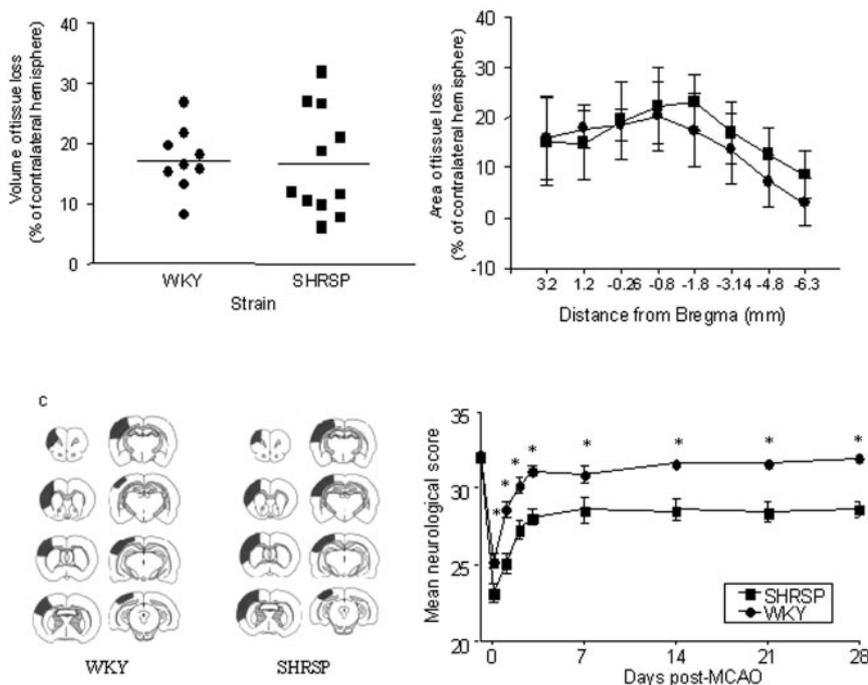


Figure 4. Study 3. Distribution and amount of tissue loss and neurological deficit in SHRSPs (n=11) and WKYs (n=9). a, Total tissue loss. b, Area of tissue loss at each coronal level. c, Distribution of tissue loss in the median animal from each strain. d, Neurological scores. WKYs scored higher than SHRSPs from 2 hours to 28 days after middle cerebral artery occlusion ($P<0.05$).

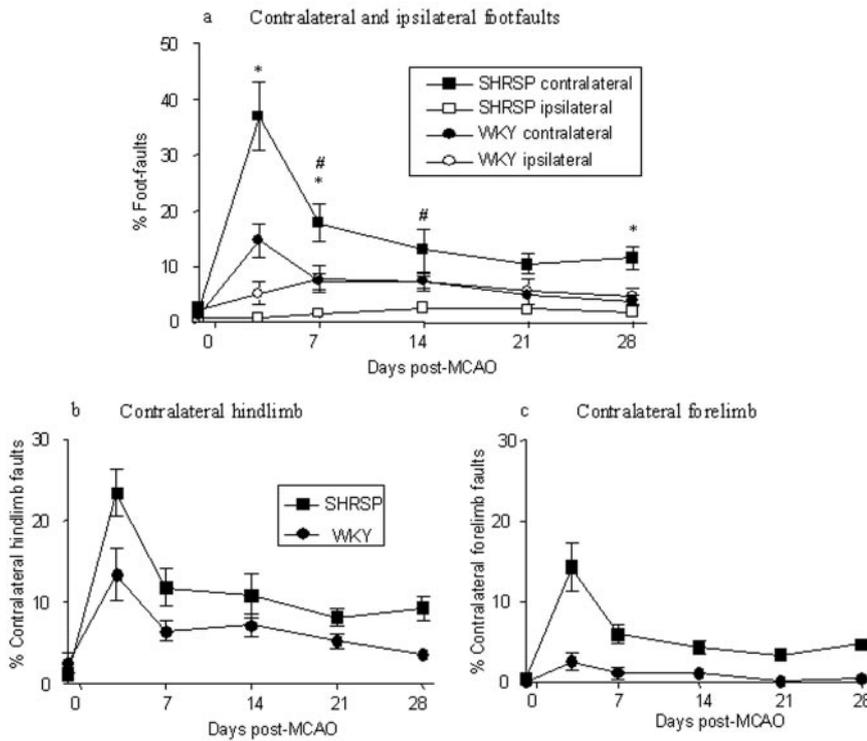


Figure 5. Tapered beam walk test. a, Contralateral and ipsilateral foot faults in SHRSP and WKY. SHRSPs make significantly more contralateral foot faults than WKYs at days 3, 7, and 28 after middle cerebral artery occlusion (* $P < 0.05$). WKYs make significantly more ipsilateral foot faults than SHRSPs at days 7 and 14 after middle cerebral artery occlusion (# $P < 0.05$). b, Contralateral hind limb faults. c, Contralateral forelimb faults.

paws used at any time point. However, in both strains there was evidence for a significant difference in the percentage use of the paws after stroke. Repeated-measures ANOVA revealed a significant effect of paw use ($P = 0.0005$), with the percent number of both movements less than that for either independent placement of the right or left paw at all time points after middle cerebral artery occlusion in both strains.

Discussion

Investigating recovery after middle cerebral artery occlusion in the SHRSPs and WKYs has revealed 2 important results. Extending middle cerebral artery occlusion distally in WKYs increased the amount of tissue loss and incorporated more medial areas of the motor cortex, including the forelimb and hind limb regions. This is particularly important when investigating functional recovery using behavior tests, such as the cylinder and beam walk tests, because the same brain regions must be affected in both strains to a similar degree for valid conclusions to be drawn about functional recovery. When WKYs and SHRSPs displayed similar volumes and distribution of ischemic damage, SHRSPs demonstrated an impaired ability to recover compared with WKY and were still significantly impaired 28 days after middle cerebral artery occlusion. This was shown with the 33-point neurological score and tapered beam walk test. Interestingly, the cylinder test did not reveal any significant degree of impairment in either strain, even at 3 days after middle cerebral artery occlusion. This might be explained, in part, by the areas of the brain affected by the distal middle cerebral artery occlusion. A distal middle cerebral artery occlusion results mainly in cortical damage, with little if any damage to the striatum. There is evidence to suggest that asymmetrical limb use in the cylinder test is a consequence of both striatal and cortical

damage,²⁰ and as such the cylinder test might not be sensitive to focal cortical lesions.

Another interesting finding was that WKY rats made significantly more ipsilateral limb foot faults, primarily with the hind limb, compared with SHRSP rats in the beam walk test. This surprising result might be explained, in part, by compensatory mechanisms the rats are using. In WKYs, there was improvement in the contralateral limbs over time, as shown by the decrease in the number of faults. However, although there was recovery, the contralateral limbs in the WKYs may have been unable to support the animal's full weight, therefore resulting in the ipsilateral limbs being improperly placed on the beam and being classed as a foot fault. However, it is important to note that the number of ipsilateral foot faults in WKYs was small and never more than the number of contralateral foot faults.

From 33-point neurological score data at 28 days after middle cerebral artery occlusion, the SHRSPs showed long-term deficits in paw placement, grip strength, horizontal bar, and general condition (body weight, general demeanor, coat appearance) compared with WKYs. The SHRSPs were slower to regain preoperative weight, even on supplemented diet (given to all animals). Some were less active and adopted a hunched posture, even at 28 days. Although there was a degree of recovery of contralateral limb grip strength in SHRSPs, it was noticeably weaker than the ipsilateral limb. Consequently, SHRSPs were unable to grip the horizontal bar properly and pull both hind limbs up onto it, resulting in a lower score in this test. A long-lasting deficit in the inclined plane was seen in both strains. However, this may be a consequence of habituation because the animals' motivation to turn and

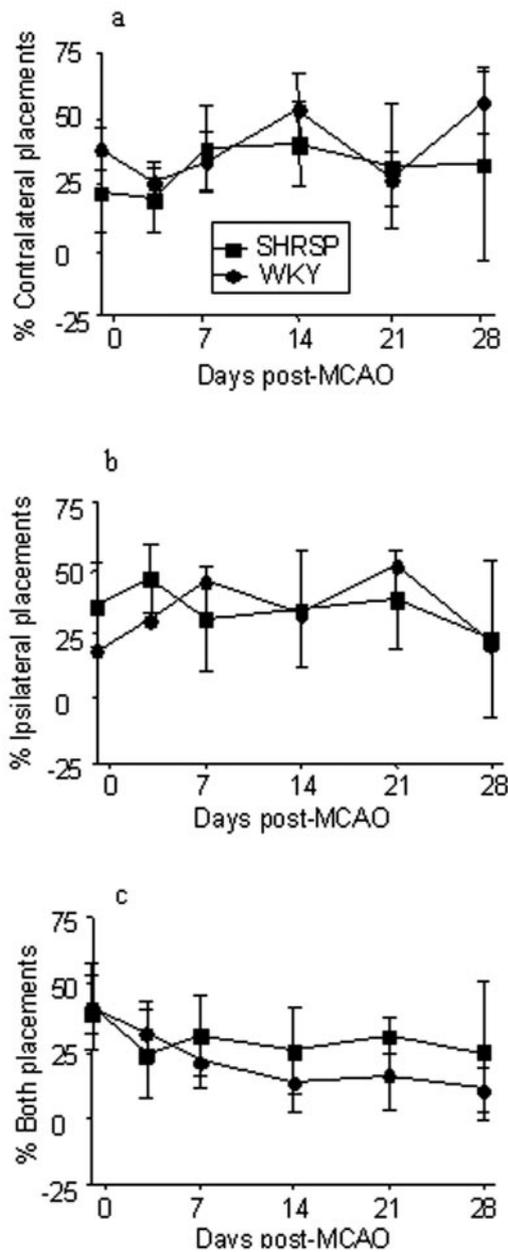


Figure 6. Cylinder test. Contralateral, ipsilateral, and both forelimb use in the cylinder test after middle cerebral artery occlusion. Neither strain shows any significant degree of impairment in contralateral, ipsilateral, or both forelimb use at any time point.

face the uphill position became less as time progressed, despite being physically capable of completing this test. Thus, the lower scores in this test could reflect motivation rather than sensorimotor impairment.

Overall, these studies have demonstrated that when the amount and distribution of tissue loss in SHRSP and WKY is similar, SHRSPs clearly have an impaired ability to recover compared with WKYs. To our knowledge, this is the first study reporting such a finding.

In support of our results, a recent study by Maguire et al¹⁹ has demonstrated a difference in functional outcome between SHRSPs and the genetically related spontane-

ously hypertensive rats. Despite both strains displaying similar volumes of damage, SHRSPs showed long-term impairment, up to 28 days after middle cerebral artery occlusion compared with the spontaneously hypertensive rats, which showed improved recovery by day 18 after middle cerebral artery occlusion. This might suggest, similar to our studies, that there is some inherent, perhaps genetic, basis for impaired recovery in the SHRSP. Interestingly, the SHRSPs in this study also displayed higher blood pressures compared with the spontaneously hypertensive rats. It is therefore possible that this elevated blood pressure and subsequent disturbances in cerebral autoregulation after middle cerebral artery occlusion in the SHRSPs may play a part in their impaired recovery. This has been shown to be important in human stroke. Several studies have shown that early lowering of high blood pressure after ischemic stroke is associated with better functional outcome.¹⁸ Current studies are underway to investigate the effect of hypertension in the SHRSP on functional outcome after stroke. Another plausible explanation for the differences in functional recovery between the 2 strains is that neuroplasticity in the SHRSP brain may be compromised. Although the exact mechanisms involved are poorly understood, there is overwhelming evidence supporting a role for plasticity in functional recovery after stroke in humans and rats.^{6,7,14} Work is currently underway to determine if there are any differences in neuroplasticity between SHRSPs and WKYs, which may underlie impaired recovery in the SHRSP.

In conclusion, the results of the present studies show that when a similar size and distribution of ischemic infarct are induced in the 2 strains, SHRSPs have an impaired ability to recover motor function, compared with their reference strain, the WKYs. This difference has been demonstrated up to 28 days after middle cerebral artery occlusion using a 33-point neurological score and tapered beam walk test.

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