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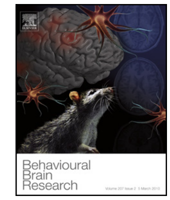

Single pellet grasping following cervical spinal cord injury in adult rat using an automated full-time training robot

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HIGHLIGHTS

- The single pellet grasping (SPG) task is used to study skilled forelimb movement.
- The SPG task is time-consuming and can yield results with high variability.
- Automated pellet presentation (APP) systems can train and test rats in the SPG task.
- We test whether rats with spinal cord injury can be trained using the APP system.
- Automation yields a bigger therapeutic windows and less variable results.

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ABSTRACT

Task specific motor training is a common form of rehabilitation therapy in individuals with spinal cord injury (SCI). The single pellet grasping (SPG) task is a skilled forelimb motor task used to evaluate recovery of forelimb function in rodent models of SCI. The task requires animals to obtain food pellets located on a shelf beyond a slit at the front of an enclosure. Manually training and testing rats in the SPG task requires extensive time and often yields results with high outcome variability and small therapeutic windows (i.e., the difference between pre- and post-SCI success rates). Recent advances in automated SPG training using automated pellet presentation (APP) systems allow rats to train ad libitum 24 h a day, 7 days a week. APP trained rats have improved success rates, require less researcher time, and have lower outcome variability compared to manually trained rats. However, it is unclear whether APP trained rats can perform the SPG task using the APP system after SCI. Here we show that rats with cervical SCI can successfully perform the SPG task using the APP system. We found that SCI rats with APP training performed significantly more attempts, had slightly lower and less variable final score success rates, and larger therapeutic windows than SCI rats with manual training. These results demonstrate that APP training has clear advantages over manual training for evaluating reaching performance of SCI rats and represents a new tool for investigating rehabilitative motor training following CNS injury.

1. Introduction

It is widely acknowledged that task specific motor training is a key factor in recovery of fine motor function after CNS injury or disease. Skilled reaching tasks are important research tools for studying motor recovery in animal models of nervous system injuries such as spinal cord injury [SCI; 1,2–7] and stroke [8–11]. There are a number of manually administered reaching tasks used to study forepaw function and deliver forepaw rehabilitation in rodent models of CNS injury or disease including the Montoya staircase test [12,13], the Whishaw tray task [14], the isometric pull task [15], and the single pellet grasping (SPG) task [16]. However, because these reaching tasks rely on individual researcher interaction with animals they are subject to high degrees of variability between experiments, researchers, and laboratories. Moreover, these tests can be time consuming and tedious

Abbreviations: SPG, single pellet grasping; APP, automated pellet presentation; SCI, spinal cord injury.

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to administer (e.g., SPG, Montoya, and isometric pull tasks), especially in animals with forelimb dysfunction, and/or provide limited insight to kinematics and mechanisms of recovery from injury or disease (e.g., well-grasping, Whishaw tray, and isometric pull tasks).

The SPG task is frequently used to evaluate motor function and reaching motions before and after cervical SCI. It has several advantages compared to other reaching tests for evaluating forelimb motor function after cervical SCI. For example, SPG training and testing can be limited to the forepaw affected by injury and detailed analyses of reaching and grasping motions are possible. Moreover, the SPG task is a complex motor task and the SPG motions are not regularly performed in the home cage. As a result, changes in SPG performance can be attributed to training and testing within the enclosure rather than self-training in the home cage, which has been proven a problem for locomotor training [17]. Yet despite its advantages manual administration of the SPG task requires extensive one-on-one researcher-to-rat training, which is time consuming and can be a source of variation between laboratories and from day to day within the same study. Also, manual training and testing of the SPG task before and after CNS injury or disease often results in small therapeutic windows (that is, the difference in SPG performance between pre-injury baseline and post-injury final scores), thus limiting the value to studies testing the effect of rehabilitative training or other treatments.

The role of rehabilitation training is growing in animal models partly because it is standard practice in the clinic, but also because there is mounting evidence drug and cell therapies are more effective when combined with rehabilitation therapy [2,18,19]. Given the limitations of current training methods there is a need for novel high-throughput and standardized training methods that accurately test the recovery of reaching and grasping function in animal models of CNS injury and disease.

We recently described an automated pellet presentation (APP) system to present pellets to rats 24 h a day, 7 days a week, which allowed APP trained rats to perform an automated version of the SPG task ad libitum [20]. Rats with APP training were successfully trained to perform the SPG task and employed similar grasping motions as manually trained rats. A key difference between the APP SPG task and the manual SPG task is that for the manual task pellets are presented from a small notch in a presentation shelf and can be scooped or dragged to the enclosure without grasping the pellet, whereas for the APP system there is a gap between the pellet presentation pedestal and the enclosure which precludes scooping or dragging pellets. Scooping and dragging is a common compensatory strategy used by rats with cervical SCI for obtaining pellets without grasping [21,22]. However, whether APP trained rats with SCI could perform the SPG task without access to compensatory scooping or dragging and whether limiting compensation improves functional recovery remains unknown.

The purpose of the present study was to explore whether rehabilitative APP training would allow for a simplified and more standardized training of the SPG task, thus opening the door for systematic exploration of the effects of task specific rehabilitative motor training in rats with SCI. For this we tested whether rats could perform the SPG task using the APP systems following unilateral cervical SCIs that affected forelimb motor function. We found that rats with APP training were able to obtain pellets using the APP system after SCI, but with lower success and attempt rates than pre-injury baseline. Importantly, APP trained rats had larger therapeutic windows than manually trained rats, indicating that APP training will be a useful tool for identifying treatment effects that could not be detected using manual training approaches.

2. Materials and methods

Eighteen female Lewis rats (Charles River Laboratories, Wilmington, MA, USA) weighing 210–240 g were trained to perform the SPG task either manually ($n = 10$ rats) or using an APP system ($n = 8$ rats). All animals were individually marked on their tails and housed in groups of 2–5 and kept on a 12/12 h light/dark cycle. Both manual and APP trained rats were housed in standard static home-cages with a PVC tube and small cedar block ($\sim 3 \times 3 \times 3$ cm) for enrichment. Additionally, the static cages of the APP trained rats had a small hole with a tube connecting the home-cage to the APP task enclosure. All procedures were approved by the Health Sciences Animal Care and Use Committee of the University of Alberta.

2.1. Manual SPG training

Manual SPG training followed the same training protocol as previously described [21,22] and is consistent with similar training protocols for this task [1,2,5,7,16]. Briefly, upon arrival to the animal facility the rats had ad libitum access to rat chow and water. Several days prior to the start of SPG training the average food intake per rat per day was measured. On the day prior to each training session food was restricted to 95% of the average food intake, usually between 9 and 11 g of food per rat per day, otherwise rats were fed ad libitum. Rats were weighed daily and their weight was maintained at about 95% the weight of ad libitum fed animals (c.f., APP trained animals who were fed ad libitum; Fig. 1E) by adjusting the amount of home-cage food provided.

To begin each training session, a rat was placed at the back of a standard acrylic SPG training chamber (40 cm long, 12.5 cm wide, 45 cm tall) with a 1 cm wide and 10 cm tall vertical slit in the front wall. Since each rat has a preference to use either their right or left paw to perform grasping tasks, the manual SPG task enclosures had two fixed pellet presentation wells located about 0.5 cm left and 0.5 cm right relative to the center of the slit. In the first few training sessions banana flavored sugar pellets (45 mg, TestDiet, 5TUT sucrose tab, St. Louis USA) were placed on both wells of the pellet presentation shelf at the front of the chamber. Once the rat had approached the front of the chamber and had completed a grasp attempt, the trainer placed a sugar pellet at the back of the chamber to encourage the rat to return to the back of the enclosure. The rat then returned to the back of the enclosure, another pellet was placed on the pellet presentation shelf, and the process was repeated for the entire session. Once the rat learned to shuttle, pellets were no longer placed at the back of the enclosure. Following completion of each training session the rat was returned to their home-cage. The preferred paw was determined by tracking which paw each rat used to obtain pellets. After a few training sessions, once paw preference was determined, pellets were presented in the left-well to rats with a right-paw preference, and vice versa.

All rats with manual training were trained to perform the SPG task for four weeks before SCI. One week following SCI, SPG training was continued for 6 weeks post-injury. For baseline and final scores in the reaching task the success rates of each animal were averaged for all training sessions in the final week before SCI and the final week of post-injury training respectively. With manual training SCI rats sometimes use compensatory strategies such as scooping or dragging the pellet to their mouth rather than grasping the pellet from the presentation shelf [21,23]. Since scooping is not possible with APP training, for this study scooped or dragged pel-