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Short communication

Spinal learning in the adult mouse using the Horridge paradigm

Devin L. Jindrich[a,b,*], M. Selvan Joseph[c], Chad K. Otoshi[c], Robert Y. Wei[c], Hui Zhong[c], Roland R. Roy[c,d], Niranjala J.K. Tillakaratne[c,d], V. Reggie Edgerton[c,d,e]

[a] Department of Kinesiology, 551 E. Orange Dr., Arizona State University, Tempe, AZ 85287-0404, USA
[b] Center for Adaptive Neural Systems, 551 E. Orange Dr., Arizona State University, Tempe, AZ 85287-0404, USA
[c] Department of Physiological Science, University of California, Los Angeles, USA
[d] Brain Research Institute, University of California, Los Angeles, USA
[e] Department of Neurobiology David Geffen School of Medicine at UCLA 73-235 CHS Box 951763 Los Angeles, CA 90095-1763, USA

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ABSTRACT

The spinal cord is endogenously capable of several forms of adaptive plasticity and learning, including functional re-training, instrumental, and Pavlovian learning. Understanding the mechanisms of spinal plasticity could lead to improved therapies for spinal cord injury and other neuromotor disorders. We describe and demonstrate techniques for eliciting spinal learning in the adult mouse using the Horridge paradigm. In the Horridge paradigm, instrumental learning occurs when a nociceptive leg stimulus is made to be contingent on leg position and the spinal cord learns to maintain the ankle in a flexed position. Using fine-wire intramuscular stimulating electrodes, an inexpensive real-time video tracking system, and DC current stimulation, we were able to elicit instrumental spinal learning from mouse lumbrosacral spinal cords that were functionally isolated from the brain. This technique makes it more feasible to use the powerful genetic manipulations available in mice to better understand the processes of spinal learning, memory, and plasticity.

1. Introduction

Spinal cord plasticity is critical for restoring sensorimotor function after spinal cord injury (Edgerton et al., 2008). Even complete, spinal cord transected mammals can be re-trained to perform different tasks with the hind legs, including standing and stepping, and to overcome perturbations to normal locomotion (Heng and de Leon, 2007). Although simple plastic processes such as habituation or sensitization could contribute to re-training, the spinal cord is also capable of more complex instrumental learning (Grau et al., 2006). Consequently, understanding the mechanisms of spinal plasticity and learning is important for improving strategies for restoring function after spinal cord injury.

A useful technique for eliciting spinal learning was developed by Horridge (1962a,b). The legs of headless insects exposed to the noxious stimulus of an electric shock exhibit a flexion withdrawal reflex. If the leg shock was made contingent on leg position by only occurring if the leg dropped below a constant threshold, after 15–30 min the legs held themselves above the threshold. The threshold was implemented by placing a saline solution at a set distance below the leg. When the leg contacted the saline solution, it completed a circuit and received a shock. To verify that the elevation of the leg was due to learning, a second animal was yoked to the first (master) animal, i.e., the leg in the yoked animal was shocked simultaneously with the leg of the master animal regardless of its position. Shock was not contingent on leg position for the yoked animal. Whereas legs receiving contingent leg shock in the master animal showed sustained elevation, the legs of the yoked animals did not, demonstrating that the elevation response was not an artifact of stimulation but reflected learning.

The Horridge paradigm has been used to study spinal learning in many different animals, including insects, rats, and frogs (Buerger and Chopin, 1976; Horridge, 1962a,b). Most recently, the experiments of Grau and colleagues have used the method to better understand the physiological and biochemical mechanisms underlying spinal learning, and to relate these findings to therapies such as step training and functional electrical stimulation (Bigbee et al., 2007; Ferguson et al., 2008; Hook and Grau, 2007).

The laboratory mouse is currently an important model organism for studying the molecular mechanisms of neural function due to the wide array of genetic manipulations that have been developed in mice (Zheng et al., 2006). Consequently, we sought to develop an experimental system to study spinal learning in the mouse using the Horridge paradigm. We show that by using fine-wire electrodes, an inexpensive real-time video point-tracking system, and DC current stimulation, it is possible to elicit spinal learning in adult spinal mice.

* Corresponding author. Tel.: +1 480 727 7382; fax: +1 480 965 8108.
E-mail address: devin.jindrich@asu.edu (D.L. Jindrich).

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2. Materials and methods

We closely followed the procedures described by Grau et al. (1998). All surgical and experimental procedures in these experiments were approved by the Chancellor's Animal Research Committee. The spinal cords of 4-week-old Swiss-Webster mice ($N = 68$) were completely transected at the T7–T8 vertebral level as described previously (Roy et al., 1992). Briefly, under 2% isoflurane anesthesia, a dorsal midline skin incision was made from T6 to T9 and the musculature covering the dorsal vertebral column was removed to expose the spinal laminae. A partial laminectomy of the T7 and T8 vertebrae was performed to expose the spinal cord. The spinal cord, including the dura, was transected completely using stainless-steel microscissors, and the completeness of the lesion was verified by separating the cut ends of the spinal cord with small cotton pellets and passing a fine glass probe through the lesion site. The skin incision was closed using small surgical staples. After surgery, the wound sites were treated with triple antibiotic ointment (Bacitracin) and the mice were given lactated Ringer's solution (1.5 ml/30 g body weight) sub-cutaneously. Preventing leg extension during recovery has been shown to facilitate subsequent learning in rats. Consequently, the hindlegs were bound with the knee and ankle joints fully-flexed (Grau et al., 1998). The animals recovered in an incubator maintained at 37 °C until fully awake and then returned to their home cages. Opioid analgesics (e.g., Buprenex) commonly used following surgeries can negatively affect learning (Hook et al., 2007). Consequently, the mice were given acetaminophen (300 mg/kg body weight, orally) as an analgesic (Jenkins, 1987), and allowed to recover for 20–24 h before testing.

The general design of the experimental setup is shown in Fig. 1. Two mice, master and yoked, were tested simultaneously during each trial. The mice were restrained loosely in small cloth harnesses (Fig. 1C). The front of each harness was closed, providing a dark enclosure in which the mice could rest undisturbed. Two slots were cut from the end of the harness, allowing both hind legs to hang freely. To minimize the effects of upper body movements on leg position, the midsection of each mouse was secured gently by using a clip to snugly close the harness. The hind legs were shaved using a small electric clipper (#50 clipper blade) and colored black using a non-toxic marker to optimize camera tracking. A small white mark was placed on the fifth metatarsal-phalangeal (MTP) joint using non-toxic paint (Fig. 1C inset).

Fine-wire hook electrodes were constructed by removing the insulation from the tips (~1 mm) of 50 μm nylon-insulated single-strand stainless steel wires (California Fine Wire Co., Grover City, CA). The skin over the tibialis anterior (TA) muscle was probed with a needle electrode to find the point most sensitive to electrical stimulation, i.e., where a single shock elicited the largest muscle twitch response as assessed visually. This point was typically ~1 mm distal to the ventral aspect of the knee and laterally adjacent to the tibia. Using a 32-gauge needle, one electrode (anode) was inserted into the TA as close as possible to the identified point. A second electrode (cathode) was attached to the dorsal surface of the foot adjacent to the lateral malleolus (Fig. 1A). To attach this electrode, a small bolus of Spectra 360 electrode gel (Parker Laboratories, Inc., Fairfield, NJ) was placed on the dorsal surface of the paw, and the de-shielded tip of the electrode inserted into the gel. A thin coating of rubber cement was used to cover the gel and electrode, and to secure the electrode wire to the dorsal surface of the foot. The electrodes were attached to a Stimulator (S88, Grass Product Group; W. Warwick, RI), through a stimulus isolation unit (SIU5) and a constant current isolation unit (CCU1). In contrast to the AC current used by Grau et al. (1998), the stimulator used for these experiments used 50 ms square wave direct current pulses.

After the electrodes were implanted, we conducted a series of stimulation trials to establish the relationship between current and force for each animal. Only moderate levels of stimulation have been shown to elicit spinal learning in rats (Grau et al., 1998). Consequently, we sought to stimulate the mice at levels approximately one-half of the amount of current necessary to produce a maximum contraction. The animals were suspended by firmly attaching the harness clip to a horizontal bar in a position where ankle dorsiflexion resulted in nearly vertical movement of the MTP marker. One end of a piece of thread was tied firmly around the distal end of the metatarsals immediately proximal to the MTP joint, and the other end was attached to a force transducer (Dual Mode Muscle Lever 300BLR, Aurora Scientific Inc., Aurora, Ontario, Canada). The position of the transducer was adjusted so that the thread was taut, but the force did not exceed ~0.1 N. Force resulting from a series of single 50 ms square-pulse stimuli from 0 to 1.0 mA, in increments of 0.1 mA, with a 30 s delay between each stimulus, were recorded and displayed by a custom-written LabVIEW program. When the stimulus series was complete, the thread was cut and removed from the foot.

The two-dimensional position of the foot marker was monitored using an inexpensive video-based point tracking system (CMUCam2; Carnegie Mellon University; Fig. 1D). The CMUCam2 can perform automatic, hardware-based point tracking of region fitting user-specified color and size criteria and report the centroid of the region to the computer. The CMUCam2 communicated with a computer using a serial connection. For these experiments, custom software was written in LabVIEW (National Instruments, Inc.; available at http://www.limblab.org/publications/code/learning.zip) to control the CMUCam2 and collect tracking data from the camera. Full-frame images from the camera were captured and used to correctly position the foot marker in the field of view of the camera. The software then calculated the range of colors associated with the marker. We found that using a high-contrast (white on a black background) marker produced the best results. By identifying all pixels in the image within the defined color range, we then could remove any regions on the animal or in the background that could interfere with marker tracking.

Once we established the desired parameters, they were used to configure point tracking by the CMUCam2 and the LabVIEW software. The speed of point tracking by the CMUCam2 is limited by the image size, but we found that slightly less than half-size images (125×125 pixels) were adequate to measure the foot motions, and could be collected at a sampling frequency of 30 Hz. The camera had a field of view of 15 mm × 15 mm, or a resolution of 8 pixels/mm with the camera positioned 40 mm from the foot.

After the stimulation parameters were set, a resting position was defined as the foot position following 3 rapid priming stimulations. Muscle stimulation could cause an increase in resting position, and the priming stimuli allowed for determination of a resting position in a post-stimulus state. Assigning a stimulation threshold position, i.e., the vertical distance above the resting position that the foot must exceed to prevent shock, is very important. The threshold determines the difficulty of the task that the spinal cord must learn. Basing the appropriate threshold on the methods used for rats is complicated by two issues. First, mice are approximately one-tenth the weight, and one-half the linear dimensions of rats. Second, Grau et al. (1998) measured the position of a rod attached to the foot of the rat. In this configuration, movements of the rod will be larger than movements of the foot itself. Consequently, we estimated that the appropriate threshold for mice should be at least one-half the 2 mm threshold used for rats to measure comparable learning behavior. We used a threshold of 1 mm above the resting position, which was set for both the master and yoked mice with a threshold parameter in the LabVIEW program.