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Sensorimotor Behavioral Effects of Endothelin-1 Induced Small Cortical Infarcts in C57BL/6 Mice

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Abstract

Mouse models have not paralleled rat models of stroke in advances in sensitive, species appropriate measures of neurological and behavioral recovery. Most available tests of mouse sensorimotor function are adaptations of those originally developed in rats and may not be as sensitive in detecting behavioral deficits after small cortical lesions in mice. Our purpose was to test the use of a vasoconstricting peptide, endothelin-1 (ET-1), to produce focal infarcts of the mouse sensorimotor cortex and to establish a behavioral test battery sensitive to resulting sensorimotor deficits. Young adult (3–5 month old) male C57BL/6 mice received intracortical infusions of ET-1 that produced unilateral lesions of the forelimb region of the sensorimotor cortex, intracortical infusions of sterile saline, or sham surgeries. Pre-operatively and at various time points over 3 weeks post-surgery, they were administered a test battery that included measures of sensorimotor asymmetry (Corner and Bilateral Tactile Stimulation Tests), coordinated forepaw use (Cylinder and Ladder Rung Tests), and dexterous forepaw function (Pasta Matrix Reaching Test). ET-1 infusions resulted in consistently placed, focal cortical infarcts and forelimb impairments as measured with the Ladder Rung, Bilateral Tactile Stimulation, and Pasta Matrix Reaching Tests. On the Bilateral Tactile Stimulation and Pasta Matrix Reaching Tests, impairments persisted throughout the time span of observation (26 days). These results support ET-1 as a viable option for creating small, reproducible lesions of anatomical subregions in the mouse neocortex that result in lasting functional impairments in the forelimb, as observed with sufficiently sensitive measures.

Keywords

motor skills; reaching; somatosensory asymmetry; upper extremity impairment; stroke; brain injury; murine model

1. Introduction

Due to the increased availability of transgenic lines of mice, and the applicability of many of these mouse models to stroke research, there has been a surge in interest in developing sensitive behavioral assays of recovery of function in mouse models of stroke (Branchi &

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Ricceri, 2002; Bu an & Abel, 2002; Zhang et al., 2002; Li et al., 2004; Farr et al., 2006; Bouët et al., 2007). However, many mouse studies still use lesion size as their sole outcome measure. Although lesion size often correlates with functional deficits (e.g. Grabowski et al., 1993; Peeling et al., 2001; Hsu & Jones, 2006), it is not a sufficient indicator of recovery. For example, Binkofski et al. (2001) found in human stroke survivors that the amount of spared motor function was a better predictor of eventual recovery than lesion size. It is therefore essential for their clinical applicability that animal models of stroke include functional assessments, as noted by many previous investigators (e.g. Zivin & Grotta, 1990; Hunter et al., 1995; Corbett & Nurse, 1998; Jones et al., 1999; Cenci et al., 2002; Kleim et al., 2007). Most of the behavioral measures currently used with mice are adapted from those used with rats and their effectiveness in detecting mouse deficits has not been well determined. Thus, there is a need to more firmly establish sensitive behavioral test batteries for mouse models of stroke, including tests that detect lasting functional deficits.

There is also a need to develop different focal infarct models in mice. Most mouse stroke studies have induced lesions by occlusion of the middle cerebral artery (MCAo), which may not model many aspects of smaller, more survivable, strokes (Carmichael, 2005) and, for many basic research questions, are less desirable than lesions in well defined anatomical subregions (e.g., Nudo & Milliken, 1996; Nudo et al., 1996; Conner et al., 2003; Kleim et al., 2003). In rats, endothelin-1 (ET-1), a powerful vasoconstricting peptide, can be applied to the cortical surface (Fuxe et al., 1997; Adkins et al., 2004) or injected intracortically (Fuxe et al., 1992; Hughes et al., 2003; Gilmour et al., 2004) to produce small lesions that are generally restricted to desired regions of the cortex (Windle et al., 2006). The period of vasoconstriction can last up to 16 hours and is followed by a gradual reperfusion over the next 48 hours (Biernaskie et al., 2001) that resembles the reperfusion time-course that occurs after some human strokes (Domingo et al., 2000). ET-1 induced lesions of the forelimb area of the sensorimotor cortex have been shown to produce reaching deficits in rats similar to those produced by aspiration and excitotoxic lesions (Bury & Jones, 2002; Voorhies & Jones, 2002; Gilmour et al., 2004; Luke et al., 2004; O'Bryant et al., 2007) and behavioral deficits and neuroplastic changes in the contralesional hemisphere that resemble those produced by similarly placed electrolytic lesions (Adkins et al., 2004; Jones & Schallert, 1992).

ET-1 has also been used as an alternative to MCAo and photothrombosis to produce cerebral ischemic lesions in the mouse brain. Wang et al. (2007) found that intracortical infusions of ET-1 into C57BL/6 mouse brains caused a 70–80% reduction in blood flow, followed by the formation of a small lesion that was restricted to cortex. Mice showed Rotorod and neurological score deficits when tested at 1 hour, but not 3 days, post-ischemia. Horie et al. (2008) found that neither cortical nor striatal infusions of ET-1 produced significant lesions in C57BL/6 mice unless combined with occlusion of the common carotid artery (CCAO) and co-administration of the nitric oxide synthase (NOS)-inhibitor, *N*(*G*)-nitro-L-arginine methyl ester (L-NAME). The mice given striatal infusions of ET-1 and L-NAME did show deficits in the Schallert Cylinder Test at 2 days post-infarct, and the addition of CCAO caused an even greater deficit. These somewhat ambiguous results suggest that there is a need for further characterization of the behavioral deficits that result when using the ET-1 model in mice.

The current study investigated sensorimotor impairments caused by intracortical infusions of ET-1 into the forelimb area of the mouse sensorimotor cortex. A battery of sensorimotor tests was used in an attempt to characterize the nature of resulting deficits over time after the lesion. This included measures of sensorimotor asymmetry, using the Corner (Zhang et al., 2002; Li et al., 2004; Bouët et al., 2007) and Bilateral Tactile Stimulation Tests (Schallert et al., 1982; Schallert & Whishaw, 1984; Starkey et al., 2005; Wells et al., 2005; Bouët et al., 2007). The Cylinder (Schallert et al., 2000; Baskin et al., 2003; Fleming et al., 2004; Li et al., 2004; Starkey et al., 2005; Wells et al., 2005) and Ladder Rung Tests (Metz & Whishaw, 2002; Riek-Burchardt et al., 2004; Farr et al., 2006) were used as measures of coordinated forepaw use. Finally, the Pasta Matrix Reaching Test, originally developed for rats (Ballermann et al., 2001; see also Teskey et al., 2003; Chiken & Tokuno, 2005), was newly adapted for mice in the current study as a measure of dexterous forepaw function.

2. Materials and Methods

2.1. Subjects

A total of 19 well-handled 3–5 month old male C57BL/6 mice were housed in groups of three to four with standardized housing supplementation (a small piece of PVC pipe, a cardboard roll, and small wooden objects) on a 12:12 light/dark cycle. Animals were maintained on a restricted feeding schedule (3 g/day) to prevent satiation and motivate reaching performance. Ten mice received intracortical infusions of ET-1 (Fig. 1A), three mice received intracortical infusions of 0.9% sterile saline, and six mice received sham procedures. One of the 10 mice given ET-1 lesions died during recovery from perioperative anesthesia and another failed to show evidence of a lesion in behavioral and histological analyses and was omitted from the study. An additional 13 animals used in a different study were used for limb length analysis. The animals were matched for age, sex, and strain. Reaching data from a subset of these animals (n=8) was used to determine the average number of days until asymptote and final success level on the Pasta Matrix Reaching Task. Animal use was in accordance with a protocol approved by the University of Texas at Austin Animal Care and Use Committee.

2.2. Intracortical infusion of ET-1

Following preoperative testing on the sensorimotor battery, mice were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). When fully anesthetized, as verified by tail/foot pinch and corneal response, the scalp was shaved and cleaned with providone-iodine. Each mouse was then placed into a mouse stereotaxic frame (Stoelting, Wood Dale, IL), lidocaine (2 mg/kg, s.c.) was injected into the scalp, and a midline incision was made. A small burr hole was drilled through the skull over the center of the forelimb region of the sensorimotor cortex at coordinates of 2.25 mm lateral to midline and + 0.6 mm anterior to Bregma. The dura was punctured and a Hamilton syringe with a 26 gauge needle was lowered into the cortex to a depth of 700 μ m. Four μ l of ET-1 (American Peptide; 320 pmol, 0.2 μ g/ μ l in sterile saline) was injected into the cortex over the course of 10 min, and the syringe was left in place for 5 min following infusion to prevent backflow. In rats, topical application of ET-1 results in little horizontal spread beyond the craniectomy borders (Adkins et al., 2004). The burr hole was then filled with gelfoam and covered with UV

curing dental cement (wave A2; Southern Dental Industries, Victoria, Australia), and the wound was sutured and covered in antibiotic ointment. The animal was allowed to fully awaken in a heated chamber before returning to its home cage. Of the two sham groups, one group received all surgical procedures up to the skull opening and the other received a skull opening and infusion of vehicle (0.9% sterile saline) into the forelimb area of the sensorimotor cortex.

2.3. Behavioral Methods

In order to acclimate mice to the behavioral testing procedures, they were exposed to the behavioral apparatus at least once during the 2 weeks prior to the initiation of training. For the Pasta Matrix Test, mice first received pieces of capellini pasta in their home cages over several days to reduce neophobic responses. Mice were then trained daily on the Pasta Matrix Test for 15 days before surgery. They were tested on the remainder of the sensorimotor battery once per week for two weeks prior to surgery, and on days 2, 4, 10, and 20 post-surgery. Daily post-operative training on the Pasta Matrix Test was initiated 4 days after surgery.

2.3.1. Pasta Matrix Reaching Test—This task involves training mice to reach for and break small pieces (3.2 cm in height and 1 mm diameter) of vertically oriented, uncooked capellini pasta (DeCecco brand, Fratelli De Cecco di Filippo Fara San Martino S.p.A., Italy), arranged in a matrix distal and lateral to the reaching chamber aperture (Fig. 2A). The animal must change its reach trajectory in order to obtain pasta pieces further from the reaching aperture. The methods used were adapted from rat versions developed by Ballermann et al. (2001) and used by Teskey et al. (2003) and Chiken & Tokuno (2005). The chamber was composed of four Plexiglas walls (20 cm tall, 15 cm long, and 8.5 cm wide) with an open top and bottom. The matrix was positioned in front of the reaching aperture, a center slit (13 cm tall and 5 mm wide) cut into the front wall of the chamber. The matrix itself was composed of a heavy-duty plastic block (8.5 cm long, 5 cm wide, and 1.5 cm tall) with 1 mm diameter holes drilled completely through. There were a total of 260 holes, beginning 2 mm from the reaching window with 2 mm between each hole. We expected the matrix size to exceed the maximum reaching distance of the mice.

In order to successfully retrieve a pasta piece, the mouse must break the pasta by grasping and pulling forward. The matrix is designed so that the pasta piece extends through the entire depth of the matrix stage so that approximately half the piece is exposed. This prevents the mice from grasping and pulling the pieces out of the matrix, and forces them to break the pieces in order to retrieve them. The movement sequence of the rat performing the Pasta Matrix Reaching Task was described by Ballermann et al. (2001) as a six step sequence: aim, digits open, pronation, grasp, withdrawal, and eat. Mice display approximately the same sequence of movements with the exception that they typically show little pronation of the paw before grasping the pasta. While rats grasp the pasta from the top, around the tip, mice grasp the pasta from the side, around the middle. See supplemental Movie 1 for examples of mice performing the Pasta Matrix Reaching Task.

Mice were trained on the Pasta Matrix Reaching Test for 15 days before surgery to establish the skill and then were tested on it daily beginning 4 days after surgery. For pre-operative training, mice first underwent 3–5 days of shaping in order to become accustomed to the reaching task. During this time, the matrix stage was completely filled with pasta, allowing mice to reach for pasta with both limbs. Training began when mice reached at least 10 times in 15 min, and at least 70% of the time with one limb (termed the “preferred” limb). Two mice did not learn how to reach and were excluded from testing on the Pasta Matrix Reaching Task but were included in the remainder of the sensorimotor battery. One of the two mice would not eat pasta (before lesion induction). Of the mice that learned how to reach, 9 had a left limb preference and 7 had a right limb preference. Lesion and sham-operation procedures were contralateral to the preferred limb. Mice were trained to reach with the preferred limb by filling with pasta only the half of the matrix contralateral to this limb. Daily training sessions consisted of up to 100 reaches or 15 min, whichever occurred first. The average number of reach attempts per session was 65.68 ± 1.38 and was not different between Lesion and Sham groups post-operatively. In order to encourage reaching and focus the mice, the experimenter held a piece of pasta perpendicularly oriented against the back of the most easily accessed piece of pasta in the matrix (see supplemental Movie 1). The number of pasta pieces successfully broken was recorded, as was the area of the matrix that the mouse cleared of pasta. Data were pooled across three day blocks in order to simplify presentation.

2.3.2. Schallert Cylinder Test—This test measures the use of the forepaws for postural support behavior during exploratory movements. Mice were placed into a Plexiglas cylinder (12.7 cm in diameter, 25.4 cm tall) and allowed to vertically explore the cylinder walls while being videotaped with a digital camcorder (Canon XL1S). This test has been used previously in mice (Baskin et al., 2003; Fleming et al., 2004; Li et al., 2004; Starkey et al., 2005; Wells et al., 2005) and was adapted from the rat version of the test (Schallert et al., 2000). Mice were removed from the cylinder after completing 10 rears (approximately 5 min). Rears were counted to ensure that a sufficient number of behavioral observations could be obtained from video playback (because, on average, each rear is followed by numerous instances of postural support with the paws against the cylinder wall). On average 39.4 ± 0.8 observations of forepaw use were recorded per test session using this method. Videotapes were analyzed in slow motion, and the number of times each paw was used to contact the cylinder wall and push off from or land on the floor were recorded for up to 30 total contacts. An asymmetry score was calculated for each movement type using the following formula (using contacts as an example): $(\# \text{ of ipsilesional contacts} + 1/2 \text{ bilateral contacts}) / (\# \text{ of ipsilesional} + \text{contralesional} + \text{bilateral contacts}) \times 100$.

2.3.3. Corner Test—This test is sensitive to responsiveness to somatosensory stimulation in mice (Zhang et al., 2002; Li et al., 2004; Bouët et al., 2007). Mice were allowed to walk into a corner formed by two Plexiglas boards, each 20 cm x 30 cm and fused into a 30 degree angle. As the mouse neared the corner, the two boards delivered bilateral vibrissae stimulation, and the mouse would rear up and turn out of the corner. The direction of rearing and turning was recorded for 10 trials. For a trial to be considered complete, the turn must have been preceded by a rear (Zhang et al., 2002). Trials in which

the mouse turned without rearing were excluded from analysis. The percentage of ipsilesional turns was calculated as: (ipsilesional turns / 10) x 100.

2.3.4. Ladder Rung Test—Mice were videotaped while walking across a horizontal ladder (Fig. 4A). This test was originally developed for use with rats (Metz & Whishaw, 2002; Riek-Burchardt et al., 2004) and was later adapted for use with mice (Farr et al., 2006). The ladder rung apparatus was composed of an elevated horizontal ladder (80 cm long and 12 cm in elevation) with the home cage at the far end of the ladder. The rungs (121 in total) were 1 mm in diameter and evenly spaced 5 mm apart, and the ladder had Plexiglas sides (15 cm tall) to prevent the mouse from turning around or jumping off. The mouse was placed on the end of the ladder away from the home cage and videotaped while walking across the ladder rungs towards the home cage. Videotapes were scored using frame-by-frame analysis (Final Cut software) for step length (measured as the number of rungs crossed over) and qualitative score. Each step onto a ladder rung was qualitatively scored on a 3 point scale, abbreviated from that used by Farr et al. (2006): 1 = paw slips between or off a rung (Fig. 4B); 2 = paw is placed on a rung and readjusted on the same rung or placed onto a different rung; 3 = paw is well-placed onto the rung (Fig. 4A). The percent errors (slips), adjustments, and correct placements per step were calculated, along with average step length and average qualitative score.

2.3.5. Bilateral Tactile Stimulation Test—This test was adapted from those previously used with mice (Starkey et al., 2005; Wells et al., 2005; Bouët et al., 2007) and rats (Schallert et al., 1982; Schallert & Whishaw, 1984). Each mouse was placed into a shallow transparent plastic container (8.5 cm tall, 18 cm in diameter) with an open top and allowed to habituate for 1 min. The mouse was picked up and lightly restrained by the scruff while a 1.27 cm long piece of 3 mm wide tape (crepe art tape, Office Depot, Delray Beach, FL) was placed onto the ventral side of each paw (Fig. 5A). The mouse was then placed back into the container and allowed to remove each piece of tape using its teeth. The latency to contact and remove each piece of tape was recorded for 5 trials, allowing 30 seconds of rest between each trial. The percentage of trials in which the ipsilesional stimulus was contacted or removed first was calculated using the following formula: $([\# \text{ of trials on which the ipsilesional stimulus is the first contacted} + \# \text{ of trials on which the ipsilesional stimulus is the first removed}] / 10) \times 100$. Average removal time for each stimulus was calculated using the following formula: latency to remove – latency to contact, averaged across five trials (Starkey et al., 2005).

2.4. Histological Euthanasia and Tissue Processing

Mice were euthanized with an overdose of sodium pentobarbital (175 mg/kg, i.p.) and perfused intracardially with 0.1 M phosphate buffer (PB) and 4 % paraformaldehyde. After perfusion, the length of the forearms (digit tip to elbow) was measured in a subset of mice (n=13) to relate to reaching distances in the Pasta Matrix Reaching test. Brains were stored in 4 % paraformaldehyde and sliced into 50 µm thick sections using a vibratome. Every sixth section was mounted onto gelatin-coated slides and Nissl stained with toluidine blue.

2.5. Analysis of Remaining Cortical Volume

Neurolucida software was used to estimate the volume of remaining cortex. Coronal sections were viewed at a magnification of X51. The cortical areas of 6 coronal sections from approximately 2.0 mm anterior to 0.5 mm posterior to Bregma, each 300 μ m apart, were measured by tracing their cortical boundaries. The sensorimotor cortex fell within the area of tissue measured, and no lesions extended outside of this area. Cavalieri's method was used to calculate total remaining cortical volume by multiplying the sum of the section areas by the distance between sections (Henery & Mayhew, 1989; Mayhew, 1992). Lesion volume was indirectly calculated by subtracting the volume of the damaged hemisphere from the volume of the intact hemisphere.

2.6. Statistical Analyses

SPSS software was used to conduct repeated-measures analyses of variance (ANOVAs) for all behavioral measures, with day as a within-subjects variable and group as a between-subjects variable. A t-test was conducted to compare the lesion extents of the Sham and ET-1 groups. The two Sham groups did not significantly differ from one another and were combined for all statistical analyses, except for the Corner Test analyses (see results). Following histological analysis, one animal in the ET-1 group was excluded from all analyses due to lack of an evident lesion. This animal also failed to show any behavioral deficits. An α level of 0.05 was considered significant for all analyses.

3. Results

3.1. Analysis of Lesion Extent

ET-1 induced lesions ($n=9$) resulted in damage to the sensorimotor cortex in 8 out of 9 mice. ET-1, which was injected 700 μ m below the cortical surface, caused a pattern of damage that extended approximately 1 mm from anterior to posterior, and approximately 1 mm from medial to lateral surrounding the infusion site (Fig. 1A). None of the lesions produced damage to the underlying white matter or striatum. There was some damage to layer I of the cortex within the perilesion area (~ 250 μ m in distance around the perimeter of the lesion), but there is no visible evidence of damage to the superficial layers of the cortex beyond this area, nor is there evidence of damage in the contralateral hemisphere. There was no difference in the volumes of the intact cortex of Lesion and Sham animals (14.8 ± 0.5 and 14.7 ± 0.6 mm, respectively). Sham (saline) infusion procedures ($n=3$) resulted in minor damage associated with the cannula tract that was evident in coronal sections. As shown in Figure 1B, ET-1 ($n=8$) significantly increased the measured volume difference between the intact hemisphere and the ischemic hemisphere, as compared to sham operates ($n=9$) ($t(15) = -3.03$, $p=0.004$). There was no significant difference in interhemispheric volume between the Sham subgroups that received saline infusions versus no infusions.

3.2. Pasta Matrix Reaching Test

Mice became proficient on the Pasta Matrix Reaching Test during 15 days of pre-lesion training (Fig. 2B). Mice with ET-1 induced lesions ($n=7$) showed a deficit in skilled reaching on the Pasta Matrix Reaching Test as compared to sham operates ($n=8$) (Fig. 2C). There was

a significant effect of day ($F(21,252)=2.81$, $p=0.00007$) and a significant day x group interaction ($F(21, 252)=1.96$, $p=0.008$). There was no significant main effect of group (Lesion versus Sham, $F(1,12)=2.02$, $p=0.18$). Post-hoc analyses indicated that the number of pasta pieces broken by the Lesion group was significantly different from the number broken by the Sham group during the first two weeks of post-operative training but was not significantly different from Sham levels in the final week of training. The Lesion group's post-operative performance was also significantly different from pre-operative performance during the first half of post-operative training and returned to pre-operative performance levels during the second half of training. Thus, after 21 days of training, mice with ET-1 induced lesions seemed to show some behavioral recovery of reaching skill.

Not only did lesions cause a decrease in the number of pasta pieces broken, but the pattern of breakage was altered after lesion induction (Fig 3A). Prior to lesion induction, the majority of mice were able to reach pasta pieces located far anterior and far lateral to the reaching aperture. Mice were able to reach pieces as far as 1.2 cm (6 pieces) away from the reaching aperture. This corresponded well to the average length of the mouse forelimb that can be extended beyond the reaching aperture (1.96 ± 0.03 cm from elbow to digit tips). Early after lesion induction, the extent to which mice were able to successfully retrieve pasta pieces from the anterior and, especially, the lateral area of the matrix was limited. With time, most of the mice broadened the area of the matrix cleared. Examples of the areas cleared by a single mouse over the course of pre-lesion training and post-lesion testing are presented in Fig. 3B.

Performance of the Pasta Matrix Reaching Task can also be measured by calculating the percentage of breaks per total reach attempts (percent success). The pattern of behavioral results following lesions was the same when calculated as % success because the average number of attempts on each day is not altered post-operatively. The behavioral deficit in the first two weeks after the ET-1 injection is not due to a generally reduced use of the affected limb because there is no decrease in the number of reach attempts made by the affected limb after lesion induction. In the number of reach attempts made per session, there was no significant change in pre- versus post-operative time points and no significant difference between sham and lesion groups. Pre-operatively, sham operates made 69.2 ± 11.0 attempts and the lesion group made 75.7 ± 12.5 attempts. Post-operatively (averaged over time points) sham-operates made 62.9 ± 7.6 and lesion groups made 72.1 ± 9.5 attempts.

3.3. Ladder Rung Test

Prior to lesion induction, $3.60 \pm 0.91\%$ of total steps made by the Lesion group and $3.25 \pm 0.61\%$ of total steps made by the Sham group resulted in an error on the Ladder Rung Test. Two days after ET-1 induced lesions, mice showed an increase in the number of errors (Fig. 4B). However, this recovered quickly. When mice were tested at later time points, they no longer showed an increase in the number of errors made with the contralesional paw compared with the preoperative performance. There was a significant effect of day ($F(4,27)=5.87$, $p=0.002$) and a significant day x group interaction ($F(4,27)=4.95$, $p=0.004$), but no main effect of group. Post-hoc analyses indicated that on post-operative day 2 contralesional errors were significantly increased relative to Shams (Fig. 4C) and relative to

the pre-operative performance level ($p = 0.019$). Errors with the ipsilesional paw were not significantly increased after the lesion compared with the pre-operative time point or compared with Shams. On the other measures of performance on this test there were no significant differences between Sham and Lesion groups, or between pre-operative and post-operative performance. Averaged over groups and days, the step distance (measured in number of rungs crossed), percent correct placements and percent adjustments per step were 5.13 ± 0.06 , $82.62 \pm 0.59\%$, and $11.24 \pm 0.47\%$, respectively. The average qualitative score was 2.73 ± 0.01 .

3.4. Bilateral Tactile Stimulation Test

ET-1 induced lesions resulted in an asymmetry in responsiveness to tactile stimulation applied to the forelimb (Fig. 5B) as well as in a slight increase in time to remove the contralesional stimulus (Fig. 5C) on the Bilateral Tactile Stimulation Test. These changes are likely to reflect sensory and motor deficits, respectively, as discussed below.

The Sham group tended to respond equally to either stimulus, while the Lesion group consistently responded to the stimulus on the ipsilesional paw before the stimulus on the contralesional paw at all post-operative timepoints. There was a significant effect of group ($F(1,15)=5.27$, $p=0.037$), but no effect of day ($F(4,60)=1.76$, $p=0.15$) or day x group interaction ($F(4,60)=1.31$, $p=0.28$) in response asymmetry.

On days 2 and 4 post-lesion, the Lesion group took longer on average than Shams to remove the stimulus from the contralesional paw. However, when the Lesion and Sham groups were compared, the effects of group ($F(1,15)=4.39$, $p=0.053$), day ($F(4,60)=2.46$, $p=0.055$), and day x group interaction ($F(4,60)=1.69$, $p=0.16$) in the time to remove the contralateral stimulus all failed to reach significance in the removal time measure. Prior to lesion induction, there were no significant differences between the Lesion and Sham groups in responsiveness to the ipsilateral stimulus (% ipsi first responses = 41.88 ± 8.55 and 59.44 ± 7.63 , respectively) or time to remove the contralateral stimulus (7.80 ± 1.35 sec and 6.49 ± 1.95 sec, respectively).

3.5. Other Behavioral Measures

There were no significant lesion effects on either the Schallert Cylinder Test or the Corner Test (Table 1). The Lesion group showed no differences between pre- and post-operative performance (as measured on days 2, 4, 10, and 20 post-infarct with a one-way within-subjects analysis for time) in any of the Cylinder Test measures (contacts, push-offs, and landings, $F's(4,28)=0.48-0.62$, $p's=0.75-0.65$), or in performance on the Corner Test ($F(4,28)=0.59$, $p=0.67$). There were no significant Lesion versus Sham main effects or group x day interactions in the % use of the ipsilesional forepaw for any measure of the Schallert Cylinder Test (group by day: $F's(4,60)=0.23-1.56$, $p's=0.92-0.20$). Similarly, there was no significant group or group x day interaction in performance on the Corner Test (group by day: $F(4,60)=0.36$, $p=0.84$); however, this analysis was complicated by a post-operative difference between the two Sham groups. The Sham – Infusion group had a contralesional bias (turning ipsilesionally $26.7 \pm 3.3\%$ of the time compared with $58.3 \pm 7.0\%$ in the other Sham subgroup). The direction of the bias is opposite to that which would be predicted

based on any injury in the saline-infused hemisphere (Zhang et al., 2002) and may reflect small sample size effects. Overall, the pattern of results suggests that these two tests are insensitive to the effects of these lesions.

4. Discussion

In this study, we tested the suitability of ET-1 for producing focal infarcts in mouse cortex and also assessed the sensitivity of a mouse battery of behavioral tests for detecting sensorimotor impairments. Intracortical infusions of ET-1 into the forelimb area of the sensorimotor cortex caused small, focal lesions that were approximately 1 mm³ in volume, centered around the infusion site and restricted to the cortex, causing no damage to the underlying white matter or striatum. The damage resulted in both acute and relatively long-lasting behavioral deficits on a subset of the tests of sensorimotor function.

The results support that intracortical infusions of ET-1 are a viable option for creating focal lesions of the mouse cortex for studies of cerebral ischemia. In addition to the ability to create anatomically defined lesions, a benefit of ET-1 stroke models is the gradual reperfusion that occurs, which may represent the time-course of some types of human stroke better than other reperfusion injury models (Domingo et al., 2000). However, this lesion method is not without its limitations. The ET-1 model differs from most human strokes in that it involves vasoconstriction rather than occlusion of vasculature. ET-1 is known to have direct effects on neurons and glia (reviewed in Rubanyi & Polokoff, 1994). These effects cannot be said to account for the damage caused by ET-1 application because co-administration with vasodilators prevents the injury (Fuxe et al., 1992). However, they may influence later cellular responses to the injury. Finally, as used in this study, the lesion method requires a small craniotomy and disruption of the dura, which together can create behavioral deficits in the absence of a lesion (Adams et al., 1994), as well as damage due to the insertion of an infusion needle into the cortex. However, as measured in the present study, vehicle infusion did not result in sensorimotor impairments contralateral to the infusion or loss of cortical volume.

Although some behavioral testing has been done on mice following ET-1 lesions, we are the first to document behavioral deficits that persisted beyond 3 days post-infarct. The same concentration and volume of ET-1 that can create large lesions in the rat brain (Windle et al., 2006) creates smaller lesions in the mouse cortex (Wang et al., 2007; Horie et al., 2008). Wang et al. (2007) found that ET-1 created small lesions of the cortex that resulted in deficits in neurological and Rotorod scores at 1 hour post-infarct, but not at 3 days post-infarct. Horie et al. (2008) found that only when ET-1 infusions were combined with the administration of L-NAME could a lesion be detected. These animals showed a significant impairment on the Cylinder Test at 2 days post-lesion, and additionally occluding the CCA resulted in an even greater deficit. Our findings show that ET-1 alone produces significant loss of tissue that results in clear behavioral deficits when measured with sufficiently sensitive tests of forelimb function. The present results also suggest that Horie et al.'s (2008) failure to find more lasting behavioral deficits may be because the test that they used (Cylinder Test) is not very sensitive to these small cortical infarcts in mice.

Mice with cortical lesions in the current study had relatively long-lasting impairments on the Pasta Matrix Reaching Test, which measures skilled motor function, in particular dexterous function of the paws and digits. In rats and non-human primates, reaching tasks have served as important tools for studying neural mechanisms of motor skill learning and motor rehabilitation (Nudo & Milliken, 1996; Nudo et al., 1996; Kleim et al., 1998; Maldonado et al., 2008). Thus, in addition to providing a highly sensitive measure of impairments in skilled motor function after cortical lesions, this test may be useful for studies of neural plasticity in response to skilled motor training. After 13 days of daily post-lesion training, mice with sensorimotor cortex lesions tended to show some recovery of motor skill, making the Pasta Matrix Reaching Test a possible rehabilitative training task for mice after ischemic lesions of the forelimb area of sensorimotor cortex. However, because the current study did not include an untrained control group, there is no way to tell if this recovery was spontaneous or due to the motor skill training, and this requires further investigation. Also, the design of the Pasta Matrix apparatus requires further refinement. Mice were only able to reach a maximum of 18 pasta pieces when the matrix was filled only on the side contralateral to the trained limb. Decreasing the distance between pasta pieces so that there are more pieces within the normal reaching distance of the mouse may further increase the sensitivity of the test. Also, the continued improvement of the Sham group following sham-operative procedures suggests that mice had not reached asymptotic performance prior to lesion induction. It is expected that the group differences would be retained on this task even after training to asymptote because of the deficits seen on other motor tasks that do not require any previous training (the Ladder Rung Task and the Bilateral Tactile Stimulation Test). However, it is possible that greater pre-injury training would further increase the sensitivity of the task to lesion-induced impairments. Schubring-Giese et al (2007) found that, when forelimb sensorimotor cortical infarcts affect a previously established skill in rats, the speed of “re-learning” the task is slowed compared with rats that did not possess the skill prior to the injury. Thus, for the purpose of maximizing the sensitivity of this measure, future studies will establish a pre-operative performance criterion of several days of training past asymptotic performance based on individual learning curves.

The group difference in response asymmetry on the Bilateral Tactile Stimulation Test likely reflects a sensory bias towards the ipsilesional limb (Schallert et al., 1982; Schallert & Whishaw, 1984). The Lesion group showed no evidence of recovery from the response asymmetry over the days of testing, indicating that this test is very sensitive to relatively long-lasting impairments in sensory function. Removal time probably mainly reveals a motor bias, since only the time *after* the stimulus was first contacted was taken into consideration in determining the latency to remove (Bradbury et al., 2002; Starkey et al., 2005). The measures of removal time approached significance. It is possible that placing smaller pieces of tape on the paw would reveal a greater deficit, as smaller pieces are more difficult to detect (Schallert et al., 1983) and may require greater dexterity to remove (though they may also require greater dexterity on the part of the experimenter to place correctly).

In the current study, the Ladder Rung Test detected transient impairments in the contralesional forelimb. Mice no longer showed a significant increase in the number of errors made with the contralesional paw when tested beyond two days post-lesion. It is very likely that variations in the test would detect more persistent deficits. For example, we did

not vary the spaces between the ladder rungs, a method that has been shown to more sensitively detect fore- and hindlimb deficits (Metz & Whishaw, 2002). Furthermore, performances on similar grid walking tests are known to be sensitive to behavioral compensation, whereby animals appear to recover lost function on the task but are actually largely relying on other types of body movement (Bury & Jones, 2002). Thus, it is possible that the quick “recovery” on this test reflects behavioral compensation.

The Schallert Cylinder Test and the Corner test did not reveal sensorimotor deficits following intracortical infusions of ET-1 into the forelimb area of the mouse sensorimotor cortex. Previous studies with mice have used these tests to detect deficits caused by MCAo (Zhang et al., 2002; Li et al., 2004; Bouët et al., 2007). Our lesions, however, did not damage the striatum, as is commonly seen after MCAo, and these tests may be more sensitive to striatal damage than to damage restricted to the cortex. For instance, in rats, lesions that caused nigrostriatal damage produce chronic post-operative asymmetries on the Cylinder Test, whereas lesions restricted to the forelimb area of the sensorimotor cortex (SMC) show most severe impairments on this measure early after the lesion, returning to more symmetrical forelimb use during the chronic phase (Schallert et al., 2000). However, the Cylinder Test is sensitive to focal ET-1 induced lesions of the forelimb area of the SMC in rats (Adkins et al., 2004) and, therefore, we are surprised not to find a similar effect on this measure in mice. This could be because these lesions in mice simply didn’t result in sufficient impairments in postural support behaviors with the contralesional forelimb. Alternatively, it could be that a more challenging way of testing is needed to reveal this asymmetry. It could also be due to a species difference in performance on the test. Postural support challenges during upright exploratory movements may be greater in rats due to their morphology and larger size, while mice might be better able to compensate for any postural support deficits.

Although apparently less potent in producing lesions in the mouse brain than in the rat brain, the intracortical infusion of ET-1 into the mouse sensorimotor cortex does produce a small cortical infarct that results in behavioral deficits on tests of skilled motor function and sensorimotor asymmetry. We have established a battery of sensorimotor tests that can be used to detect behavioral deficits following sensorimotor cortex damage in the mouse, including a newly adapted version of the Pasta Matrix test of skilled reaching. These tests can be used to further explore the differences between mouse and rat models of cerebral ischemia, and aid in advancing the mouse as a model organism for the study of stroke.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A, Representative Nissl stained coronal sections of an endothelin-1 (ET-1) induced sensorimotor cortex lesion. Brain tissue was collected 26 days after lesion induction. Numbers to the right indicate approximate distances from Bregma in mm. Scale bar = 1 mm.

B, ET-1 lesions of the sensorimotor cortex resulted in a significant loss of sensorimotor cortex in the ipsilesional hemisphere as measured by the interhemispheric volume difference. * $P < 0.01$ significantly different from Sham.

Figure 2.

A, A mouse performing the Pasta Matrix Reaching Test. The mouse reaches for pasta arranged in the side of the matrix contralateral to the trained reaching limb. **B**, Mice received 15 days of pre-lesion training during which time they became proficient in performing the test. **C**, The lesion resulted in significant impairments compared to the Sham group. Impairments were most evident in the first two weeks of post-lesion testing. Data are means $\pm$ SEM change from pre-operative performance (post-operative - pre-operative number of pieces). * $P < 0.05$ significantly different from Sham.

Figure 3.

A, Three-dimensional surface plots reflecting the percentage of animals in the Lesion group ($n=7$) that successfully broke a pasta piece at each location in the matrix on a given day. Horizontal axes represent the anterior (column) and posterior (row) number of the matrix, with anterior 1, lateral 1 being closest to the reaching aperture. *B*, An example from a single mouse of the area of the matrix cleared over the course of initial learning of the task (pre-operative) and post-operative performance. The position of the reaching aperture in relation to the pasta-containing portion of the matrix is indicated by a gray bar. The mouse reaches through this aperture for pasta pieces placed contralateral to the reaching limb. Scale bar = 5 mm.

Figure 4.

A, A mouse performing the Ladder Rung Test. Both paws are placed correctly on the ladder rungs. *B*, Example of an error on this test. The arrow indicates the limb that has slipped through the ladder rungs. *C*, Lesions caused a transient increase in errors of the contralesional forelimb. Data are means $\pm$ SEM change from pre-operative performance (post-operative - pre-operative % errors/step). * $P < 0.05$ significantly different from Sham.

Figure 5.

A, Placement of the tape on the ventrum of the paw for the Bilateral Tactile Stimulation Test. Stimuli are placed on both paws for each trial and the order (left versus right) and latency for contact and removal are recorded. *B*, Response bias. After the lesions, mice responded to the ipsilesional stimulus before responding to the contralesional stimulus on a greater percentage of trials compared to sham operates. *C*, Response latency. Lesions tended to increase the latency to remove a stimulus from the contralesional forepaw, but this failed to reach significance (see text for details). Data are means $\pm$ SEM change from pre-operative performance of the contralesional limb (post-operative - pre-operative % ipsilateral, *A*, and time, *B*).

Table 1.

Behavioral performance on the Cylinder and Corner Tests

Test	Measure	Pre-Operative		Post-Operative	
		Sham	Lesion	Sham	Lesion
Cylinder	% Ipsi Contacts	54 ± 2	49 ± 2	51 ± 2	53 ± 2
	% Ipsi Pushoffs	42 ± 8	44 ± 6	40 ± 5	39 ± 9
	% Ipsi Landings	43 ± 4	47 ± 3	59 ± 4	50 ± 4
Corner	% Ipsi Turns	46 ± 6	51 ± 7	48 ± 7	63 ± 6

There were no significant differences in the performance of Sham and Lesion groups on the Cylinder and Corner Tests. Data are presented as the mean ± SEM performance at pre-operative and day 2 post-operative timepoints.