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A Straight Alley Version of the BBB Locomotor Scale

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Abstract

We describe here an alternative procedure for assessing hindlimb locomotor function after spinal cord injury that uses the BBB scale, but tests animals in a reward-baited straight alley rather than an open field. Rats were trained to ambulate in a straight alley and habituated to the open field typically used for BBB open field testing. Three groups of rats were tested. Sprague-Dawley rats received either 200kD (n=19) or 300kD contusions (n=9) at T9 with the Infinite Horizon device. Fisher rats (n=8) received moderate contusions (12.5mm) at T8 with the NYU impactor. BBB scores were assessed at different post-injury intervals in the open field and the straight alley, and scores were compared by correlation analyses. BBB scores in the open field vs. the straight alley were highly correlated ($r=0.90$), validating the use of the straight alley for locomotor assessment. Rats exhibited a larger number of bouts of continuous steps in the straight alley vs. the open field (termed passes), providing more opportunities to score hindlimb use and coordination over the 4 minute testing interval. Comparisons of scores across days revealed higher day-to-day correlations in the straight alley vs. the open field (r^2 values of 0.90 and 0.74 for the straight alley and open field respectively), revealing that the straight alley yielded more reliable scores.

Keywords

Spinal cord injury; hindlimb motor function; recovery of function

Introduction

The BBB open field locomotor scale has become an industry standard for assessing hindlimb motor function after spinal cord injury in rats (Basso et al., 1995). The scale was developed and validated to assess motor function after contusion injury at the thoracic level, but has also been used to assess function after transection and other injuries (Basso et al., 1996), as well as injuries at lumbar and cervical levels (Magnuson et al., 1999; Webb et al., 2002).

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There are many advantages of the BBB scale including its simplicity and the fact that it can assess the full range of function from complete paralysis to normal use including inter-limb coordination. One practical disadvantage, however, is that the assessment depends on the animals' willingness to walk about in the open field. This is especially critical at the range of the scale where inter-limb coordination is a key measure. Assessment of inter-limb coordination requires that animals take several steps in a continuous un-interrupted sequence (termed a "pass"). If the animals fail to take several continuous steps, they cannot demonstrate inter-limb coordination, and thus cannot score above a "12" on the scale. Often, investigator intervention of some sort is required to encourage a sufficient number of continuous steps and scorable passes to adequately assess coordination. This required intervention by the investigator is an uncontrolled variable that could vary across labs and influence scoring, and can also startle and/or distract the animals. This can be especially problematic when animals are just at the threshold of coordination where the critical measure is the proportion of passes in which all steps are coordinated.

In the present study, we sought to develop a hindlimb locomotor assessment test in which animals' locomotion would be more standardized. We used the simple approach in which animals were trained to walk from one end of a runway to a darkened goal box baited with a food reward. Animals quickly learned to cross the runway by taking a continuous series of steps. Hindlimb use during locomotion was assessed as in the open field version of the BBB. We demonstrate a high correlation between the measures of hindlimb motor function in the open field vs. the straight alley and show that the straight alley version yields more consistent scores across days of testing.

Methods

Experimental animals

Three separate groups of animals were tested. The first experimental group consisted of 9 female Sprague-Dawley rats weighing 270g-350g at the time of injury that received 300kD contusion injuries at T9. These animals were initially injured to provide groups for training students in an intensive summer course in spinal cord injury techniques. Rats were tested between 1 day post injury (DPI) and 14DPI to evaluate hindlimb motor function during the acute phase of recovery. Specifically, hindlimb motor function was evaluated by traditional BBB in an open field (a 119.5cm diameter round metal stock tank) at 1DPI, 6DPI, and 13DPI and in the straight alley at 2DPI, 7DPI, and 14DPI.

The second experimental group consisted of 19 female Sprague-Dawley rats weighing 230g-260g at the time of the surgery that received 200kD contusion injuries at T9. The rats were tested in the chronic post-injury period when hindlimb motor function should have reached a plateau. Specifically, hindlimb motor function was evaluated by traditional BBB in an open field at 77DPI, 207DPI, and 220DPI and in the straight alley at 110DPI, 203DPI, and 222DPI.

A third experimental group consisted of 8 female Fisher rats weighing 165g-200g at the time of injury that received moderate contusion injuries with the NYU impactor at T8. The Fisher rats belonged to one control group in a study that aimed to repeat the experiment by Pearse et al., 2004, who reported that combined treatment with rolipram, intraspinal injections of dibutyryl cAMP, and Schwann cell transplants promote axonal growth and functional recovery after spinal cord injury. At the time of injury, 2 empty model 2001 200- μ l Alzet minipumps (Durect Corp.) were implanted subcutaneously. Hindlimb motor function was evaluated by traditional BBB in an open field at 2DPI, 6DPI, then weekly for 8 weeks, and in the straight alley at 4DPI and 6DPI. In this current study, we compared BBB data from 2DPI in the open field to data from 4DPI in the straight alley, and BBB data from both apparatuses at 6DPI.

Training Procedures

Prior to injury, the animals were exposed to both the metal tank used for traditional open field testing and the straight alley. The straight alley is made of black plexiglass measuring 28cm × 175cm (Fig. 1). We found that the animals had difficulty moving along the smooth surface of the plexiglass and therefore a plastic liner with a rough surface was placed on the floor of the alley to minimize slipping. The plastic liner also allowed us to more accurately assess toe clearance. At one end of the alley is a darkened goal box (28cm×28cm) baited with Froot Loops® cereal. Each rat was placed at the opposite end from the darkened goal box and allowed to ambulate towards the baited goal box. Once the rat reached the goal box, they were allowed to explore and eat a Froot Loop. After approximately 20 seconds, rats were picked up and placed at the starting position at the opposite end of the alley. For each session, the total time spent in the straight alley was 4 minutes, which is the length of time required to assess hindlimb motor function using the BBB locomotor rating scale. The Sprague-Dawley rats were noticeably more active than the Fisher rats during the 4 minute session. This likely reflects inherent strain differences in behavior as Fisher rats tend to be more timid and less exploratory than the Sprague-Dawley rats.

In the metal tank, the animals were allowed to ambulate for 4 minutes. If the animals were either stationary or walking against the edge of the tank, the rat was picked up and placed in the center of the tank to encourage locomotion. Pre-testing sessions continued for a minimum of 7 sessions, at which time the rats were accustomed to both testing apparatuses, as well as being picked up and moved by the examiner.

SCI Surgery

In two separate experiments, Sprague-Dawley rats were anesthetized with ketamine (80mg/kg) and xylazine (10mg/kg). A laminectomy was performed at the ninth thoracic vertebra and the rats received either a moderate (200kD) or severe (300kD) contusion with the Infinite Horizon (IH) device at T9. In the third experiment, Fisher rats were anesthetized with ketamine (50mg/kg) and xylazine (10mg/kg). A laminectomy was performed at the eighth and ninth thoracic vertebrae and the rats received a moderate contusion with the NYU impactor (10g weight dropped from a 12.5mm height) at T8.

Following surgery, the rats were placed on a water jacketed heating pad maintained at 37°C. Post-operatively, rats received subcutaneous injections of buprenorphine hydrochloride (Buprenex, 0.03mg/kg) for 3 days, baytril (2.5mg/kg) for 7 days, and 10ml lactated ringers for 7 days. Bladders were manually expressed twice daily until urine retention at the time of expression was minimal (generally 7-10 days) indicating recovery of reflex bladder function.

Post-injury Testing Procedures

Hindlimb motor function was assessed using the BBB locomotor rating scale. For both testing apparatuses, two examiners were positioned across from each other to observe both sides of the animal. When tested in the straight alley, the animals were returned to the starting position immediately after consuming the food reward in the baited goal box to maximize the number of scorable passes they could achieve during the 4 minute testing session. Testing procedures in the metal tank were the same as described for pre-testing procedures. With the exception of the 6DPI time point for the Fisher rats, we did not test rats in the straight alley and the metal tank on the same day in order to avoid the possibility of fatigue influencing BBB scores.

Statistical Analyses

The relationship between BBB scores obtained in the open field and BBB scores obtained in the straight alley was determined by the Pearson correlation coefficient. The consistency of

BBB scores obtained in the same apparatus across testing sessions was determined by linear regression analyses. Analyses were performed using GraphPad Prism, version 5.01.

Results

By the end of the training sessions, the Sprague-Dawley rats no longer showed signs of fear in both the straight alley and the metal tank, as indicated by a lack of freezing. The Fisher rats remained less active than the Sprague-Dawley rats, and were more inclined to groom themselves, suggesting that they had grown accustomed to the apparatuses. One major difference between the two apparatuses is that the animals made a noticeably greater number of passes in the straight alley than in the metal tank. The Sprague-Dawley rats achieved an average of 9 passes (S.D. = 3.7) in the straight alley but only an average of 5 passes (S.D. = 2.1) in the metal tank. Although the Fisher rats made fewer passes overall, they also achieved a greater number of passes in the straight alley versus the metal tank; an average of 5 (S.D. = 2.2) and 3 (S.D. = 1.3) passes respectively. Another difference is that the animals tended to move in the middle of the straight alley, whereas in the metal tank, the animals tended to move along the walls, making it difficult for the examiners to assess hindlimb motor function on both sides of the body. When the animals were placed into the straight alley, they would immediately move towards the baited goal box, suggesting that they remembered that the goal box contained a food reward.

With respect to the different aspects of the BBB scale such as limb movement, paw placement etc., there were no major differences in the way hindlimb motor function was assessed. One minor difference, however, is that toe scrapes against the metal tank sound different than toe scrapes against the plastic liner of the straight alley. However, toe scrapes against the plastic liner could be distinguished, and toe clearance in the straight alley was assessed in the same way as in the metal tank.

Overall, BBB scores obtained in the straight alley were highly correlated with BBB scores obtained in the metal tank (Fig. 2). The correlation coefficient for all groups of rats was 0.90. Analysis of the Sprague-Dawley rats and Fisher rats independently revealed correlation coefficients of 0.95 and 0.91 respectively. In the majority of instances where BBB scores in the metal tank and the straight alley were not identical, the BBB scores tended to be slightly higher in the straight alley than in the metal tank. This was especially true of the Fisher rats. This may be attributed to the fact that in many cases, hindlimb motor function was assessed in the metal tank one day before evaluation in the straight alley; at early post-lesion intervals, this additional time could be sufficient for some degree of spontaneous recovery to occur. However, in the group of Sprague-Dawley rats that were tested over 77 days post injury, BBB scores also tended to be higher when assessed in the straight alley as compared to the metal tank. One explanation for this trend is that with a greater number of passes achieved in the straight alley, the examiners had a better chance of observing limb movements, allowing a more accurate assessment of coordination than in the metal tank where the animals spend a longer period of time either stationary, or moving along the walls. Alternatively, it is possible that the higher degree of motivation to locomote across the straight alley (as a result of the food reward) led to better locomotor performance. In either case, the differences were relatively small, and were primarily seen in animals performing at the higher end of the BBB scale (in the chronic Sprague-Dawley group and acute Fisher group).

Regression analyses were performed to compare BBB scores obtained in the same apparatus on different days (Fig. 3). The r^2 values ranged from 0.63-0.88 for Sprague-Dawley rats in the open field and 0.91-0.93 for the straight alley. Values for Fisher rats were 0.66 in the open field and 0.96 in the straight alley. Combining the two strains and the different experiments, the overall r^2 values were 0.90 in the straight alley and 0.74 in the open field. Thus, BBB scores

obtained in the straight alley were more consistent across days regardless of strain of rat or time post-injury.

Discussion

The present study revealed a high correlation between hindlimb locomotor function scores obtained in a reward-baited straight alley and the open field used for BBB testing. The principal advantage of the straight alley version is that rats ambulate reliably across the alley without experimenter encouragement taking a continuous series of steps. Rats in all 3 experimental groups ambulated more reliably in the straight alley than in the open field as measured by the number of multi-step passes over the 4 minute testing interval. Correlation analysis revealed a high correlation between hindlimb locomotor function scores obtained in the straight alley and open field.

Having a greater number of passes is an important advantage for scoring because the intermediate and higher end of the BBB locomotor rating scale relies heavily on predominance judgments of certain aspects of locomotion. For instance, in order for an animal to be classified as frequently coordinated (a score of 13 on the BBB scale), 51-94% of the total number of passes must be coordinated. Consistent coordination requires that 95-100% of the passes be coordinated. Using the mean values reported in this study as an example, this means that all passes must be coordinated. A larger number of total passes improves accuracy and reliability in these predominance judgments.

In addition to this crucial advantage, assessing rats in the straight alley does not require a significantly longer period of training than in the metal tank. After only a few exposures to the straight alley, rats learn that the darkened goal box in the straight alley contains a food reward. This learning is inferred from the observation that when placed into the straight alley, the majority of rats will immediately move towards the baited goal box, and they continue to show a preference for the baited end throughout the 4 minute session. Presumably, it is this learning of an association between the straight alley and a food reward that underlies the higher degree of motivation to locomote. It is possible that increased motivation may be responsible for the instances where BBB scores obtained in the straight alley were slightly higher than those obtained in the metal tank (as seen in the chronic Sprague-Dawley group). Further studies will be needed to determine whether increased motivation actually improves locomotor performance or whether it is simply that the higher number of passes increases the likelihood of observing more joint movements, plantar steps, or coordinated passes (all of which would contribute to a higher BBB score). However, since the same animals were assessed in the straight alley and in the metal tank, it is unlikely that the slight discrepancies in BBB scores are due to superior hindlimb motor function in the straight alley as compared to the metal tank.

Overall, the high correlation between BBB scores in the open field and straight alley validates the feasibility of adopting the straight alley version of the BBB. Future studies that report BBB data using the straight alley can be reasonably compared to past studies with BBB data collected in the open field. There are several practical advantages of using the straight alley. One is that the straight alley is much smaller than the metal tank used for open field analyses, which means that less space is required, and the apparatus can be easily transported between testing rooms. Another advantage is that hindlimb motor function is more easily observed in the straight alley as the rats tend to move in the center of the alley as opposed to along the walls. The straight alley version of the BBB offers a standardized testing situation in which the accuracy and reliability of the BBB data are improved as a result of the higher number of total passes that can be scored. A final technical advantage of the straight alley is that other functional analyses could be carried out simultaneously. For example, footprint analysis could be carried out by inking the animals' paws and having them walk along paper placed on the floor of the alley,

or video kinematic analyses could be carried out by capturing video images of the animal from below the alley.

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Figure 1.

A photograph of the straight alley apparatus. The straight alley measures 28cm × 175cm and is lined with a plastic liner to minimize slipping.

Figure 2.

The scatterplot shows the relationship between BBB scores of individual animals in the straight alley vs. the open field for all 3 groups: acute Sprague-Dawley rats (n=9), chronic Sprague-Dawley rats (n=19), and acute Fisher rats (n=8). The correlation coefficient for all groups combined is 0.90.

Figure 3.

The scatterplot shows the linear regression for BBB scores obtained in (a) the straight alley or (b) the open field during an initial session vs. a repeat session for all groups: acute Sprague-Dawley rats (n=9), chronic Sprague-Dawley rats (n=19), and Fisher rats (n=8) during the acute post-injury period. Two different pairs of days are represented for the acute Sprague-Dawley rats. For all groups combined, $r^2=0.90$ for the straight alley and 0.74 for the open field.