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So you think you can jump? A novel long jump assessment to detect deficits in stroked mice

Nitish Mittal¹, Jie Pan^{2,3}, Julie Palmateer³, Lianna Martin⁴, Arushi Pandya⁴, Sungita Kumar⁴, Adaora Ofomata⁴, Patricia D. Hurn^{3,5}, and Timothy Schallert⁴

¹College of Pharmacy, University of Texas at Austin, Austin, TX

²Nanjing University Medical School, Nanjing, PR China

³Department of Neuroscience, University of Texas at Austin, Austin, TX

⁴Department of Psychology, University of Texas at Austin, Austin, TX

⁵University of Texas System, Austin, TX

Abstract

Background—Stroke survivors suffer from persistent disability, as well as severe sensorimotor and cognitive deficits. The preclinical assessment of such deficits is important for the development of novel interventions and therapeutics.

New Method—The aim of this study was to develop a quantitative behavioral measure of hindlimb functionality in rodents, which could be used to assess deficits after a neural injury, such as stroke. Here we introduce a test to measure long jump behavior in mice.

Results—Using this test we first showed that while male and female mice exhibited no differences in jump success rate, the female mice showed lower baseline jumping latencies. Next we demonstrated that the induction of a cerebral stroke via middle cerebral artery occlusion (MCAO) for 45 minutes did not affect the jump success rate in either group; however, it did significantly increase jump latencies in both male and female mice. Finally, we used therapeutic interventions to explore mechanisms that may be involved in producing this increase in jump latency by administering the anti-depressant fluoxetine prior to the long jump assay, and also tested for potential changes in anxiety levels after stroke.

Comparison with Existing Methods—Other methods to assess hindlimb functionality are not specific, because they measure behaviors that rely not only on hindlimbs, but also on forelimbs and tail.

Corresponding Author: Nitish Mittal, PHR 5.224, Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, 107 W. Dean Keeton Stop C0875, Austin, TX 78712, mittal.n@utexas.edu, Phone: +1 512 471 6141.

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Conclusions—This study introduces a novel assay that can be used to measure a stroke induced behavioral deficit with great sensitivity, and raises interesting questions about potential mechanisms regulating this effect.

Keywords

Stroke; MCAO; Behavior; Anxiety; Depression

1. Introduction

Stroke is a debilitating neural injury resulting in persistent disability, as well as severe sensorimotor and cognitive deficits (Bouët et al. 2007). Interventions directed at treating these outcomes are important in reducing the overall impact of stroke in patients and in restoring their ability to carry out normal day-to-day functions (Schallert 2006). Rodents have served as important models in studying the pathophysiology of, and the functional behavioral deficits arising from a variety of neural injuries, such as cerebral stroke. The middle cerebral artery occlusion method of stroke induction in rodents has been an invaluable tool in studying the short and long term neurological deficits resulting from cerebral stroke (Rosell et al. 2013). The preclinical assessment of such deficits allows for a better understanding of the progression of this injury and is important for the development of novel interventions and therapeutics.

Many behavioral assays have been designed to evaluate a wide array of functional outcomes following focal cerebral ischemia induced injury in rats and mice (Schaar et al. 2010). Some such tests include composite scores of general welfare such as the Bederson Neurological Scoring Scales, the Modified Neurological Severity Scores (Bederson et al. 1986; Zhang et al. 2002), and tests for cognitive deficits such as the Modified Morris Water Maze and the Radial Arm Maze (Volpe et al. 1984; Schallert 2006). Furthermore, a battery of tests have been developed to measure sensorimotor deficits, some of which are specific for forelimb use, such as: the Cylinder Test, the Reaching Pellet Retrieval Task, the Staircase Test, the Vibrissae Elicited Forelimb Placing Test, the Corner Test, the Accelerated Rotarod Test and the Adhesive Removal Test (Schallert et al. 2000, 1982; Montoya et al. 1991; Markgraf et al. 1992; Baird et al. 2001; Chen et al. 2001; Gharbawie et al. 2004; Schallert 2006; Allred and Jones 2008; Allred et al. 2008; Bouet and Freret 2012); and others that are specific for hindlimb use, such as: the Grid Walking Test, the Ledge Tapered Beam Test and the Ladder Rung Walking Test (Metz and Whishaw 2002; Schallert and Woodlee 2005; Allred and Jones 2008). While it is apparent that there are various tests measuring deficits in forelimb use, there are far fewer tests that measure hindlimb functionality. Moreover, these hindlimb assays are not very specific, because they measure behaviors that rely not only on hindlimbs, but also on forelimbs and tail.

Improved limb mobility, and walking in particular, are considered to be the primary goals of many patients recovering from stroke (Bohannon et al. 1991; Mayo et al. 1999). Therefore, it is important to develop reliable and valid assays of hindlimb function in animals, which can then be useful for designing therapeutic interventions directed at achieving these goals. One of the difficulties with designing ways to measure hindlimb use is that rodents usually do not use their hindlimbs, as often as they use forelimbs, for complex motor functions.

Moreover, the assays that have been shown to be associated with hindlimb use, typically measure balance and walking ability, both of which are markedly different in rodents. This is because they are able to use their forelimbs as well as their tails for balance, something that is not applicable to humans. Identification of behaviors that rely primarily on hindlimbs, and that cannot be easily compensated with forelimbs or tail, may be required for the development of novel assays to measure hindlimb function. One such behavior may be the innate ability to jump in mice and rats alike (Hart et al. 2009). Jumping requires the animal to kick off with the hindlimbs in a fashion that cannot easily be compensated. As such, it may be possible to quantify the jumping ability of rodents and measure changes in this ability following a neural injury.

Here we introduce a novel test to measure long jump behavior in mice. We hypothesized that the induction of stroke will produce a deficit in the jumping ability of mice, and that this deficit will represent a loss of hindlimb functionality. In order to test this idea, we quantified the long jump ability in mice using two variables over four distances: 1) the percent of successful jumps at each distance and 2) the latency to initiate a jump per trial. Using this test we first established baseline measures of jumping ability in mice, and sought to identify any basal sex differences that may exist. Next, we tested the mice for potential deficits in these measures after the induction of stroke via middle cerebral artery occlusion (MCAO). Lastly we sought to identify potential neural mechanisms that may regulate long jump behavior in mice.

2. Materials and Methods

2.1. Animals

All mice in the study were purchased from Charles River (Wilmington, MA); male and female C57BL/6 mice between 18–24 grams of weight were used. The animals were group housed in a 12/12 hour light/dark cycle, and the housing room temperature was maintained between 75–85 °F. For behavioral studies, the experimenter was blinded to the treatment. All experimental procedures were conducted in accordance with, and pre-approved by the *Institutional Animal Care and Use Committee*.

2.2. Ischemia

Transient focal cerebral ischemia was induced via right middle cerebral occlusion (MCAO) as previously described, with some modification (Pan et al. 2014). Reversible right MCAO was induced for 45 minutes, using the intraluminal filament technique (6-0 suture) under isofluorane anesthesia. Core and temporalis muscle temperatures were maintained between 35.5 and 37.5 °C throughout the procedure with the use of a heating lamp. Occlusion, and subsequent reperfusion, were confirmed via laser-doppler flowmetry (LDF). Sham operations were conducted in a similar fashion, with the exception of cauterizing the common, external, and internal carotid arteries. The animals were monitored for food and fluid consumption, as well as general health status, for three days following the procedure.

2.3. Cylinder Test

The cylinder test was used to confirm stroke induced forelimb bias in animals, as previously described (Schallert et al. 2000). The animal was placed in a transparent plastic cylinder (H: 15 cm, D: 7 cm), and independent use of right or left forelimb to touch the cylinder during a full rear was measured using video recordings. The test was carried out for at least 15 touches or a maximum of 10 minutes. Data are represented as the percent of right forepaw touches following the MCAO or sham procedure vs. the baseline touches. A student's t-test was used for statistical analyses (StatView, Cary, NC).

2.4. Elevated plus maze

The elevated plus maze test (EPM) was used as previously described (Pellow et al. 1985), with some modifications. The apparatus was a '+' shaped maze, which was raised 52 cm from the floor. The four arms were 34 cm long and 9.5 cm wide. The 'closed' arms were surrounded by plywood walls 14 cm in height, while the open arms had no walls. The animals were placed in the middle of the maze facing either the open or the closed arm at random. Each test was carried out for 5 minutes. The total time spent in the open and the closed arm, and the number of entries into each arm were measured. The data are represented as the percent of time spent or entries into the open arm, over total time spent and entries into both arms. For male vs. female experiments, a student's t-test was conducted to determine significant differences. For the treatment experiments, a repeated measures ANOVA, with Fisher's PLSD post hoc test was used to assess group by interaction differences. A higher ratio suggests increased anxiety, while a lower ratio suggests decreased anxiety according to previous research sources (Pellow et al. 1985).

2.5. Long Jump Test

The long jump test was performed using a wooden beam with dimensions H: 1.5 cm × W: 4.5 cm × L: 100 cm. The beam was raised 80 cm from the floor using a saw horse, and one end of the beam was covered with a surgery drape to provide additional padding for the jump. An empty plastic housing cage was placed on its side, on a table, at level with the beam (80 cm from the floor), such that the open end was facing the beam. A bag of bubble wrap and packing peanuts was placed on the floor between the beam and the cage to provide cushion for any potential falls due to failed jump attempts. The cage was covered to occlude the mice's view of the surrounding areas. The bottom edge of the cage was lined with a strip of drape to allow the mice to better grip the cage after a successful jump. All the cage mates, along with saw dust from the home cage and a food pellet coated with peanut butter, were included to make the cage more appealing for the mice.

All mice underwent a training session, during which they were trained to jump up to 30 cm. Ten minutes before the beginning of the session, all the cagemates were placed in the testing cage for habituation. Then, at the beginning of the training session, the beam was placed in a way such that it was touching the cage and the mice were placed on the beam such that they could simply walk into the cage. After three successful entries at each distance, the beam was slowly moved further away from the cage in 5 cm increments (see schematic in Fig 1a). Two days after the training session, a pre-test was conducted to acquire baseline data. Success rate and jump latency data were measured at 10, 20, 25 and 30 cm distances. At

each distance the mice were given a maximum of five attempts to complete three successful jumps. The success rate at each distance was calculated as the total number of successful jumps divided by the total number of attempts. The latency to jump was measured from the moment the mouse reached the edge of the beam until the initiation of a jump. The mice were gently tapped, at the base of their tail, at 30 second intervals to encourage them to jump. A maximum cut off time of 105 seconds was used. If a mouse did not initiate a jump within 105 seconds, the base of their tail was lightly tapped repeatedly until a jump was made, and the mouse received a latency score of 105 seconds. The mice were retested using the same parameters 3 days after induction of stroke. The data are represented as the percentage of successful jumps and the average latency to jump in seconds. A repeated measures ANOVA was used, with Fisher's PLSD post hoc test for multiple comparisons (StatView). The statistical comparisons are shown as group by interaction effects for all experiments. For a visualization of the long jump test, please refer to the video provided in the online supplements.

3. Results

3.1. Focal cerebral ischemia and cylinder test

Focal cerebral ischemia was successfully induced via middle cerebral artery occlusion for 45 min. Occlusion decreased local blood flow to $10.66 \pm 0.26\%$ of basal levels, as confirmed by laser doppler flowmetry (LDF). The core and temporalis muscle temperatures were maintained at 36.3 ± 0.1 °C. Please refer to Table 1 for the total number of animals that underwent MCAO or sham procedure, and their respective day 3 survival rates. Three days after the ischemia procedure all mice underwent long jump testing. On post-surgery day 4, one day after the long jump test, cylinder test was performed as a marker of functional deficits produced by stroke. As expected, stroke produced a significant increase in ipsilateral forelimb use in both female (MCAO vs. sham: $p < 0.01$, $F_{1,11} = 10.766$; Fig 1b), and male mice (MCAO vs. sham: $p < 0.01$, $F_{1,17} = 10.648$; Fig 1c). There were no sex differences in stroke-induced forelimb asymmetry (male vs. female: $p = 0.140$, $F_{1,18} = 2.380$). Moreover, treatment with either saline (untreated vs. saline: $p = 0.432$, $F_{1,23} = 0.640$), or fluoxetine (untreated vs. fluoxetine; $p = 0.263$, $F_{1,22} = 1.132$) prior to long jump test on day 3 did not affect the functional outcome of stroke, as measured by the cylinder test on day 4. It should be noted that since our focus was on behavioral and functional outcomes of stroke, we did not perform histological analysis of ischemia induced infarction in the present study. We have previously shown that the occlusion measured by LDF, and the behavioral deficits measured by the cylinder test are at least sufficient functional markers of stroke, and correlate well with infarctions produced by the MCAO procedure (Li et al. 2004; Craft et al. 2006; Akiyoshi et al. 2011; Pan et al. 2014).

3.2. Basal sex differences in long jump

We first sought to identify potential sex differences in baseline jumping ability in mice. While we did not expect to find any sex differences, we thought it was prudent to collect these data, so that they are available as reference for future studies. For this purpose, we measured the basal success rate and latency to jump in these mice. As expected, male ($n=19$) and female ($n=15$) subjects exhibited similar success rates across all distances (male vs.

female: $p = 0.504$, $F_{1,32} = 0.457$; Fig 2a). However, they markedly differed in their latency to jump. Female mice demonstrated a shorter latency to jump as compared to male mice, at 20, 25 and 30 cm distances (male vs. female: $p < 0.001$, $F_{1,32} = 15.420$; Fig 2b). These mice were then re-tested on the same task after the MCAO procedure to assess stroke-induced deficits.

3.3. Stroke induced deficits in long jump

Although the stroke procedure successfully produced a functional deficit, as measured by the cylinder test, no significant deficits in jump success rate were observed three days after the MCAO ($n=21$) or sham ($n=13$) surgery (sham vs. MCAO: $p = 0.193$, $F_{1,30} = 1.769$; Fig 3a). Furthermore, there were no significant sex X procedure interactions on this measure (sex*success rate: $p = 0.6018$, $F_{1,30} = 0.278$). However, the induction of stroke did produce a significant increase in the latency to jump as compared to the sham mice (sham vs. MCAO: $p < 0.001$, $F_{1,30} = 40.554$; Fig 3b). Moreover, this increase was seen in both male and female subjects, and no further sex X procedure interactions were observed (sex*latency: $p = 0.673$, $F_{1,30} = 0.182$). These data suggest that the long jump test is not able to sufficiently assess potential stroke-induced deficits in hindlimb functionality in mice. However, the lack of a deficit in success rate, coupled with the increase in jump latency may reflect a cognitive or affective, rather than a sensorimotor, deficit in these mice.

3.4. Acute fluoxetine treatment does not alleviate stroke induced deficits in long jump

One affective pathology that has frequently been associated with stroke is post-stroke depression. Therefore, we tested whether the acute administration of an anti-depressant, fluoxetine, before the long jump test would be sufficient to blunt the increase in jump latency produced by stroke. Given the basal sex differences in jump latency, all treatment studies were conducted in male mice in order to remove any confounding effects of sex on stroke induction or fluoxetine administration.

On post stroke day 3, male mice were treated with either saline ($n=13$) or fluoxetine (10 mg/kg, $n = 14$) 30 minutes before the long jump test. For statistical analyses, the data for all three post-stroke groups (untreated, saline treated or fluoxetine treated) were combined, and a three way repeated measures ANOVA was used. There was no effect of treatment on success rate (treatment*success rate: $p = 0.8874$, $F_{2,36} = 0.120$; Fig 4a). However, a main effect of treatment was observed in the latency to jump (treatment*latency: $p = 0.022$, $F_{2,36} = 4.232$; Fig 4b). Post-hoc analyses revealed that while the latency to jump for the untreated group was significantly higher than both the saline treated ($p < 0.05$) and fluoxetine treated ($p < 0.01$) groups, there were no further differences between the saline and fluoxetine treated mice (saline vs. fluoxetine: $p = 0.5073$).

3.5. No differences in the Elevated Plus Maze test

While these data suggest that the change in jump latency may not necessarily be a marker of post-stroke depression, it is possible that this represents a shift in another affective modality, such as anxiety. To explore this possibility, we used an EPM test to measure differences in anxiety levels. First we tested for potential sex differences in basal anxiety between male ($n=12$) and female ($n=12$) mice. Both groups spent an equal time exploring the arms (total

time: males: 253.8 ± 5.1 seconds, females: 252.1 ± 4.1 seconds) and there were no significant differences in either the ratio of time spent in the open arms (males: $30.21 \pm 3.21\%$, females: $25.01 \pm 2.41\%$; $p = 0.204$; Fig 5a) or the ratio of entries into the open arm (males: $40.50 \pm 2.16\%$, females: $37.12 \pm 1.41\%$; $p = 0.203$; Fig 5b). Next we explored the effect of ischemia induced stroke on anxiety levels.

In a cohort of male mice, we observed no effect of stroke in either the time spent (baseline: $30.21 \pm 3.21\%$, MCAO: $37.91 \pm 8.14\%$; Fig 5c), or the entries (baseline: $40.50 \pm 2.16\%$, MCAO: $46.45 \pm 4.55\%$; Fig 5d) into the open arm. Moreover, fluoxetine treatment did not further affect EPM behavior. All groups spent an equal amount of time exploring both arms (baseline: 253.8 ± 5.1 sec; MCAO + saline: 246.5 ± 8.5 sec; MCAO + fluoxetine: 248.9 ± 10.0 sec). There was no effect of procedure or treatment on the ratio of time spent in the open arm (procedure*treatment: $p = 0.635$, $F_{2,38} = 0.460$), nor the ratio of entries into the open arm (procedure*treatment: $p = 0.330$, $F_{2,38} = 1.141$).

4. Discussion

The aim of this study was to develop a novel long jump task which could be used to assess deficits in hindlimb functionality in rodents after a neural injury, such as stroke. First we demonstrated that although male and female mice exhibited no differences in jump success rate, the female mice showed lower baseline jumping latencies at all distances measured. Moreover, this difference in jump latency was not associated with any differences in basal anxiety levels between the male and female mice. Next we tested the effect of stroke on this behavior and found that while the induction of stroke did not affect the jump success rate in either group, it significantly increased jumping latencies in both male and female mice. We then sought to identify the factors contributing to this increase in jump latency by administering anti-depressants prior to the long jump assay, as well as testing for potential changes in anxiety levels after stroke. Contrary to our hypothesis, the long jump test was unable to detect stroke-induced sensorimotor deficits in hindlimb function. However, it did detect a potential psychological or cognitive deficit, resulting in an increased latency to jump, with great sensitivity. Although we were unable to attribute this stroke-induced increase in jump latency to either depression or anxiety in the current study, these results raise interesting questions about the potential mechanisms regulating this change that may be addressed by future investigations.

The presence of a stroke induced deficit in jump latency, but not success rate, was an interesting finding. One explanation for this phenomenon might be that the stroke induced by a unilateral 45 min MCAO procedure was not sufficient to induce measurable damage to the hindlimbs, and therefore did not affect the ability to jump in these mice. Although, previous studies have shown that strokes produced by transient and focal MCAO procedures can sufficiently induce measurable hindlimb damage, we did not use a specific assay to directly measure hindlimb deficits in the current study (Schallert et al. 2000; Lim et al. 2008). Therefore, it is possible that future studies using longer occlusion times or a bilateral occlusion may be able to detect changes in jump success rate. Furthermore, the change in jump latency, but not success rate, may also suggest that the stroke induced damage could have produced a cognitive rather than a sensorimotor alteration. Indeed, studies have shown

that ischemic insults can result in cognitive decline (Shih et al. 2013). Therefore, it is possible that this shift in jump latency may be a marker of altered cognitive function, resulting from the induction of ischemic stroke, in these mice.

Post-stroke depression has been shown to increase cognitive impairment and mortality after stroke. Furthermore, it has also been shown to be the single strongest correlate of impairment in activities of daily living (Robinson and Spalletta 2010; Cojocaru et al. 2013). As such, we sought to determine whether the increase in jumping latency may be a marker of post-stroke depression. Fluoxetine is one of the most widely used selective serotonin reuptake inhibitors (SSRI) for the treatment of depression. There is also evidence, both clinical and preclinical, that fluoxetine treatment may induce neuroprotection and attenuate stroke-induced spatial cognitive deficits in rodents (Jolkkonen et al. 2000; Windle and Corbett 2005; Li et al. 2009; Lim et al. 2009), as well as, improve motor recovery in human patients (Pariente et al. 2001; Fruehwald et al. 2003; Choi-Kwon et al. 2006; Chollet et al. 2011) alike. Moreover, acute fluoxetine treatment has been shown to produce antidepressant effects in rodents (Liu et al. 2010). As such, we tested whether the administration of fluoxetine 30 minutes prior to the long jump test was sufficient to blunt the increase in long jump latency in mice. We did not find an effect of acute fluoxetine on long jump latency at a 10 mg/kg dose. While the saline and fluoxetine treated mice displayed similar jump latencies, the untreated mice had higher jump latencies, than both the saline and fluoxetine treated mice. This alteration may be a result of stress caused by the i.p. administration of treatment solutions 30 minutes before the experiment. This observation further sheds light upon the sensitivity of this test to potential changes in stress and anxiety levels following the induction of ischemic stroke. In an additional cohort of animals we tested the effects of a double dose of fluoxetine (20 mg/kg) on this measure, but this dose was also without effect on the stroke-induced increase in jump latency (data not shown).

While these results may suggest that the change in jump latency is not a marker of post-stroke depression, it is also possible that a single administration of fluoxetine may not be sufficient to eliminate all depression-like symptoms in these mice. Indeed, fluoxetine is usually prescribed chronically for the treatment of clinical depression and it often takes several weeks for the anti-depressive effects to manifest themselves (Blier 2003; Dulawa et al. 2004). Such chronic treatment would entail daily fluoxetine injections over 14 or 21 days, which was not feasible in the current study due to the low survival rates in post-ischemic mice over such a long timeline. It is possible that the use of a rapid acting anti-depressant such as ketamine (Browne and Lucki 2013; Kavalali and Monteggia 2015) may yield better results in the post-stroke mice. However, known models of depression, such as social defeat, learned helplessness or chronic mild stress (McArthur and Borsini 2006), which are not nuanced by added sensorimotor deficits, and can allow for chronic pharmacological treatment may serve as better tools to investigate the role of depression in the long jump test.

Several studies have reported post-stroke anxiety as a widely recognized disorder with a prevalence of 12–28% (Sharpe et al. 1990; Burvill et al. 1995; Astrom 1996). Moreover, there is also evidence of sex-based differences in various measure of anxiety in rodents (Johnston and File 1991; Palanza 2001; Vöikar et al. 2001), which could potentially explain the baseline differences in jump latency between male and female mice. Therefore, we

sought to investigate whether the differences in jump latency can be explained by concomitant differences in anxiety levels in these mice. At baseline, we found no differences between the male and female mice in either the ratio of time spent, or the ratio of entries, into the open arms. Moreover, there were no measureable effects of stroke induction or fluoxetine treatment on the anxiety levels of these mice. Thus, it is likely that the increase in jump latency cannot be explained by potential changes in anxiety levels. However, it is also well known that tests of anxiety can vary in their reliability, and that despite its popularity as a classical test of anxiety, the EPM test is certainly not a comprehensive measure of all types of anxiety. Some deficiencies of the EPM test are discussed in the cited reviews (Ramos 2008; Haller and Alicke 2012). Thus it is possible that in this case the EPM test may not be sensitive enough to identify stroke-induced differences in anxiety, and that such differences may be detected with greater sensitivity by other assays such as the open field test or the light/dark box test (Ramos 2008). However, given that MCAO induced stroke is not typically considered to be a reliable model of anxiety, we did not pursue further investigation of a potential role of anxiety in regulating long jump latency with this model. Future studies employing established models of anxiety, or pharmacological manipulations, aimed at increasing or decreasing anxiety, will be better suited for this purpose.

In this study, we introduced a new test to measure long jump behavior in mice. We showed that male and female mice differ in their latency to initiate a jump. We found that while stroke did not produce differences in jumping ability, it increased the latency to jump in both male and female mice. We then tested whether these differences could be attributed to post-stroke depression, using pharmacological anti-depression treatment, or to anxiety, by measuring open arm exploration on the EPM test, but we were unable to attribute the differences in latency to changes in these measures. It is clear that the long jump test measures a stroke-induced behavioral change with great sensitivity. However, we have not yet identified the mechanisms responsible for this change. Additional studies are needed to systematically explore the mechanisms involved in the jump-making process. Once fully understood, the long jump test may serve as an important addition to the battery of assays used to assess neurological functions in rodents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- The Long Jump Test can assess potential psychosomatic deficits produced by stroke.
- Male and female mice differ in latency to jump, but not in jump success rate.
- Cerebral stroke does not affect jump success rate, but increases latency to jump.
- Higher jump latencies could not be explained by increased anxiety.
- Acute fluoxetine did not have any effect on jump latency or success rate.

Figure 1.

Stroke induced functional deficits and the long jump test. **a)** A schematic of the long jump test procedure. On training day mice were placed on a beam that was touching the cage such that they could simply walk into the cage. After three successful jumps at each distance, the beam was slowly moved further away from the cage in 5 cm increments, until a maximum distance of 30 cm was achieved. **b)** Stroke-induced increase in ipsilateral forelimb use in female mice (sham vs. MCAO: $p < 0.01$, $F_{1,11} = 10.766$). **c)** Stroke-induced increase in ipsilateral forelimb use in male mice (sham vs. MCAO: $p < 0.01$, $F_{1,17} = 10.648$). No measurable effect of treatment with either saline (untreated vs. saline: $p = 0.432$, $F_{1,23} = 0.640$), or fluoxetine (untreated vs. fluoxetine: $p = 0.263$, $F_{1,22} = 1.132$) administered prior to the long jump test on day 3, on the cylinder test conducted on day 4. Student's t-test was used for statistical analyses. Data are represented as mean $\pm$ SEM. Line represents pre-stroke baseline.

Figure 2.

Long jump test baseline sex differences. **a)** Male (n=19) and female (n=15) subjects exhibited similar success rates across all distances measured (male vs. female: $p = 0.504$, $F_{1,32} = 0.457$). **b)** Female mice demonstrated a shorter latency to jump as compared to male mice, at 20 cm (male: 29.7 ± 2.6 vs. female: 17.4 ± 1.6 sec), 25 cm (male: 33.9 ± 3.4 vs. female: 16.5 ± 2.0 sec) and 30 cm (male: 32.6 ± 3.8 vs. female: 16.4 ± 2.4 sec) distances (male vs. female: *** $p < 0.001$, $F_{1,32} = 15.420$). Repeated measures ANOVA was used to determine statistical differences with a student's t-test for post hoc measures. Data are represented as mean $\pm$ SEM.

Figure 3.

Stroke induced changes in the long jump test: **a)** There were no significant deficits in jump success rate were observed, three days after the MCAO (n=21) or sham (n=13) surgery (sham vs. MCAO: $p = 0.193$, $F_{1,30} = 1.769$). There was also no significant sex X procedure interactions on this measure (sex*success rate: $p = 0.6018$, $F_{1,30} = 0.278$). **b)** The induction of stroke did produce an increase in the latency to jump as compared to the sham mice (sham vs. MCAO: $***p < 0.001$, $F_{1,30} = 40.554$). However, this increase was seen in both male and female subjects, and no further sex X procedure interactions were observed (sex*latency: $p = 0.673$, $F_{1,30} = 0.182$). Repeated measures ANOVA was used to determine statistical differences with a student's t-test for post hoc measures. Data are represented as mean $\pm$ SEM.

Figure 4.

Fluoxetine treatment does not alleviate stroke induced deficits in the long jump test. **a)** In male mice, there was no effect of saline ($n=13$) or fluoxetine (10 mg/kg, $n=14$) treatment on success rate ($p = 0.8874$, $F_{2,36} = 0.120$). **b)** A main effect of treatment was observed in the latency to jump ($p = 0.022$, $F_{2,36} = 4.232$). Post-hoc analyses revealed that while the latency to jump for the untreated group was significantly higher than both the saline treated (untreated vs. saline: $*p < 0.05$) and fluoxetine treated (untreated vs. fluoxetine: $**p < 0.01$) groups, there were no further differences between the saline and fluoxetine treated mice (saline vs. fluoxetine: $p = 0.5073$). For statistical analyses, the data for all three post-stroke groups (untreated, saline treated or fluoxetine treated) was combined, and a three way repeated measures ANOVA was used. Post hoc differences were determined using a student's t -test. Data are represented as mean $\pm$ SEM. Gradient represents untreated post stroke male group from the Fig 3b.

Figure 5.

Differences in the long jump test are not associated with differences on the EPM test. Both male (n=12) and female (n=12) mice spent an equal time exploring the arms (total time: males: 253.8 ± 5.1 seconds, females: 252.1 ± 4.1 seconds) and there were no significant differences in either **a**) the ratio of time spent in the open arms (males: $30.21 \pm 3.21\%$ vs. females: $25.01 \pm 2.41\%$; $p = 0.204$) or **b**) the ratio of entries into the open arm (males: $40.50 \pm 2.16\%$ vs. females: $37.12 \pm 1.41\%$; $p = 0.203$). In male mice, all treatment groups spent an equal amount of time exploring both arms (baseline: 253.8 ± 5.1 sec; MCAO + saline: 246.5 ± 8.5 sec; MCAO + fluoxetine: 248.9 ± 10.0 sec). There was no effect of procedure or treatment on **c**) the ratio of time spent in the open arm (baseline: $30.21 \pm 3.21\%$, MCAO + saline: $37.91 \pm 8.14\%$, MCAO + fluoxetine: $36.61 \pm 5.10\%$; $p = 0.635$, $F_{2,38} = 0.460$), or **d**) the ratio of entries into the open arm (baseline: $40.50 \pm 2.16\%$, MCAO + saline: $46.45 \pm 4.55\%$, MCAO + fluoxetine: $47.70 \pm 3.05\%$; $p = 0.330$, $F_{2,38} = 1.141$). Repeated measures

ANOVA was used to determine statistical differences. Data are represented as mean $\pm$ SEM. Gradient represents male baseline group from panels a and b respectively.

Table 1

Post-stroke survival rate following 45 minute MCAO procedure.

n	Total Sham	Total MCAO	Day 3 Sham	Day 3 MCAO	Day 3 MCAO Survival Rate
Male	7	64	7	45	70.3 %
Female	6	14	6	9	64.3 %