Long-term spinal reflex studies in awake behaving mice

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Abstract

The increasing availability of genetic variants of mice has facilitated studies of the roles of specific molecules in specific behaviors. The contributions of such studies could be strengthened and extended by correlation with detailed information on the patterns of motor commands throughout the course of specific behaviors in freely moving animals. Previously reported methodologies for long-term recording of electromyographic activity (EMG) in mice using implanted electrodes were designed for intermittent, but not continuous operation. This report describes the fabrication, implantation, and utilization of fine wire electrodes for continuous long-term recordings of spontaneous and nerve-evoked EMG in mice. Six mice were implanted with a tibial nerve cuff electrode and EMG electrodes in soleus and gastrocnemius muscles. Wires exited through a skin button and traveled through an armored cable to an electrical commutator. In mice implanted for 59–144 days, ongoing EMG was monitored continuously (i.e., 24 h/day, 7 days/week) by computer for 18–92 days (total intermittent recording for 25–130 days). When the ongoing EMG criteria were met, the computer applied the nerve stimulus, recorded the evoked EMG response, and determined the size of the M-response (MR) and the H-reflex (HR). It continually adjusted stimulation intensity to maintain a stable MR size. Stable recordings of ongoing EMG, MR, and HR were obtained typically 3 weeks after implantation. This study demonstrates the feasibility of long-term continuous EMG recordings in mice for addressing a variety of neurophysiological and behavioral issues.

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1. Introduction

Identification of the sites and mechanisms of the neuronal plasticity underlying learning and memory requires study of animals during the acquisition and maintenance of defined behaviors. Acute studies require anesthetized or surgically reduced preparations, which are incapable of expressing the behavior of interest and/or afford periods of study too brief to capture the time course of gradually evolving behaviors. These factors limit the utility of acute studies in connecting specific mechanisms of plasticity with specific behaviors. More complete information can be gleaned and greater insight achieved when behavior is studied continuously in unrestrained animals. Chronic recording of electromyographic activity (EMG) and nerve stimulation using implanted electrodes can provide information on motor activity in awake freely moving animals. The reduction in stress and other perturbations permits recording during naturally occurring motor (Belanger et al., 1996; Fortier et al., 1987; Hoffer et al., 1987; Leblond et al., 2003), respiratory (Trelease et al., 1982), sexual (Affanni et al., 2001; Miura et al., 2001), and gastrointestinal (Atobe et al., 1980; Dwinell et al., 1994) behaviors in rats, cats, primates, and other species. In addition, the ability to repeatedly or continuously stimulate nerve and/or record muscle activity for extended periods in the same animal permits powerful within-subject experimental designs.

The continuing development of new mutant and transgenic mice provides powerful tools for exploration of the mechanisms underlying memory and learning (Morgan, 2003; Vaillant et al., 2002). Such tools are ideally suited to
establishing links between specific behaviors and molecular mechanisms. Application of these tools to chronic studies has been limited by the lack of continuous recording methodologies in mice. Implantation and data transmission hardware used in earlier studies involving chronic nerve stimulation and/or EMG recordings in mice were designed for brief epochs of stimulation and/or recording at periodic intervals with the mice disconnected between recording sessions (Leblond et al., 2003; Milner and Hoffer, 1987; Valatx, 1971; Warren et al., 1998). Development of very lightweight nerve cuffs, wires, and tethers to permit long-term continuous (i.e., 24 h/day, 7 days/week) instrumentation of the animals would greatly facilitate joint application of these two powerful methodologies.

Continuous chronic stimulation and recording technology has been an essential tool in our laboratory’s study of learning and memory in the spinal cord using an H-reflex (HR) operant conditioning paradigm (Wolpaw and Tennissen, 2001). Originally developed in non-human primate (Wolpaw, 1987) and subsequently transferred to rats (Chen and Wolpaw, 1994, 1995) and humans (Wolf and Segal, 1990), HR conditioning is associated with altered motoneuron excitability and other spinal cord plasticity. Exploring the mechanisms underlying HR conditioning using genetic variants (which are not widely available in rats) would require transferring our HR conditioning paradigm to mice. As the first step in establishing the HR conditioning paradigm in mice, this report describes methodology for construction and implantation of EMG electrodes and a stimulating nerve cuff in the mouse. It also demonstrates the feasibility of continuous long-term recording of spontaneous and stimulus-evoked EMG. Portions of this study have been reported previously (Carp et al., 2004).

2. Materials and methods

2.1. Implant and tether assembly

The cable assembly consists of a short implanted segment and a longer external segment that connects the animal to the recording equipment (Fig. 1A). Each assembly is constructed from Teflon-insulated stainless steel wires (iw) forming two pairs of EMG wires and one pair of stimulating wires in a nerve cuff (cu) that are braided together and woven into a biocompatible mesh (me). The braided wires are secured to the mesh with sutures (su) and with silicone medical adhesive (gl). The uninsulated end (uw) of the insulated wire (iw) penetrates the cuff (cu), forming an omega-shaped electrode that is secured to the cuff with three sutures (su) and silicone medical adhesive (gl). A second suture wrapping around the cuff and sewn next to the edges of the longitudinal slit is used to close the cuff around the nerve (cc). The braided insulated wires (iw × 6) descend through the spring (sp), weave through the mesh (me), and travel caudally above the back muscles subcutaneously. The incision for introducing the mesh is closed with a purse-string suture tightened around the spring. Four sutures (su; only two shown for clarity) anchor the subcutaneous mesh to the external skin button (bu).
To construct the stimulation cuff (Fig. 1A, with enlarged views in insets A1 and A2), two wires are trimmed by 1 cm, and the two other wires are used for the stimulation cuff. The wires are secured by suturing them to the mesh with 6/0 polyester mesh (Mersilene, Ethicon, Somerville, NJ). Four of the six wires are designated for recording electromyographic activity (EMG), and the two other wires are used for the stimulation cuff.

To design and external portions are sutured together with 7/0 silk suture to travel along the long axis of the cuff. The two wire leads are sutured to the cuff at a point 0.5 mm from the end. Excess stripped wire protruding from the other side of the cuff is trimmed and all external wires and sutures are insulated and secured with medical adhesive. After the adhesive cures, the cuff is slit longitudinally along one side between the gaps in the electrodes. A 7/0 nylon suture is sewn around the back of the cuff at its midpoint through each of the slit edges. Two 2-cm lengths of suture are left protruding beyond the cuff to facilitate application and closure of the cuff around the nerve.

The ends of the >30-cm lengths of wire emerging from the mesh (i.e., the connector end of the assembly) are temporarily attached to a thin stainless steel wire with 7/0 nylon sutures and cyanoacrylate glue, and carefully pulled through a 30-cm-long x 1.5 mm o.d. x 1.0 mm i.d. stainless steel spring (0.25 mm wire diameter). After detaching the guide wire, the spring is secured to the mesh by spiraling the cut spring end through the holes in the mesh and gluing it in place with the medical adhesive. A skin button made from a nylon washer (8.7 mm o.d. x 2.6 mm i.d.) with eight 0.4-mm diameter holes around its perimeter is then threaded onto the cable assembly from the connector end.

The wires extending beyond the spring at the connector end are trimmed to about 1 cm, stripped, and crimped into small gold connectors (E63-0, Plastics One, Roanoke, VA). The free end of the spring is straightened to match the length of the wires and is crimped into the same type of connector. The uncrimped ends of the seven connectors are slid onto the pins of an 8-position circular transistor socket and soldered. The connector end of the cable is then potted in epoxy from the wired side of the circular socket to the first 5 mm of the spring to protect the fine wires and prevent the cable from twisting when the animal moves about its cage.

The portion of the cable assembly directly attached to the mouse (i.e., nerve cuff, wires, mesh, and skin button) weighs 0.12 g. The entire cable assembly weighs about 3.5 g, but this load is largely supported by the recording hardware from which it is suspended. Based on the horizontal load vector when the mouse maximally deflects its cable plus implant and slack cable weight, we estimate the maximum load experienced by the animal to be ±0.9 g.

2.2. Animals and preparation

Mice (Swiss Webster, male, 9–13 weeks) are each implanted with a nerve cuff on the right tibial nerve, a pair of recording electrodes in the gastrocnemius muscles (GAS; one wire in the medial head and one wire in the lateral head) to record spontaneous EMG and evoked responses. All animal procedures are in accordance with the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Academy Press, Washington, DC, 1996), and Department of Health, Education and Welfare (DHEW) Publ. No. 0309-05377-3, “Guide for the Care and Use of Laboratory Animals.”

Surgical procedures are modified from those described for use in rats (Chen and Wolpaw, 1994, 1995). Mice are anesthetized with a mixture of ketamine and xylazine (120 mg/kg and 8 mg/kg i.p., respectively) supplemented as needed to maintain deep surgical anesthesia. Additional pre-surgical injections include glycopyrrolate (0.02 mg/kg i.p.) and penicillin G (10,000 units/kg i.p.). The head is secured with a nose clamp and tooth plate, and medical tapes hold the right hindlimb in an extended position. Body temperature is maintained using a heating pad under the animal and by radiant heat from a lamp above the animal. A thin layer of petroleum jelly on the eyes covered with gauze prevents desiccation during surgery.

Surgical techniques are used throughout surgery. The areas for incision (i.e., the dorsal and lateral aspects of the right hindlimb and the midline of the upper back) are shaved and the skin is cleaned with 70% ethanol and an iodine-based antiseptic solution (Xenodine, Veterinary Products Laboratories, Phoenix, AZ). The areas surrounding the incision sites are covered with sterile drapes.

A 5 mm long incision is made at the nape of the neck and a purse-string suture is inserted loosely around its perimeter for later use. This incision permits introduction of six wires from the implanted end of the cable, which travel under the skin above the back and thigh to emerge at the incision point. The wires are brought out of the skin above the back and thigh to emerge at the incision point.
exposing the lower half of the back of the right thigh. For eliciting the H-reflex, the tibial nerve is exposed, carefully isolated from the sural and peroneal nerves, and encircled by the silicone rubber nerve cuff just proximal to the GAS. For recording EMG, the ends of the four stainless steel electrodes are stripped of insulation, twisted, inserted into a 30-ga needle, and bent to form a hook; they are then inserted in pairs in the median and lateral GAS (3–4 mm stripped length) and in the SOL (2–3 mm stripped length). After electrode implantation, the subcutaneous incision is closed with 7/0 nylon sutures. All wires are sutured to muscle fascia about 1–2 cm rostral to their implantation sites to provide strain relief and to minimize the pressure exerted by the wires on the overlying skin. The incision area is flushed with saline and 1–2 drops of a long acting local anesthetic (bupivacaine; 0.25%) is applied to the incised tissue. The skin incision is closed with tissue adhesive (Nexaband, Closure Medical Corp., Raleigh, NC) and painted with nitrofurazone ointment.

At the cable exit site, each 6/0 nylon suture is used to secure the mesh by which the wires are anchored to the skin. This is accomplished by inserting a 25-ga needle through skin, mesh, and then skin again. After introducing a suture through the needle, the needle is removed, leaving the suture in place. The process is repeated for the other three sutures. The exit site is flushed with sterile saline and installed with 1–2 drops of the local anesthetic. The skin incision at the nape of the neck is closed with a purse-string suture around the spring. Nitrofurazone is applied to the incised skin edges. Each pair of four suture ends is passed through adjacent holes in the skin button and tied to secure the mesh to the skin of the back. The knots were then lightly coated with tissue adhesive to prevent slippage.

After surgery, the mouse receives an analgesic (Demerol, 3 mg/kg i.p.) and is transferred to a recovery cage. The custom-built cages are 30.5 cm high on an octagonal base (16–17 cm between opposing sides; 209–243 cm² floor space). Compared to standard rectangular cages, the near-circular footprint and increased height-to-width ratio minimizes cable slack when the mouse is in the center of the cage. The cage is placed on a thermostatically regulated warming table to maintain the litter at 30–32 °C for 3–4 h. Because the cable is temporarily attached to the mouse (i.e., no connector at the skin button), a fluid bearing positioned atop the cage supports the connector end of the cable. The bearing consists of a buoyant inner ring floating in a slightly larger water-filled outer ring. The cable passes through a hollow tube in the center of the outer ring to attach to a mating connector secured to the center of the inner ring. This device permits the mouse to turn freely with its attached tether in its cage with minimal torque demands.

Each mouse receives penicillin G on days 2 and 4 post-surgery. For the first two post-surgery days, chow softened by soaking in water is placed in each cage. Mice that do not eat either wet or dry chow receive a nutritional supplement (Nutri-Cal, Evsco Pharmaceuticals, Buena, NJ). Mice are monitored for signs of stress or discomfort, and receive analgesia (acetaminophen at 20 mg per 100 ml drinking water) whenever necessary. Mice showing signs of dehydration (e.g., weight loss, skin tenting) receive saline s.c.

2.3. Data collection and analysis

Once mice exhibit normal feeding, grooming, and locomotor behaviors, the fluid swivel atop the cage is replaced with a low-torque electrical commutator (SL-88-10, Dragonfly R&D, Ridgeley, WV). The wires connect via the commutator to amplifiers (gain = 1000, bandwidth 10–3000 Hz) and an isolated stimulator.

Data are collected using PC-based multifunction boards (National Instruments, Austin, TX). The software system for data acquisition and analysis (ELIZAAN 3.0, based on prior versions used for controlling monkey and rat experiments (Chen and Wolpaw, 1995; Wolpaw and Herchenroder, 1990)) was written in-house (G.S.) to run the online experimental protocol and provide offline analysis capabilities. One multifunction board is dedicated to each animal. The online software controls nerve stimulation and gathers EMG signals from the animals continuously (i.e., 24 h/day, 7 days/week). Cage and animal maintenance require only brief interruption of data collection. Data are stored using the open-source database mySQL. Additional components permit offline extraction of data from individual trials or averaging of data for any period from any animal while online data collection continues. In its present configuration, each animal’s data collection system supports up to two stimulus channels and up to 16 EMG channels at sampling rates ≥10kHz/channel for multiple animals. The amount of data that can be collected and stored is limited only by available hard drive space.

Each online data acquisition system monitors the absolute value of the ongoing EMG 24 h/day. When the ongoing EMG remains within an operator-defined range for 3.0–3.6 s, the computer stores the most recent 50 ms (defined as the background EMG (HEMG) interval), delivers a tibial nerve stimulus, and continues to collect and store EMG for another 100 ms. The data stored in the database for each evoked response defines a single trial. The computer calculates the average absolute value of the EMG in the M-response (MR) interval (typically 1–3 ms post-stimulus) and the HR interval (typically 4–6 ms post-stimulus). Time intervals include approximately 90% of the center of the MR and HR EMG envelopes (see examples of time ranges used in one animal in inset of Fig. 2). The computer then increments or decrements the D/A converter that controls the stimulus amplitude, depending on whether the MR is below or above a target value. This provides feedback control over the size of the MR, such that the computer regulates the MR size by adjusting stimulus intensity. Typically, we selected a target MR value corresponding to a stimulation level at which the HR is maximal (see below). The average MR amplitude ± standard deviation (S.D.) produced by this feedback-control method was 26 ± 15% (range, 16–48%) of the maximum MR amplitude (determined in four animals from the average of the five
The purpose of this study was to develop methodology in unanesthetized, unrestrained mice for performing continuous (i.e., 24 h/day, 7 days/week) recording of spontaneous EMG and to elicit and record the HR. Towards this end, we have implanted each of six mice with a tibial nerve cuff and GAS electrodes. All animals recovered quickly from surgery, exhibiting normal behaviors (e.g., grooming, locomotion, rearing, and climbing the bars of its food hopper with all four limbs) within 24 h. No motor impairment was observed during quadrupedal stance or locomotion. However, immediately after surgery, two of the six animals did not appear to support their body weight fully with the operated hindlimb during rearing and climbing. This deficit disappeared within 1 week after surgery.

The mice weighed 36–59 g at time of surgery. Body weight decreased between 2 and 7% within the first 4 days after surgery. Body weight stabilized within 10 days in four mice; in the two remaining mice, it stabilized at 16–18% below pre-surgery weight after 4–6 weeks. After body weight stabilized, it increased again in all six mice. One mouse irreparably damaged its skin button and implanted wires, and was euthanized 60 days after surgery.

3. Results

3.1. Recovery from surgery

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3.2. Daily HR recording

Recordings were initiated in all mice within 2 weeks after surgery. Data were recorded from SOL in all six mice and from GAS in five mice (a large stimulus artifact precluded analysis of GAS data in one mouse). Stable recordings (i.e., <10% variation in mean daily hEMG and <25% day-to-day variation in the mean daily MR) were obtained beginning from 17 to 39 days (median 20 days) after surgery. After stabilization, a 10-day period was selected for each mouse for further analysis. After collecting these baseline data, the mice entered other studies. Data continued to be recorded from each mouse for a total of 18–92 days (median 34 days). Recordings were continued intermittently, spanning a total of 25–130 days (median 64 days). In all, the six mice carried their implants for 59–144 days (median 97 days). During the 10-day baseline period, an average of 3643–11045 trials/day were recorded in each animal (median 7111). The means of each animal’s latency to the beginning and the end of the HR interval were 6.2 (±0.3) ms, respectively. The means of each animal’s latencies to the beginning and the end of the HR interval were 6.2 (±0.3) ms, respectively.

In the four mice in which the maximum MR was assessed (see Section 2.3), the maximum HR was calculated as the value at the 99th percentile of the distribution of HR values during each animal’s 10-day baseline period. The mean HR:MR ratio ± S.D. was 0.22 ± 0.01 (range, 0.20–0.23).
Table 1 shows two measures of day-to-day reproducibility of the mean daily bEMG, MR, and HR for SOL and GAS for all six mice recorded during the 10-day period after stabilization: the average coefficients of variation (i.e., S.D./mean) and the intraclass correlation coefficient (as assessed using SPSS 10.0, using the 10 daily averages as the within-subject measurements). Day-to-day variability of the SOL bEMG is low, as is that of the SOL MR. These results reflect the requirement for stable background EMG within a defined range and the computer’s control over the SOL MR (see Section 2).

The average daily variation in SOL HR is larger than that observed in the SOL MR. Fig. 3 shows average evoked responses from SOL from one mouse recorded on 3 days at 5-day intervals. Each trace represents the average of all trials within a narrow range of bEMG values (3568–4798 trials in each day out of the 9241–12468 total daily trials). This bEMG restriction allows comparison of the daily average responses at nearly identical levels of background excitation (i.e., the pre-stimulus EMG levels are matched). These data illustrate the small day-to-day variation in the MR and the modest day-to-day variation in the HR.

Table 1 also shows that the GAS bEMG also exhibits low variability. The GAS MR and GAS HR vary more than do SOL MR and SOL HR, which is consistent with the independence of GAS MR from computer control (i.e., the computer adjusts the nerve stimulus in response to the SOL MR).

3.3. Dependence of HR on bEMG and MR

The natural variation in bEMG and MR permitted evaluation of the dependence of the HR on these two factors. Because we were not able to normalize each animal’s EMG data to its maximum MR (determined in only four of the six mice; see Section 2.3), we adopted an alternate normalization procedure based on the z-transform to address inter-animal differences in recording conditions. The bEMG data from each animal were normalized by subtracting the animal’s mean bEMG value, and then dividing by the S.D. of the animal’s bEMG. The resulting distribution of normalized bEMG values had a mean = 0 and a S.D. = 1. The MR and HR data from each animal were normalized simply by dividing by the S.D. of the animal’s mean MR and HR values, respectively.

The average values of MR and HR were not subtracted from each of the individual MR and HR values in order to preserve the meaning of the point where MR = 0 and HR = 0, i.e., the thresholds for the MR and HR. The normalized data were then sorted and binned into groups with equal numbers of trials for several ranges of bEMG and MR. The means of each of these groups defined by the bEMG-MR range combinations were averaged for all six animals.

The average normalized HR amplitudes of all six animals are shown as a function of eight different ranges of average normalized MR amplitudes for the three different levels of normalized bEMG in Fig. 4A. Because the bEMG has been subtracted from the MR, the zero point on the abscissa is by definition the MR threshold, i.e. the input level just above which the first motor axon is activated by the stimulus (dotted vertical line in Fig. 4A). Note that the HR and MR values, which were normalized by dividing by each animal’s S.D., are in units of S.D. (e.g., an HR value of 0.5 is half of one S.D. above the threshold value). Negative (i.e., subthreshold) MR values on the abscissa reflect the random variation in EMG during the bEMG and MR intervals.

In Fig. 4A, the HR is evident at stimulus intensities at or below motor threshold, which presumably reflects a lower...
Fig. 4. Dependence of SOL HR upon bEMG and MR. For each animal, normalized (see text for description of normalization procedure) bEMG, MR, and HR values from individual trials during the 10 days of stable recordings were binned into subsets of the data pool according to the magnitude of their MR and bEMG values. (A) SOL HR values ± S.E. are shown as a function of MR octiles at three different levels of bEMG (defined in legend in figure) for a total of 24 data subsets. For increasing levels of bEMG, the input–output relationship shifts to the left for values of MR at or below motor threshold (i.e., MR ≤ 0; dotted line at MR = 0). For input levels above motor threshold (i.e., MR > 0), the HR increases with increasing MR at low bEMG levels, but reaches a plateau with higher bEMG levels. (B) SOL HR values ± S.E. are shown as a function of bEMG octiles at three different levels of MR (defined in the figure legend) for a total of 24 data subsets. At low or intermediate levels of MR, the HR increases with increasing bEMG to a plateau. For all levels of MR, the HR decreases with further increases in bEMG.

average threshold of Ia afferent fibers than that of motoneuron axons. At these low input levels, increasing bEMG is associated with a leftward shift in the input-output curve (i.e., less input is required to evoke the HR as background activity increases). The leftward shift is more pronounced between the lowest and middle bEMG ranges than it is between the middle and highest bEMG ranges. The HR increases with increasing suprathreshold MR values, and then tends to decrease again at the highest MR values. This presumably reflected a reduction in the HR due to the increased recruitment of motor units contributing to the MR and to the bEMG and/or increased recurrent inhibition (Ashby, 1995). Data on the HR at higher MR levels where the HR is expected to decrease substantially or at lower input levels near HR threshold (see Fig. 2) is not available using this mode of data collection. The computer’s control over stimulus intensity that maintains the MR near the target value prevents mapping the uppermost and lowermost regions of the MR–HR input–output relationship.

Fig. 4B shows the average HR amplitudes of all six animals as a function of eight different ranges of average bEMG amplitudes for the three different levels of MR amplitudes. At low and intermediate levels of MR, the HR increases with increasing bEMG to a plateau, and then decreases at the highest bEMG levels. At high levels of MR, the HR is already large, even at very low levels of bEMG, but decreases rapidly.
HR trials were not evenly distributed throughout the day, but rather exhibited a marked diurnal variation. Fig. 5A shows the average daily time course of the number of trials collected every hour (expressed as a percentage of the daily mean number of trials per hour). There were significant differences in number of trials collected per hour among the 24 different time periods ($p < 0.0001$ by ANOVA). Ongoing EMG met the criteria for initiation of an HR trial most often in two time periods: during the dark between 00:00 and 04:00 h, and during the light between 09:00 and 11:00 h. Fewer than average numbers of trials per hour were collected between 04:00 and 08:00 h and between 13:00 and 18:00 h. Fig. 5B shows a similar diurnal variation in the average bEMG amplitude recorded just prior to nerve stimulation in SOL (filled circles) and GAS (open circles). There were significant differences in bEMG in both muscles among the 24 different time periods ($p < 0.0001$ by ANOVA). The pattern of diurnal variation in number of trials collected and in bEMG amplitude is similar to that in locomotor activity in mice (Ticher and Ashkenazi, 1995).

The diurnal variation in bEMG was consistent from day to day. The average coefficient of variation ± S.D. of bEMG for all 24 of the 1 h time ranges was 0.06 ± 0.02 for SOL (range: 0.03–0.11) and 0.08 ± 0.03 for GAS (range: 0.04–0.16). This level of variability is comparable to that seen for day-to-day variability in MEMG (see Table 1). The diurnal variation in the number of trials collected per hour was less consistent. The average coefficient of variation ± S.D. of the number of trials collected for all 24 of the 1 h time ranges was 0.40 ± 0.17 (range: 0.17–0.80).

4. Discussion

This report describes long-term continuous recording of spontaneous and nerve-evoked EMG in mice. Fabrication and implantation of the chronic stimulation and recording cable assembly is straightforward and readily implemented. Our custom software continuously monitors ongoing EMG 24 h/day, 7 days/week, stimulates the tibial nerve, records the evoked response, calculates the average size in the bEMG, MR, and HR intervals, and continually adjusts stimulus intensity to maintain the MR at a given size. The bEMG, MR, and HR stabilize after typically a 3-week recovery period. The implants remained viable for ≥2 months in all six animals, and ≥3 months in three animals.

The development of methodology for continuous recording in mice is advantageous for studies of learning and memory, and for behavioral studies in general. It provides detailed information on muscle activity during motor behavior. Data can be collected over prolonged periods, eliminating disruptions to animal behavior due to stress associated with handling or anesthesia during intermittent recording methodologies. These advantages permit comprehensive study of the acquisition of ongoing behaviors that would be difficult or impossible using intermittent recording techniques (e.g., diurnal variation in spinal reflexes (Chen and Wolpaw, 1994; Dowman and Wolpaw, 1989; Wolpaw and Seegal, 1982)). Of even greater benefit may be the potential for combining long-term continuous recording with the tools of molecular biology that have been developed primarily in mice. The ongoing development of mutant and genetically manipulated mice continues to offer new ways to probe the mechanisms underlying learning and memory (Morgan, 2003; Vail lend et al., 2002).

Development of continuous recording methodology in mice specifically benefits our studies of spinal cord plasticity by enabling development of the HR conditioning paradigm in mice. Use of genetically modified mice in this paradigm could help to identify the molecular basis underlying operantly conditioned change in the HR. In addition, application of the recently developed slice preparation of mouse spinal cord (Carp et al., 2003; Hirt et al., 2001) will permit us to perform in vitro recordings from the spinal cord at any point during HR conditioning.

The present study is the first report of spinal reflex recordings in unrestrained awake mice. Thus, direct comparisons cannot readily be made between the present study and previous studies in mice, all of which describe H-reflex recordings from other muscles in anesthetized or sedated and restrained mice (Chandran et al., 1991; Elias et al., 1998; Turski and Stephens, 1993). For example, the ratio of the maximum HR to the maximum MR in the present study in SOL (0.22) was lower than that reported previously for intrinsic muscles of the foot in mice (0.61 in Chandran et al., 1991). The HR:MR ratio can vary widely among muscles, typically being higher in muscles with a higher proportion of slow-twitch fibers (Messina and Cotrufo, 1976; Palmieri et al., 2002). The higher HR:MR values in the latter muscles could reflect stronger afferent input to their motoneurons and/or greater intrinsic motoneuron excitability. The SOL fiber-type composition of the Swiss-Webster mice used in the present study is unknown. The percentage of SOL muscle fibers with myosin heavy chain type I (i.e., slow-twitch) varies from 20 to 70% among other strains of mice (Carlson et al., 1999; Stelzer and Widrick, 2003). Thus, inter-strain and/or inter-muscle differences in motor unit type distribution could account for the observed difference in the HR:MR ratio between the present study and that of Chandran et al. (1991).

It is not surprising that the HR exhibits modest day-to-day variability. This degree of variability exceeds that seen in recumbent humans, but is comparable to that seen in standing humans (Hopkins et al., 2000; Palmieri et al., 2002). Unlike the well-controlled clinical setting, no postural restrictions...
are placed on the animal during recording other than those resulting indirectly from the limitations imposed on on-going EMG. The HR variability is evident even with on-line computer control of data collection and stimulus intensity and off-line matching of MEMG and MR values. This presumably reflects variation in the supraspinal control over pre-motoneuronal elements (e.g., presynaptic inhibition ofafferent input to spinal motoneurons (Capaday, 1997)) or over intrinsic motoneuron properties that affect reflex gain (e.g., neuromodulator-dependent persistent currents (Lee et al., 2003)).

The input-output relationship of the SOL HR is comparable to that observed in other species. HR size varies not only with input magnitude (as assessed by MR size or stimulus amplitude), but is highly dependent upon the level of ongoing EMG (as assessed by the prestimulus MEMG level). Similar dependencies of the HR on bEMG have been demonstrated in humans (Verrier, 1985). The ability to record continuously over many days in an unrestrained behaving animal facilitates evaluation of the relative contributions of changes in net synaptic drive to motoneurons. With additional implanted electrodes, it may also be possible to evaluate other determinants of the HR such as presynaptic inhibition.

Unlike experimental designs in which the tether is removed from a connector on the mouse between recording sessions (Leblond et al., 2003; Milner and Hoffer, 1987; Valax, 1971; Warren et al., 1998), in our experiments the animal is connected to the data acquisition system at all times. The implanted portion of the cable assembly is very light, due in part to the absence of an intermediate connector. Most of the weight of the cable is directly supported by the commutator. The mouse only experiences the weight of the unsupported slack cable (typically <2 cm) and any radial load when located away from the center of the cage. Compared to standard rectangular cages, the near-circular cage design minimizes the slack in the cable that the animal needs to access all parts of its cage. The mass of the cable’s protective stainless steel spring was minimized by using the finest gauge wire that was stiff enough to rotate the commutator without twisting itself. The commutator used in this study has a lower torque requirement than any other device from other sources that we have assessed (e.g., Plastics One, Airflyte Electronics). In order to achieve a further reduction in the torque requirement (and thus further reduce spring weight), we are developing a commutator that incorporates the fluid bearing used here for maintaining cable integrity prior to recording.

The outbred Swiss-Webster mice used in the present study are substantially larger than many of the inbred strains commonly used for genetic manipulations. Future studies with smaller or less robust strains may require further miniaturization of the cable assembly and/or reduction in the torque requirements of the commutator.

The alternative to hardwired data transmission is telemetry, which removes the requirement for any physical connection between the animal and the recording equipment. This has facilitated chronic EMG recordings in many species larger than mice (Herzog et al., 1993; Kramer and Kinter, 2003; Marques and Dutourne, 1977; Winter and Quanbury, 1975), but the need to implant a power source (usually a battery) along with transmission and/or receiving hardware limits its use in small animals to short duration recordings. Telemetry has been performed for acquisition of EEG, heart rate, blood pressure, and/or temperature data (Ishii et al., 1996; Kramer and Kinter, 2003), but not with higher bandwidth signals such as EMG and/or those requiring nerve stimulation.

In summary, the data show the feasibility of continuous long-term EMG recording and HR study in mice. Development of this methodology will permit us to transfer the HR conditioning paradigm to mice. Thus, the mouse may provide an important new model for defining the spinal cord plasticity associated with HR operant conditioning.

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