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Refining forelimb asymmetry analysis: Correlation with Montoya staircase contralateral function post-stroke

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HIGHLIGHTS

- A refinement of Forelimb Asymmetry or the Cylinder test analysis is presented, through the separation of palm and fingertip touches.
- This refinement allows better correlation of contralateral function following stroke with that determined with the Montoya Staircase.
- Every animal can easily perform Forelimb Asymmetry, without the necessity of pre-stroke training and diet restriction used in Montoya Staircase.

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ABSTRACT

Background: Forelimb Asymmetry Test is a simple test of motor function, using exploration behavior of a rat in a novel environment and counting the number of times that a rat touches the wall with either forepaw. Our lab has noticed, however, that there appears to be an increased number of fingertip touches to the wall following a stroke in the impaired forelimb.
New method: We counted the number of times that the animal either laid its palm flat against the wall of the chamber or touched the wall with only its fingertips, for both the left and right forepaws. We also separated bouts of exploration, so we could clearly determine if fingertip touches normally were associated with a transition from resting state to exploration state.
Results and comparison with existing methods: Fishers exact test indicated that there were significant differences in the way that the animals touched the wall pre-stroke compared to post-stroke, with more fingertip touches occurring post-stroke. Counting palm touches as normal and fingertip touches as abnormal increases the sensitivity of the Forelimb Asymmetry analysis and gives a good correlation with the contralateral functional deficits determined by Montoya Staircase post-stroke. If we counted every wall touch as normal (palm touches and fingertip touches), we see a loss of sensitivity and a poor correlation with contralateral function as determined by Montoya Staircase.
Conclusions: This refinement of the Forelimb Asymmetry analysis improves correlation with Montoya Staircase contralateral function after stroke.

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1. Introduction

As individuals run pre-clinical tests on animal function before and after a brain injury, it is important that the functional tests chosen bear some resemblance to the tests that will typically be used in clinical trials, especially if we expect the results from animal studies to translate well into clinical trials. For stroke, there are a limited number of functional tests that would translate well into clinical trials: Montoya Staircase and Forelimb Asymmetry are the functional tests that are often used. In Montoya Staircase, a rat must grasp and hold onto a sucrose pellet, which is similar to a human holding onto a utensil, such as a fork. In Forelimb Asymmetry, we evaluate the use of the forepaws to explore a new environment, which is comparable to any activity in which a human uses his/her hands to support their weight, for example, while rising from a chair.

In a Forelimb Asymmetry test, a rat is placed into a clear vertical tube and is videotaped for five minutes. During this time, the rat will typically rear up on its hind paws and touch the walls of the cylinder with its forepaws. Before a brain injury, the rat generally uses both its right and left paw to touch the wall equally. After an injury, such as a stroke, the rat will generally use less of its contralateral paw and increase the usage of its ipsilateral paw, resulting in a

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forelimb asymmetry in paw use (Schallert et al., 2000). In a recent paper (Roome and Vanderluit, 2015), the analysis of this method was refined in mice to account for a paw-dragging behavior, which correlated well with cortical injury, however they only counted 20 wall contacts with each session, which limits their data and interpretation. We have noticed that a normal rat places weight against its palms as it explores, most often using its fingertips to touch the wall when it is transitioning from a resting state to an exploration state (upright on hind legs) and back to a resting state. However, after a unilateral ischemic stroke, we see increased use of fingertips in the impaired contralateral limb. This paper will assess the use of the rat palm to touch the walls of the cylinder versus the use of both the rat palms and fingertips to touch the walls of the cylinder and determine which analysis correlates better with results obtained for the contralateral limb function using Montoya Staircase.

2. Methods

2.1. Animals

Retired Breeder female Sprague Dawley rats (10–12 months old) were used in this study. All procedures using animals were approved by the IACUC at Wright State University. Animals had normal 12 h lights (light phase) on and 12 h lights off (dark phase) and ad lib rat chow except for trials using the Montoya Staircase (see Section 2.2 below). All of our animal studies are blinded, with animals only identified by a number until all analysis is completed. Results are then keyed to specific treatment groups, using the animal ID.

2.2. Montoya staircase

All rats were weighed the day after they arrived at the animal facility and their rat chow was also weighted at that time. No further chow was added for two days, then the remaining chow weighed so that we could determine the ad lib amount of chow eaten by each rat in the study per day. In preparation for the Montoya Staircase training (Corbett et al., 2015; Pagnussat Ade et al., 2009), we fasted the rats overnight, then began training in the dark phase, allowing the rats to be placed in the Montoya Staircase apparatus for 15 min. Maple extract painted sucrose pellets (3 pellets per stairwell, 7 stairwells for each side) were previously placed in the Staircase, and we counted the number of pellets obtained by the left and right forepaws at the end of the 15 min time. Rats had previously been exposed to the maple extract painted pellets in their home cages, so they would associate the smell with something good to eat. At the end of the first day's training, rats were given 85% of their ad lib feed for a day (restricted feeding). Training continued for a total of 13 days, with rats generally obtaining 15–18 pellets with each paw by the end of training. Restricted feeding always occurred at the finish of the days training. The performance over the last three days of training was used for pre-stroke performance. The best performance (total pellets taken) over that three day period was used for that rat's pre-stroke function with each paw, averaging the amounts of pellets over two days if two different days had the same total number of pellets taken. An example of these calculations is given in the following table, with the gray highlight showing the best performance (total pellets taken) in those last three days of training:

Rat ID	Day 11	Day 12	Day 13	Pre-stroke L	Pre-stroke R
801	12L, 15R	14L, 17R	16L, 15R	15	16
802	13L, 10R	14L, 15R	15L, 16R	15	16
803	15L, 16R	15L, 13R	17L, 17R	17	17

At the end of the training period, rats are returned to ad lib feed.

Following stroke induction, the animals are tested on post-stroke days 3, 4 and 5, again during the dark phase, to determine contralateral and ipsilateral function post-stroke. Prior to the start of this three day trial, the animals are fasted overnight, and then fed their restricted feed after that day's trials are complete. At the end of the trials on the third day, the animals are returned to ad lib food. An example of some values that might be obtained over that three day period are shown in the table below.

Rat ID	PSD 3	PSD 4	PSD 5	Post-stroke L	Post-stroke R
801	2L, 14R	0L, 15R	1L, 16R	1	16
802	3L, 15R	4L, 16R	3L, 17R	3.5	16.5
803	2L, 10R	3L, 9R	4L, 10R	4	10

Contralateral function is then determined by dividing the number of pellets obtained by the left paw post-stroke by the number of pellets obtained by the left paw pre-stroke. This normalizes contralateral function across the group of animals, so we may compare functional deficits and recoveries over time in the group. In the same manner, Ispilateral function is determined by dividing the number of pellets obtained by the right paw post-stroke by the number of pellets obtained by the right paw pre-stroke. Contralateral Deficit is then equal to 1–contralateral function post-stroke, with a similar calculation for ipsilateral deficit. An example of these calculations is given in the table below:

Rat ID	Pre-L	Pre-R	Post-L	Post-R	Contra function	Ipsi function	Contra deficit	Ipsi deficit
801	15	16	1	16	0.066	1.000	0.933	0
802	15	16	3.5	16.5	0.233	1.031	0.766	−0.031
803	17	17	4	10	0.235	0.588	0.765	0.412

In this table, it is easy to see that two of the stroked rats have no ipsilateral deficit, while one stroked rat (803) does have a 41% ipsilateral deficit. We most often see an ipsilateral deficit when the endothelin injection in our stroke surgery (see section 2.3) has gone into the corpus callosum and damaged it.

2.3. Endothelin-induced stroke

We use a modification of the method developed in Dale Corbett's laboratory (Windle et al., 2006) for cortical stroke. The basic surgery protocol has been described previously (Corbett et al., 2015). We use stereotactic coordinates of AP 0.0, ML 2.5 for the first drilled hole in the skull and coordinates of AP 1.5, ML 2.5 for the second hole. The second hole in the skull is centered over the forelimb motor cortex. We then inject endothelin (600 pmoles in 1.5 ul) in each position at a depth of 2.0 mm. Post-surgical procedures are as described previously (Corbett et al., 2015).

2.4. Forelimb asymmetry analysis

These studies are carried out in the light phase. A rat is placed in a clear vertical chamber and videotaped as it explores the chamber for 5 min. We have placed mirrors behind the rat, so that when it turns away from the camera we can still determine whether it is touching the wall with its left palm or fingertips as compared to its right palm or fingertips. We analyze the videotape in slow motion, separating the movements frame by frame, and separating bouts of exploration (transitioning from resting to hind legs and then back to resting) for a given rat with breaks in the assignments of letters for right paw (R), left paw (L), right fingertips(4), left fingertips(O). In this manner we can determine if fingertip touches to the wall are primarily associated with transitions in exploratory behavior or are generally associated with abnormal behavior using the impaired limb. If an animal touches the wall with both paws simultaneously, we separate for the analysis according to what portion of each paw was used. To ensure that we don't see strong habituation in this test, we wipe a little maple extract high on the wall of the cylinder:

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