Paw-Reaching, Sensorimotor, and Rotational Behavior After Brain Infarction in Rats

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Background and Purpose: Functional tests that are stable and consistent over time are an advantage for long-term evaluation of treatment in experimental stroke research. Because little information on this subject is available in rodents with focal cerebral ischemia, we investigated the outcome of three behavioral tests for a period of 3 months after the insult.

Methods: Spontaneously hypertensive rats were sham-operated (n=27) or underwent an occlusion (n=36) of the right middle cerebral artery. Before surgery all rats were tested for amphetamine-induced rotational behavior, and half of the rats were trained in a paw-reaching task. One, 2, and 3 months after surgery the tests were repeated, together with a test for sensorimotor function. Infarct size was measured morphometrically.

Results: In the lesion group, total hemisphere area was reduced by 22%, caudate putamen by 47%, and the thalamus by 24%. Contralateral to the lesion, paw-reaching was highly impaired, regardless of whether or not the rats had been pretrained, and lesion size correlated significantly to paw-reach performance. Ipsilateral rotation increased and sensorimotor function recovered with time in infarcted rats.

Conclusions: In contrast to amphetamine-induced rotation and sensorimotor behavior, the paw-reaching test provides a stable behavioral parameter after a middle cerebral artery occlusion. Moreover, the lesion-induced deficit in paw-reaching is highly correlated to the extent of the infarct, suggesting that this test is useful in evaluating treatment effects for a longer period of time. (Stroke 1995;24:889–895)

Key Words • behavior, animal • cerebral infarction • rats

We have recently described the survival, neural connectivity, and vascularization of fetal neocortical grafts implanted in the infarcted area after a middle cerebral artery occlusion (MCAO) in the spontaneously hypertensive rat (SHR). Preliminary studies in our laboratory have not been successful in establishing a behavioral test suitable for transplantation purposes. The main reason has been a spontaneous recovery in all test groups, making it impossible to evaluate a behavioral effect of the grafts.

Although an increasing body of knowledge has emerged on behavior after MCAO in the rat during the past years, only a few studies have extended behavioral observation for more than a month's time after the insult. The aim of the present study was to perform a behavioral follow-up for several months after MCAO, which is essential when evaluating therapeutic procedures, such as neural transplantation, that could be expected to exert an effect after a longer period of time.

Deficits in sensorimotor integration were highly correlated to lesion size 3 weeks after a right MCAO in the SHR. In addition, amphetamine-induced rotation discriminated between lesioned and control groups but was poorly correlated to lesion size. The outcome of these tests at later time points after MCAO has not been studied. Recently, Montoya et al developed a test measuring independent forelimb reaching and grasping abilities in rats. Since different paradigms for skillful forelimb use previously have demonstrated contralateral reaching deficits after unilateral lesions of the neocortex and the striatum regions affected by a proximal MCAO, we decided to evaluate this new test in a behavioral study after MCAO.

The present study includes testing of forelimb function, amphetamine-induced rotation, and sensorimotor integration behavior 1, 2, and 3 months after MCAO in the SHR. A rotation test was performed before surgery to determine the preoperative rotation side-bias. To monitor to what extent any lesion-induced deficits in the paw-reaching test were due merely to an inability to learn new motor skills, half of the rats were trained to a criterion level before being subjected to surgery. Lesion size was assessed morphometrically on histological sections from 12 coronal levels.

Materials and Methods

Sixty-three adult male inbred SHR of the subline BM/Mol (Mollegaard Breeding Centre, L.I. Skensved, Denmark), weighing 200 to 250 g at the start of the experiment, were housed in groups of five animals per cage on a normal 12-h/12-h light/dark cycle with free access to water. Food was restricted during the paw-reach test as detailed below.

The rats were anesthetized with ketamine (Parke-Davis, Barcelona, Spain) 50 to 100 mg/kg and xylazine...
(Bayer, Leverkusen, Germany) 5 to 10 mg/kg given intraperitoneally. The surgical procedure of occluding the middle cerebral artery has been described in detail elsewhere.\textsuperscript{5,10} The vessel was ligated using a 10-0 monofilament nylon suture (Deknatel, Hamburg, Germany). Control rats were sham operated by means of an incision of the skin and the underlying fascia of the temporal muscle, followed by suturing.

A set of 10 custom-made Plexiglas boxes modeled after Montoya et al.\textsuperscript{13} was used for measurement of forelimb function. This box contains a central elevated platform with a staircase on each side. The staircases have six steps, each baited with 45-mg chow pellets (Campden Instruments Ltd, Loughborough, England). The rat is placed on the platform and from this position may collect pellets by the mouth or tongue from the top two steps. The ipsilateral forepaw must be used to reach pellets from the lower steps. Pellets that were dropped or only scratched down from the staircase fell into a container under the box floor beyond reach of the animal. Each test period was preceded by a 48-hour period of food deprivation, resulting in about a 10% reduction of body weight. One test lasting 20 minutes was performed daily, after which the rats were transferred to their home cages and given approximately 20 g of standard food pellets to maintain body weight. Half of the rats were pretrained for 10 days before surgery, and all rats had a 10-day test period 1 month after surgery, repeated for 5 days after 2 and 3 months. On days 7 to 9 and 1 to 4 each staircase was baited with 8 pellets, making a total of 48 pellets on each side. The number of pellets consumed and the number removed from the staircase (consumed plus dropped in the container) were counted, and mean values for days 6 to 9 and 1 to 4 were used for group comparisons. Additionally, the percentage of unsuccessful reaching attempts (removed - eaten / removed \times 100) was computed. This is an approximate measure, because with the present design it was not possible to separately count removed pellets that were subsequently

**FIG 1.** Photomicrograph of coronal section showing cystic infarct affecting neocortex and caudate putamen 3 months after occlusion of middle cerebral artery in spontaneously hypertensive rat. This specimen exhibited a larger lesion (the ipsilateral hemisphere surface area was 65% of that of the contralateral hemisphere) than the mean group value. Cresyl violet stain; bar, 1 mm. **FIG 2.** Line graphs of paw-reach test, showing number of consumed pellets ipsilateral and contralateral to sham operation or middle cerebral artery occlusion (MCAO). Two-way analysis of variance showed significant group differences on both sides (contralateral: $F_{3,38}=16.02$, $P=.0001$; ipsilateral: $F_{3,38}=4.65$, $P=.006$). $^*P<.05$, $^*^*P<.01$, $^*^*^*P<.001$, $^*^*^*^*P<.0001$, differences between MCAO group and its respective control (Scheffé’s test). Values are means of performance days 6 to 9 (in prelesion and 1-month test) and days 1 to 4 (in 2- and 3-month test). Error bar is 1 SEM.
dropped on the staircase’s steps. On the last day of a test period (day 10 or 5), only the staircase contralateral to the lesion was baited. This was performed to reveal residual capacity in the contralateral paw, which we anticipated to display a paw-reach deficit.

The rotation test was performed in rotometer bowls. Rotation was induced by D-amphetamine (1 mg/kg), which was given intraperitoneally immediately before the test. The direction and number of all 180° rotations were registered during 90 minutes. All rats were tested before surgery, and the test was repeated 1, 2, and 3 months thereafter.

For the sensorimotor integration test5,21 the animals were placed on an elevated 15-cm-wide circular platform. The fur was then touched with a ballpoint pen at seven regions on each side of the body. These regions included the proximal hindleg, hindpaw, lateral chest, proximal foreleg, forepaw, lateral head, and nose. For each region the extent of orientation was rated according to the following scale: 0, no head orientation toward touch; 1, slight head turning toward touch; 2, turning of head approximately halfway in the direction of touch; 3, scratching by the hindpaw or precise localization of the head to body region contacted; and 4, precise localization of the head and biting of pen. Touching the paws could also result in a slow or fast removal of the limb, which was rated as a score of 1 or 2, respectively. The regional scores were added separately for each side. It was noticed that the more alert the rat the higher the scores were registered on both sides. Since alertness of individual rats changed during the experimental period, large variations of side scores occurred. This variation was decreased by transforming the data into the difference of ipsilateral and contralateral scores. The test was performed 1, 2, and 3 months after surgery.
After completion of behavioral testing, lesioned animals were anesthetized with methohexital (Eli Lilly and Co, Indianapolis, Ind) intraperitoneally and perfused via the ascending aorta with 0.9% saline followed by the fixative containing 4% formaldehyde in a 0.1 mol/L phosphate buffer. The brains were postfixed overnight and then stored in 20% sucrose in 0.1 mol/L phosphate buffer until sectioning. The brains were cut in 40-μm-thick sections on a freezing sliding microtome. Every 20th section was retrieved, stained with cresyl violet, and coverslipped.

The morphometrical analysis was performed using a computerized image processing program (OPTILAB, Graftek, Meudon-La-Forêt, France). Area measurements of each hemisphere were carried out at 12 coronal levels (between anterior coordinates +3.7 mm and −6.0 mm in relation to bregma). The neostriatum was measured separately on the same sections, and the area of the thalamus was calculated at one level (2.8 mm caudal to bregma according to Paxinos and Watson2). The sectional areas were added for each hemisphere, and lesion size was expressed as the percentage of operated side divided by nonoperated side sectional area.

**Results**

One rat did not have an infarction after MCAO, but only a small cortical lesion at the ligature site. The other brains exhibited cystic infarcts affecting neocortex and the caudate putamen (Fig 1). The ischemic damage resulted in a reduction of cross-sectional area of the operated hemisphere to 78±2% (mean±1 SEM) of the value of the nonoperated hemisphere. This is a smaller lesion than previously reported,57 in which we used a different subline of SHR (SHR/Mol). The caudate putamen was more severely affected, showing a reduction of surface area to 53±5%. Corresponding value for the thalamus was 76±3% because of an atrophy most prominently affecting the ventrolateral and ventroposterior nuclei. Significant correlations were found between lesion size of total hemisphere and caudate putamen (r=.75, P=.0001) and between total lesion size and thalamus (r=.69, P=.0001). The subgroups of lesioned rats in the paw-reach test had comparable infarct sizes (pretrained, 77±3%; not pretrained, 78±2%; unpaired t=0.35, not significant).

In the following presentation of the behavioral tests, the rat without histologically verified infarct was excluded from all results except for the correlational analysis between lesion size and test outcome.

Fig 2 shows the mean number of pellets consumed on the contralateral and ipsilateral side for all groups in the paw-reach test. The main findings were seen on the contralateral side, where an analysis of variance (ANOVA) from months 1 to 3 yielded a significant difference between groups (P=.0001). Both lesion groups, irrespective of pretraining, displayed poor performances at all test sessions, and intergroup comparisons revealed highly significant differences from the respective control group (P<.01). Ipsilateral to the lesion, the infarct groups also displayed a deficit, although less pronounced than on the contralateral side. The statistical analysis showed a significant difference between groups (P=.006). Post hoc Scheffé's test showed differences (P<.05) between MCAO groups and controls 2 and 3 months after the lesion.

Groups that were not pretrained in the paw-reach task were first presented to the test 1 month after surgery. Fig 3 depicts the results of 9 consecutive test days in this session. Groups that were pretrained versus groups that were not pretrained were compared to evaluate any pretraining effect. Contralateral to the lesion, a significant difference between control groups (P=.0002) was seen. The controls that were not pretrained successively acquired the task, and by day 8 the performance was not significantly different from that of the pretrained controls. The corresponding analysis for the MCAO groups only revealed a pretraining effect up

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**TABLE 1. Net Rotation (Number of Rotations in Dominant Minus Nondominant Direction)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Prelesion</th>
<th>1 Month</th>
<th>2 Months</th>
<th>3 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>52±9</td>
<td>109±17</td>
<td>198±43</td>
<td>249±56</td>
</tr>
<tr>
<td>MCAO</td>
<td>46±6</td>
<td>246±43</td>
<td>383±57</td>
<td>477±77</td>
</tr>
</tbody>
</table>

Values are mean±1 SEM. MCAO, middle cerebral artery occlusion. Analysis of variance showed a significant group (F(3,51)=5.5, P<.05) and time (F(3,153)=30.6, P<.0001) effect as well as a significant group×time interaction (F(3,153)=3.0, P<.05).

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**Fig 5.** Line graphs of amphetamine-induced rotation before and 1, 2, and 3 months after sham operation or middle cerebral artery occlusion (MCAO). Ipsilaterally, two-way analysis of variance showed a significant group effect (F(1,51)=13.3, P=.0006). The MCAO group successively increased ipsilateral rotation, which was shown by a significant time effect (F(3,153)=20.3, P=.0001) and the group×time interaction (F(3,153)=6.9, P=.0002). *P<.05, **P<.01, ***P<.001 compared with control group (post hoc Scheffé's test). Values are means; error bar is 1 SEM.
to day 2. Ipsilaterally, the curves had a similar course for both groups that were not pretrained. The MCAO group learned the task slightly more slowly, which is indicated by a shift of the curve to the right and the fact that significant differences from the pretrained MCAO group were evident up to day 8 compared with day 5 in the comparison between the control groups.

Fig 4 depicts the paw-reaching error. On the contralateral side, a significant difference for groups was found ($P = .0002$), and individual comparisons showed that the reaching error for the MCAO group with no pretraining was significantly larger than the error for the control group and the pretrained MCAO group (both $P < .05$) 1 month after surgery. No significant differences of paw-reaching error were seen between the groups 2 and 3 months after the lesion. In contrast, the MCAO groups displayed significantly higher error rates with the ipsilateral paw compared with controls at 2 and 3 months after surgery.

To evaluate whether there was a difference in performance when the staircase was baited only contralaterally instead of bilaterally, the number of pellets consumed contralaterally was compared on days 9 and 10 and on days 4 and 5. For this purpose a two-way ANOVA with factors of group and day was performed. No significant difference was noticed for the factor of day in any of the test sessions, indicating that bilateral or contralateral baiting did not influence execution of the test.

Amphetamine induced a large increase of rotations to the lesion (ipsilateral) side in the MCAO group (Fig 5), with a significant difference between groups ($P = .0006$). The repeated-measures ANOVAs for time ($P = .0001$) and group by time interaction ($P = .0002$) were significant, showing a successive increase of ipsilateral rotations for the MCAO group. The control group increased the number of rotations on both sides because of an increase of net rotation (difference between predominant and nondominant direction of rotation), which, however, was of a lower magnitude than that of the MCAO group (Table 1).

The MCAO group displayed a reduced sensorimotor function contralateral to the lesion, which partly improved with time (Fig 6). However, 3 months after surgery the MCAO group still differed significantly from the control group ($P = .0006$, Scheffé’s post hoc test).

Table 2 presents the correlation of the three behavioral tests at the different time points to the extent of the total lesion as assessed histologically. The paw-reach test exhibited overall significantly high correlation coefficients, and the result 3 months after surgery is shown in Fig 7. The outcome of the sensorimotor integration test significantly correlated to lesion size 1 and 2 months after the insult, whereas the correlation analysis never reached statistical significance for circling behavior. The rotational data were manipulated in several ways (such as forming differences and quotients between preoperative and postoperative results) without receiving any different results. The outcome of the behavioral tests also correlated to lesion extent of the caudate putamen and the thalamus. None of these computations revealed higher correlation coefficients than those presented in Table 2.

**Discussion**

The paw-reach test revealed large deficits contralateral to the infarct side when the number of pellets

![Graph](image-url)
consumed was measured (Fig 2), with no improvement during the experimental period. Except for the initial test days 1 month after surgery (Fig 3), the performance of the pretrained MCAO group did not differ significantly from the MCAO group that was not pretrained, and both groups differed from the controls throughout the study. Impaired forelimb use has previously been demonstrated after unilateral[15-17] or bilateral[22] lesions of the motor cortex, the nigrostriatal dopamine system,[16,18] and ibotenate-induced damage of the striatum.[12,16,24] With regard to the striatum, particularly the lateral part has been implicated in reaching impairments,[15,24] a region involved in the damage after a proximal MCAO.

On the ipsilateral side, both MCAO groups also exhibited paw-reaching deficits, although they were less pronounced than contralaterally (Fig 2). Unilateral lesions inducing bilateral paw-reaching deficits have previously been reported after cortical[13,15,17] and nigrostriatal[12,16] damage, proposing that both uncrossed and crossed descending pathways contribute to forelimb use. In addition, electrophysiological observations indicate involvement of the ipsilateral cortex in movement control.[25] In contrast to our results, in which a clear side difference was seen in the number of pellets consumed, Montoya et al[13] reported a very prominent ipsilateral deficit such that no significant difference between sides was detected after various cortical lesions. In our study a maximum of 48 pellets per side could be retrieved during a period of 20 minutes, whereas only 12 pellets per side were available during 15 minutes in the study by Montoya et al.[13] The apparent lack of side asymmetry in their work could possibly be explained by a relative "shortage" of pellets on the ipsilateral side.

Two and 3 months after surgery, the percentage of reaching error (Fig 4) did not differ between groups on the contralateral side, in contrast to the large differences in the amount of pellets eaten on that side (Fig 2), implying that the accuracy of reaching improved with time, whereas speed or motivation did not. Delayed movement initiation after lateral ibotenate-induced lesions of the striatum has been demonstrated in a different paw-reach test,[24] an observation that is in agreement with the present findings.

Amphetamine-induced ipsilateral circling after MCAO (Fig 5) has previously been demonstrated[5,7] and is most probably caused by damage of the striatum.[26,27] A rotation side-bias was also revealed in the control group, since net rotation (Table 1) increased between the first and second test, indicating an exaggeration of the inherent lateralized circling behavior known to exist both spontaneously and after drug treatment in rats.[26] A continuous rise in net rotation was subsequently observed for both test groups, and the previous literature provides evidence for rotational behavior to be progressively enhanced by both amphetamine[28] and classic conditioning.[29,30] In the present study (Table 2) as well as in previous studies,[5,7] neither total lesion size nor extent of striatal damage correlated to postoperative circling behavior. This time we allowed for each animal's preoperative magnitude and direction of rotation in the calculation of lesion-induced circling. This measure also did not significantly correlate to the extent of ischemic damage.

The sensorimotor integration test 1 month after surgery (Fig 6) showed a smaller side-bias score than previously reported at approximately the same time point.[7] This is consistent with the smaller lesions in the present study showing a reduction of the surface area of the infarcted hemisphere to 78% of that of the intact side compared with 62%7 and 67%5 in previous reports. At the subsequent test points, score asymmetry was reduced, indicating a partial recovery of function during the experimental period. Others have shown improvement of sensorimotor function after MCAO[6] and electrolytic cortical lesions,[31] but in those studies (using another sensorimotor test) lesioned rats reached control level 20 days after the insult, whereas our groups still were significantly different from each other after 3 months. Similar to our previous study,[7] test scores were significantly correlated to lesion size 1 month after surgery (Table 2). The correlation was weakened later in the study and became nonsignificant at 3 months. This suggests that plasticity of the nervous system, as understood from decreasing sensorimotor asymmetry, reduces the impact of lesion size in course of time.

The stable deficit in consuming pellets contralateral to the infarcted hemisphere in the paw-reach test contrasted with the recovery of sensorimotor function. The sensorimotor test measures the attentional capacity of sensory stimuli by a rather crude motor score, whereas the former test requires a very specific and skilled function of the forepaw. It may be pertinent to allude to the clinical course in human stroke, where the degree of recovery of movement at the larger joints is larger than that of the hand.

In summary, amphetamine induced significant differences in rotational behavior between controls and the MCAO group, but no correlation between lesion size and degree of circling was noted. This finding, together with increasing net rotation in both experimental groups caused by other factors (presumably drug sensitization and conditioning), restricts the application of the test in the MCAO model. The sensorimotor test correlated to lesion size early in the experiment, suggesting that it may be particularly useful for behavioral evaluation in studies lasting for shorter time periods. The paw-reach test, using absolute values of consumed pellets, provides a stable behavioral parameter that is highly correlated to lesion size. It is not necessary to train the task before the lesion is induced. The paw-reach test may be used for therapeutic trials with the MCAO model that extend for a period of several months.

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References


Editorial Comment

An increasing number of animal studies in stroke are performed in rodents. It is likely that this trend will accelerate in the foreseeable future. There are well-defined objective criteria for evaluating the consequences of cerebral ischemia in this species. These include the measurement of infarct volume, quantitative evaluation of brain edema, and measurements of cerebral blood flow. There is a dearth of useful functional studies to evaluate neurological function. Such functional studies must be stable and reproducible, and useful in evaluating the neurological status of the animals after middle cerebral artery occlusion over a period of several weeks or months. Furthermore, the results of this test are highly correlated with the size of the lesion. The test is simple to perform and does not require extensive training before the lesion is induced. It is expected that this test will complement the array of techniques available for evaluating brain function after cerebral ischemia in this species.

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