A decerebrate adult mouse model for examining the sensorimotor control of locomotion

Stan T. Nakanishi and Patrick J. Whelan
Hotchkiss Brain Institute, Department of Comparative Biology and Experimental Medicine, Department of Physiology and Pharmacology, and Department of Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada

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Nakanishi ST, Whelan PJ. A decerebrate adult mouse model for examining the sensorimotor control of locomotion. J Neurophysiol 107: 500–515, 2012. First published October 12, 2011; doi:10.1152/jn.00699.2011.—As wild-type and genetically modified mice are progressively becoming the predominant models for studying locomotor physiology, the technical ability to record sensory and motor components from adult mice, in vivo, are expected to contribute to a better understanding of sensorimotor spinal cord networks. Here, specific technical and surgical details are presented on how to produce an adult decerebrate mouse preparation that can reliably produce sustained bouts of stepping, in vivo, in the absence of anesthetic drugs. Data are presented demonstrating the ability of this preparation to produce stepping during treadmill locomotion, adaptability in its responses to changes in the treadmill speed, and left-right alternation. Furthermore, intracellular recordings from motoneurons and interneurons in the spinal cord are presented from preparations where muscle activity was blocked. Intraxonal recordings are also presented demonstrating that individual afferents can be recorded using this preparation. These data demonstrate that the adult decerebrate mouse is a tractable preparation for the study of sensorimotor systems.

treadmill; motoneuron; afferent

FOR MANY DECADES, NEUROSCIENTISTS and physiologists have studied the sensory and locomotor systems of humans, cats, rats, mice, lamprey, frogs, and many other species. Historically, decerebrate preparations, mainly cats, have been used to study the mammalian adult sensorimotor systems with the foundations of these kinds of studies established by Sherrington (1906) and Graham-Brown (1911). Later studies aimed at examining exercise physiology (Bedford et al. 1992), respiration (Fung et al. 1994), and fictive locomotion (Iles and Nicolopoulos-Stournaras 1996) led to the development of the adult decerebrate rat preparation.

More recently, advances in genetics have led to the production of hundreds of genetically modified mice, providing a potentially powerful set of tools to examine the effects of gene manipulation on locomotion and sensory and motor systems. To study locomotion in these transgenic mice, it is necessary to maintain viable tissue to record from, leading many researchers to focus their efforts on neonatal in vitro mouse spinal cord preparations, including the isolated spinal cord, brain stem spinal cord, and spinal cord slice preparations. However, in vitro preparations become less viable in older animals, making it difficult to elicit locomotor-like activity. Neonatal preparations provide a window of opportunity to study motor system development that is insightful but limited to younger mice.

However, similar to humans and many other species, the sensory and motor systems of rats and mice continue to develop and change after they are born (Nakanishi and Whelan 2010; Vinay et al. 2000). Therefore, some conclusions about spinal cord physiology drawn from neonatal preparations may not necessarily apply directly to the adult system, and comparative studies of neonatal and adult mice would help determine which aspects undergo changes during development.

For example, amyotrophic lateral sclerosis (ALS) is a human disease that is predominantly diagnosed in adults (Eisen et al. 1993; Juergens et al. 1980; Mui et al. 1995). A transgenic mouse model with a mutation of an enzyme called SOD appears to follow a disease progression similar to that seen in a subset of human patients diagnosed with ALS (Birve et al. 2010), and these SOD mice have become the main model organisms used to study ALS. Previous studies of motoneurons from SOD mice have shown changes at neonatal stages (Kuo et al. 2004; Quinlan et al. 2011) and in anesthetized adults (Jiang et al. 2009; Meehan et al. 2010b), but currently there are no reports of motoneuron properties recorded from unanesthetized adult SOD mice.

Recently, multiple research groups have performed intracellular recording of motoneurons from adult mice in vivo. A publication by Manuel et al. (2009) examined the action potential firing rates of motoneurons and the relationships between firing rates and afterhyperpolarization amplitude as well as a number of other motoneuron electrophysiological properties while the mouse was anesthetized with sodium barbitone. Soon afterward, Meehan et al. (2010a) showed that with an anesthetic combination (Hypnorm and midazolam), plateau potential-like action potential frequency-current relationships could be recorded from adult mouse motoneurons, which likely reflect persistent inward currents. In a later publication, Meehan et al. (2010b) showed that SOD mutant mice exhibit an increase in persistent inward currents, a finding that may provide some insight into understanding the neurodegenerative mechanism of ALS. In these studies, the animals were anesthetized with different drugs, with similar effects, to produce a deeply anesthetized preparation suitable for intracellular recording by altering neuronal excitability and synaptic transmission. The studies by Manuel et al. (2009) and Meehan et al. (2010a,b) are important and valuable measures of motoneuron properties in adult mice, but neither of these preparations would be capable of generating locomotion, and both intrinsic properties and synaptic transmission would be affected by the anesthetic drugs that were used.

There are clearly uses for an in vivo adult mouse preparation that are not altered by the presence of anesthetic drugs. The
goal of this study is to describe the preparation of a decerebrate adult mouse from which stepping behavior can be obtained. One of the main advantages of the decerebrate mouse compared with an intact preparation is the ability to record intra-cellularly from afferents and from neurons in the spinal cord, and examples of these approaches are described in this paper. We expect that the in vivo decerebrate adult mouse preparation will be a valuable preparation to study sensorimotor function.

METHODS

All procedures were approved by the University of Calgary Animal Resources Center. The mice used for the experiments were postpartum adult female Swiss Webster mice. Between 1 day and a few weeks after removing the pups from the cage, the adult mouse was used for the studies described below. All adult mice were housed in individual cages, provided food and water ad libitum, and kept in a dedicated mouse housing room with a 12:12-h light-dark cycle with the room maintained at approximately 23–24°C with low humidity.

General Acute Procedures

This section describes the surgical procedures common to all experiments presented in this paper. Details specific to each study are described following these general acute procedures.

Induction of general anesthesia. The mouse was placed in an enclosed plastic chamber with a tightly fitting cover. General anesthesia was induced by administering 2-bromo-2-chloro-1,1,1-trifluoroethane (halothane; Sigma, St. Louis, MO), 5% vaporized (SurgiVet) in carbogen (95% oxygen-5% carbon dioxide), delivered by a small animal respirator (MiniVent; Hugo Sachs Elektronik-Harvard Apparatus; stroke volume: 225 μl, strokes/min: 165) through the intubation tube inserted into a small hole drilled into the induction chamber. There was some variability in the time between the beginning of anesthesia administration and the animal transitioning to a deeply anesthetized, unresponsive state; typically, it took between 3 and 5 min. A deeply anesthetized state in the induction chamber was determined by the absence of a righting response when the chamber was tilted and the animal’s breathing became slow and regular. At that point, the animal was removed from the induction chamber, and its nose and mouth were placed in a nose cone connected to the respirator with anesthesia continuing at 2–3% and adjusted so that the animal maintained at approximately 36–38°C using an infrared heating lamp as needed. For fluid supplementation, lactated Ringer solution (Braun, Irvine, CA) was administered ∼0.05 ml ip every 1–2 h.

Decerebration procedure. The animal was then secured in a mouse stereotaxic head mount with ear bars and nose holder (Stoelting Mouse and Neonatal Rat Adaptor for Stereotaxic Instruments). The hair was removed from the dorsal part of the animal’s head with small hair clippers (Wahl), and then an incision was made along the midline using a scalpel from between the eyes to the back of the cranium. Next, rongeurs were used to remove part of the cranium while avoiding sutures formed between bones to minimize blood loss. A small hole was made in the middle of the left parietal bone, and this opening was extended rostrally across the coronal suture into the caudal half of the left frontal bone with care not to compromise the rostral dorsal cerebral vein rostrally or the lambda suture caudally or cross the temporal line laterally. The opening was then extended across the midline with a small amount of bleeding typical on crossing the sagittal suture; this bleeding was stopped by applying bone wax to the area (Bonewax, Ethicon; Johnson & Johnson, New Brunswick, NJ). The right frontal and parietal bones were then removed using a similar approach. The time between securing the mouse’s head and completing the craniotomy was normally about 5–10 min.

After performing the cranial opening, we aimed to perform a precordial, premammillary decerebration, similar to the decerebration procedure done in cats, which results in a cat preparation that exhibits spontaneous locomotor activity (Whelan 1996). A scalpel (no. 10 blade; Fine Science Tools, Vancouver, British Columbia, Canada) was used to make an ∼45° cut, with respect to the horizontal plane, from the caudal cortex through the brain to the base of the cranium, directed at performing a precordial, premammillary decerebration. Postexperiment observations and histology (data not shown) show that there was some variability in the location and angle of the decerebration incision through the brain tissue. The brain rostral to the cut was then removed from the cranium. Two small pieces of gel foam (∼3 × 3 × 3 mm; Surgifoam; Johnson & Johnson) were placed in the cavity, a few drops of mineral oil were added to minimize tissue desiccation, and then this opening was closed with nylon thread or 4-0 silk suture.

At this point, the anesthesia vaporizer was switched to 0%, and the animal remained on the ventilator with the carbogen source. Animals typically began to show increased muscle tone and reflex responses within 10–30 min after discontinuing the anesthesia. For the most part, the animals exhibited sufficient muscle tone to maintain weight support independently. In a minority of cases, some minimal tail support was provided manually for a subset of trials in three to four of the locomotor preparations.

Electromyogram Electrodes and Implantation, Treadmill Design, and Control System

Electromyogram (EMG) electrodes for the present study were fabricated similar to the design described by Pearson et al. (2005). The differences between methods employed by Pearson et al. (2005) and our methods were the specific wire used and the implantation procedure, described in RESULTS. Pearson et al. (2005) used Teflon-coated, seven-stranded, stainless steel wire (cat. no. 793200; A-M Systems, Everett, WA). For the results presented here, the EMG electrodes were custom-made using Teflon-insulated silver wire (76.2-μm diam-
eter bare, 139.7-μm coated; cat. no. 785500; A-M Systems) and 25-gauge needles (25 gauge, 5/8 in. in cart no. 305122; BD Precision-Glide; Becton Dickinson, Franklin Lakes, NJ). The plastic end of the needles was cut off of the steel cylinder, and the cut end of the cylinder was then sanded back to provide an opening. Two lengths of silver wire were wound together. One end of the entwined wires was slid into the needle tip and then secured in place by crimping the needle cylinder. The opposite end of the coiled wire was soldered into an array of pins that connected to the headstage for the extracellular amplifier.

The EMG signals were preamplified 10× by headstages and then amplified (200×) and band-pass filtered (30 Hz to 2 kHz, AC coupled; EX4-400: Dagan). Amplified and filtered signals were digitized (Digidata 1440A), recorded (10-kHz sampling rate) on a computer, and saved for post hoc analysis (Clampfit 10.0; Molecular Devices).

The treadmill used in a subset of experiments was constructed using plastic building blocks, servomotors, and customized plastic treads (Lego Mindstorms NXT 2.0; NXT Programs, Enfield, CT). The treadmill assembly was optimized over the course of the study, with the most recent version using a subset of pieces from two sets of NXT 2.0 (Fig. 4). The blocks were used to form a stable frame on which two servomotors were mounted and connected to the left and right sides of the split-treadmill tracks. The motors were controlled to rotate at a constant speed and/or during a slow acceleration. Also, having two separate motors, and the axes of the wheels supporting the treadmill separated, provided for the opportunity to move the right and left sides of the treadmill at identical speeds (constant velocity or acceleration) or to vary the speeds of the left vs. right side (split treadmill). The center of the tracks was supported by a small piece of Plexiglas to maintain a constant horizontal position for the treadmill tracks. In certain experiments, we made use of the programming language shipped with the system. We constructed a light-emitting diode (LED) that was driven by TTLs delivered via Clampex 10.0. The LED was read by the optic sensor in Mindstorms, allowing us to trigger a change in speed, belt direction, or coupling between the two servomotors.

Intraaxonal Dorsal Root Recording from Afferents

In a subset of experiments, action potential activity from individual afferent axons was recorded using sharp glass microelectrodes. These experiments required additional surgical procedures.

Before the decerebration, while the animal remained deeply anesthetized, the left hindlimb was dissected to remove part of the biceps femoris muscle to expose and isolate the tibial nerve. Next, a separate midline incision was made in the skin over the spinal cord from approximately the 8th thoracic segment caudal to about the level of the iliopubic eminence. The thoracolumbar fascia and erector spinae muscles were cut away from the spinous processes from approximately the 10th thoracic (T10) segment to the 2nd lumbar (L2) vertebrae. The muscle was then removed from the dorsal and lateral aspects of the costal processes over the same segments without compromising the thoracic or abdominal cavities. Then, the decerebration proceeded as described above. Next, the exposed spinal cord segments were secured into a customized spinal clamp stereotaxic frame. The original spinal clamp frame (Cunningham Spinal Stereotaxic Adaptors; Model 51690; Harvard Apparatus, Holliston, MA) was modified so that the arms approached the spinal cord at a 45° angle, which allows the use of the skin around the spinal cord to be suspended using nylon thread and form a mineral oil pool around the exposed spinal cord after performing a dorsal laminectomy. For the laminectomy, rongeurs were used to remove any muscle remaining on the T10 through L2 vertebrae. Then, starting at the caudal end, the dorsal part of the vertebrae was removed, from L2 toward T10, exposing the lumbar enlargement of the spinal cord, approximately L3–L6. During this procedure, it was important to maintain the integrity of the dura mater, in part to minimize damage to the spinal cord. Once the laminectomy was completed, mineral oil was slowly poured into the pool area. Then, a strip of dura mater was cut using small dissecting scissors over the exposed spinal cord. On the left side, large dorsal roots were gently suspended, in continuity, over a bipolar electrode. Another bipolar stimulating electrode suspended the left tibial nerve in the left hindlimb, also in a mineral oil pool formed by the leg skin. The animal was then paralyzed and maintained in a paralyzed state with injections of rocuronium bromide (~0.25 mg/kg ip with supplementary injections as needed; Sigma-Aldrich; see above).

Intraaxonal recordings were performed using sharp glass microelectrodes (approximately 60–80 MΩ, borosilicate glass with filament, 1.5-mm outer diameter, 0.86-mm inner diameter, fabricated using a Sutter P-97 electrode puller; Sutter Instruments, Novato, CA) and filled with 3 M KCl. The tibial nerve was stimulated through bipolar hook electrodes attached to a Stimulus Isolator (Model A365; World Precision Instruments, Sarasota, FL), computer controlled (Clampex 10.0) to deliver 50-μs pulses at 1 Hz. While stimulating, the dorsal roots were approached with a microelectrode with the movement controlled by a microdrive (Model 861 Motion Controller, set for minimum-sized steps; Newport, Irving, CA). In some cases, axonal impalement was accomplished by manually, gently tapping on the back of the microdrive. With the stimulation strength set to 25–50 times threshold for an observable muscle twitch to stimulate a wide variety of afferents, tibial afferents were identified by the presence of an action potential recorded by the glass microelectrode time-locked to the stimulation delivered by the peripheral bipolar electrode on the tibial nerve. The time delay between the stimulus onset and the onset of the action potential recorded intraaxonally by the glass microelectrode defined the conduction delay time; the conduction distance was measured at the completion of each experiment between the stimulation site and the recording site; conduction velocity (CV) was calculated by dividing the conduction distance by the conduction delay time (CV = conduction distance/conduction delay; Fig. 8).

Intracellular Recording in the Spinal Cord

The surgery for intracellular recording was largely identical to that described above for intraaxonal recording. In addition, a small area of pia mater was removed from the surface of the spinal cord targeted for intracellular recording; the glass microelectrodes used had a lower resistance than for intraaxonal recording (approximately 30–40 MΩ); and the tibial nerve stimulation was reduced to 2.5 times threshold for an observable muscle twitch with the goal of targeting somatic α-motoneurons. It is common to observe an antidromically initiated field potential when approaching the ventral horn region with a sharp glass microelectrode in adult rat and cat experiments; in the mouse preparation, a field potential was not observable in single sweeps, perhaps because the microelectrode resistance used in the mouse was higher than commonly used in rats and cats (for example, see Geertsen et al. 2011); it may be possible to observe a field potential in the mouse by averaging a large number of stimulus time-locked sweeps. Motoneurons were identified by the presence of a short-latency antidromic action potential time-locked to the peripheral tibial nerve stimulation. A putative Renshaw cell recording was observed and identified based on the stimulus-locked burst of action potentials following a longer delay than that observed for motoneurons. On approaching the estimated ventral horn area, various cells were impaled, many of which were quiet or exhibited activity that was not apparently associated with the stimulus timing. An unidentified, rhythmically active candidate interneuron was also impaled, and data from this cell are presented in RESULTS.
Analysis of Frequency and Coherence Using Nonstationary Analyses Techniques

Spinalcore is a wavelet-based data analysis software developed by the Lev-Tov group (Mor and Lev-Tov 2007) that we have applied to EMG signals recorded during locomotion in a subset of experiments. Briefly, EMG signals, as complex waveforms, are rectified, integrated, and low-pass filtered. The filtered EMG signal is then fit to a Morlet wavelet model and processed with respect to time to calculate power or coherence across a range of frequencies; this output is termed a “spectrogram.” The spectrograms are a graphic representation of the frequency components of the EMG signal(s) over the time course of the recording. For this paper, the x-axis represents the time course of the recording, in seconds; the y-axis represents the range of frequencies of interest, from approximately 0.1 to 10 Hz. The colors that are plotted on the graph represent logarithmic power, which shows the amplitude of a given frequency component (y-axis) at that time (x-axis) in the raw recording. High-power areas are arbitrarily assigned “warm” colors (i.e., red and orange), and low-power regions are assigned “cool” colors (i.e., green and blue). The spectrograms show which frequencies are more strongly represented in the recording and how those frequency components may change over the course of the recording. In some cases, cycle period was calculated using traditional approaches of measuring the time from the onset of one EMG burst to the next.

Animal Survival

In total, 40 adult mice were used in the process of developing and refining the preparation described in this paper. As an outline, the development of this preparation advanced in 3 stages: anesthesia, ventilation, and surgery (10 animals), treadmill locomotion (20 animals), and finally intraaxonal and intracellular recording (10 animals). Within each stage, there were different rates of survival at different stages of surgery. For the experiments on anesthesia, ventilation, and surgery, these animals mostly likely succumbed to an overdose of anesthetics or blood loss during surgery, some after surviving for >5 h as we aimed to optimize anesthesia levels and ventilator settings. For the treadmill locomotion experiments, with the goal of a preparation that would locomote on the treadmill, the initial success was quite low (20% of the 1st 10 animals) but improved substantially later (70% of the 2nd 10 animals; more details provided below). Lastly, for the intraaxonal and intracellular recording, out of the 10 animals used, 2 died during the laminectomy surgery, and 2 more died during decerebration; of the remaining 6 animals, 2 were used for intraaxonal recordings, and the last 4 were used for intracellular recording experiments.

RESULTS

EKG Recordings

During the surgery and during experiments where the animal was paralyzed, EKG data were recorded along with core body temperature to evaluate the health of the animal. EKG was recorded by placing a ground electrode on the tail and then one each on a forelimb and another either on the contralateral forelimb or hindlimb (Fig. 1A). With this recording configuration, EMG activity associated with respiration could be recorded before rocuronium administration, and EKG could be recorded throughout the experiment (EKG, black downward arrows and left inset; respiratory EMG, gray upward arrows and right inset; Fig. 1B). Various combinations of ventilator settings and anesthesia level were tested in a collection of preliminary experiments, and the effects on EKG amplitude and rate were noted. The ventilator unit settings ranged 30–325 μl for the stroke volume and 50–400 strokes/min (see Methods, above). Ultimately, the crucial measure for optimizing the ventilator settings was animal survival, and the settings used for all of the experiments presented here were approximately in the middle of the available range of settings on the ventilator unit, a stroke volume of 225 μl and a stroke rate of 165 strokes/min, with induction anesthesia levels set at 5% while maintenance levels ranged from 1.0 to 1.5%, controlled by the vaporizer.

EMG Recordings

Electrical activity from muscle fibers was recorded using custom-made EMG electrodes based on those designed and described by Pearson and colleagues (2005; Fig. 2A, and Methods, above). The EMG electrodes used for the recordings described here were implanted against the surface of various muscle groups (hindlimb ankle flexors and/or ankle extensors, specified below) through the skin and secured with suture on both sides of the EMG wires externally. Pearson et al. (2005) implanted their EMG electrodes directly into the muscles they studied, a procedure that required extensive surgery including making multiple openings in the mouse’s skin and running wires from the muscles, under the skin, to a headpiece connector. The Pearson et al. (2005) method is ideal for chronic recordings from mice over the course of weeks and for positively identifying the exact muscles that they recorded. In contrast, we aimed to record from various muscle groups over an acute time frame, over the course of a few hours; in addition, we wanted to minimize the surgery done to the mouse’s hindlimbs to minimize aberrant afferent input that could be associated with more invasive procedures to the skin or muscles. The EMG electrodes, implanted using our method, provided the opportunity to occasionally observe the spontaneous activity of putative single motor units, based on a consistent rate, amplitude, and waveform (Fig. 2B, top and left inset) during periods when the animal was not stepping. As this example recording continued, the animal began to locomote spontaneously, and rhythmic EMG bursts were observed (Fig. 2B, top and right inset; note the change in scale). One disadvantage of the EMG electrode implantation method used in this paper is that it is not ideal for identifying exactly which muscles are recorded. Rather, we could identify that we were recording from the ankle extensors or triceps muscle group but could not specify, for example, if we were recording from the medial gastrocnemius, lateral gastrocnemius, or soleus muscles. If an experiment requires the exact identification of a muscle, a more invasive hindlimb surgery and EMG implantation method like that applied by Pearson et al. (2005) would be more appropriate.

Crossed-Extension Reflexes

Pronounced crossed-extensor reflexes could be recorded in the present study using the EMG electrodes described above. A moderately strong, brief (<1-s) toe pinch to the left hindpaw (Fig. 3A), sufficient to leave a temporary indentation of the skin, was sufficient to induce repeatedly a burst of muscle activity in the right ankle extensor muscle group, lasting from 15 to 20 s (Fig. 3B). The presence of crossed-extension reflexes demonstrates that peripheral sensory afferents can be activated, communicate centrally, be processed in the spinal cord, and induce motoneuron and muscle activity on the contralateral side. All 11 animals that physiologically stabilized after the surgery and decerebration...
procedures, which were not used for experiments requiring animals to be paralyzed, showed clear crossed-extensor reflexes. Crossed-extensor reflexes were observed within 10–15 min after the anesthesia had been discontinued, regardless of whether the preparation later exhibited locomotor activity.

**Treadmill Locomotion**

After decerebration, the EMG electrodes were implanted, the anesthesia was discontinued, and the animal was placed on the treadmill (Fig. 4). Typically, the mouse was unresponsive

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**Fig. 1.** Electrocardiogram (EKG) recordings. *A:* diagram of EKG recording electrode positions on the body of the mouse, dorsal view. (-), Negative; (+), positive. *B:* example of the raw data recorded using the electrode configuration in A. 6-s record. Downward-pointing black arrows identify the 1st 6 heartbeats (~8.2 Hz; 500 beats/min), and the upward-pointing gray arrows identify the 1st 3 respiratory bursts in this record (~2 Hz). *C:* anesthesia level and breaths per minute affect EKG amplitude and EKG rate. *Left:* increasing anesthesia levels leads to a decrease in EKG amplitude. *Right:* various breaths-per-minute settings affect EKG rate.
for 15–30 min, after which, on starting the treadmill, it would take tentative or uncoordinated steps for another 30 min. After this period, robust locomotion could be initiated by starting the treadmill and tended to occur in bouts of 5 min at a time with “rest” periods of about 5–10 min between locomotor bouts. As we developed the procedures to produce an adult decerebrate mouse that could step on a treadmill, we used 20 mice over the course of 6 mo; a number of other mice were used for related experiments described below. Of the 1st 10 mice of the 20, 5 did not survive for more than a few minutes after the decerebration procedure; of the 5 that did survive, after discontinuing the anesthesia, 3 mice exhibited reflex activity ipsilateral and contralateral to toe pinches but did not exhibit locomotor activity on the treadmill, although these animals survived for 2–4 h after the decerebration procedure; of the 2 remaining mice, both walked on the treadmill in bouts of locomotion. During these early experiments, we tested only 2 treadmill speeds, slower (13 cm/s) and faster (27 cm/s). For the 2nd group of 10 mice used for the treadmill experiments, 8 of the 10 survived the surgery, and 7 of these mice walked on the treadmill and provided the results presented below, showing locomotion at various treadmill velocities, during treadmill acceleration and in response to a split-treadmill paradigm. The readily identifiable differences that made the later experiments more successful are: 1) minimizing blood loss during the surgery, including decerebration; 2) altering the angle of the decerebration so that more of the diencephalon remains intact; early experiments may have been more akin to postmammillary decerebrate cats, which generally do not exhibit spontaneous locomotion; 3) maintaining body temperature between 36 and 38°C; and 4) maintaining the preparation on carbogen (95% oxygen-5% carbon dioxide) throughout the experiment. The data presented in this paper focused on the hindlimbs, but the animal’s forelimbs also moved in a pattern that was coordinated with the hindlimb activity, and future studies could include an investigation of forelimb-hindlimb coupling.

Locomotion at various constant velocities. Once the animals had recovered from the surgery sufficiently to produce consistent locomotion, they showed adjustments to their locomotor patterns in response to the treadmill moving at different speeds. These EMG records show that the adult decerebrate mouse shows consistent patterns of EMG activity with alternating bursts of activity between ankle flexor (left tibialis anterior) and extensor muscle groups (left triceps surae) within the same hindlimb (Fig. 5, A and B; mean phase = 194.8°, Rayleigh test, P < 0.01) and alternating bursts of activity between contralateral extensor muscle groups (left triceps surae and right triceps surae; Fig. 5, A and C; mean phase = 139.6°, P < 0.01). The cross-wavelet analysis shows that during locomotion at constant velocities, the high-power bands also reflect a consistent pattern of muscle activity between muscle groups at a slower
treadmill speed (Fig. 5D; \( \sim 13 \text{ cm/s} \), frequency = 0.91 ± 0.14 Hz, Rayleigh, \( P < 0.01 \)) and faster speed (Fig. 5E; \( \sim 27 \text{ cm/s} \), frequency = 2.82 ± 0.30, \( P < 0.01 \)).

**Locomotion during treadmill acceleration.** During treadmill walking, the decerebrate mouse preparations adjust their step frequency in response to changes in the treadmill velocity. Starting from rest, and slowly increasing the treadmill velocity from 0 up to 80 cm/s over the course of \( \sim 1 \) min, an acceleration of \( \sim 1.33 \text{ cm/s}^2 \), EMG recordings of the left triceps surae during the treadmill acceleration show an increase in burst frequency corresponding to the increase in step frequency (Fig. 6A). The cycle period of each EMG burst during the treadmill acceleration decreased as the treadmill accelerated (Fig. 6B). Previous studies examining locomotion at various speeds in adult cats have shown that for each step, composed of a swing and stance phase, the duration of the swing phase is relatively constant regardless of the locomotion velocity, whereas in contrast, the duration of the stance phase decreases as the velocity of the locomotion increases (Clarke 1991; Hruska and Silbergeld 1979). The decerebrate mouse locomotor pattern showed a similar response to acceleration with a minimal change in swing duration and a decrease in stance duration as the velocity of locomotion increased per step (Fig. 6C). An auto-wavelet analysis of the EMG signal from Fig. 6A shows a prominent high-power region at lower frequencies and moves to higher frequencies as the treadmill accelerated (Fig. 6D). We examined locomotor responses to the accelerating treadmill in two animals, and both responded similarly, increasing their step frequency. Overall, these data show that the adult decerebrate mouse preparation can adjust its locomotor pattern in response to changes in treadmill velocity.

**Split-treadmill challenge.** The locomotor system is able to alter the coordination of the limbs to maintain locomotion even while the left and right sides of a treadmill are moving at different speeds (Forssberg et al. 1980), and related experiments have been conducted (Musselman et al. 2009; Reisman et al. 2007; Vasudevan et al. 2011). These kinds of experiments challenge the locomotor central pattern generator (CPG) since presumably contralateral coupling has to be reduced while maintaining ipsilateral flexor-extensor coupling. Similar to human and cat split-treadmill experiments, the adult decerebrate mouse showed the ability to adapt to the split-treadmill challenge and recoordinate its locomotor pattern (Fig. 7A).

Spectrograms of the EMG activity of the left and right triceps muscle groups show the pattern of coordination and adaptation of muscle activity to a unilateral slowing of the treadmill velocity (Fig. 7B). From rest, the treadmill was started with the left and right sides moving at the same speed (20 cm/s; Fig. 7B, *epoch 1*). Then, while the left side of the treadmill maintained a velocity of 20 cm/s, the right side of the treadmill was slowed to 15 cm/s (Fig. 7B, *epoch 2*). During *epoch 2*, the spectrogram of the left triceps surae maintained a high-power band at \( \sim 1.8 \text{ Hz} \), while the spectrogram of the right triceps surae showed a shift from a high-power band of \( \sim 1.8 \text{ Hz} \) before the treadmill velocity slowed to a lower frequency \( \sim 1.0 \text{ Hz} \) (Fig. 7B, *epoch 2*). Finally, during *epoch 3*, when the velocity of the right side of the treadmill returned to match the velocity of the left side, 20 cm/s, the spectrograms for the left and right sides returned to high-power bands at 1.8 Hz (Fig. 7, *epoch 3*). We tested the
from proprioceptive (lower threshold) through nociceptive (higher threshold) afferents, based on previous studies of afferents recorded in rats (Bichler et al. 2007; Edgley and Wallace 1989; Fitzgerald 1987), cats (Bottermann and Eldred 1982; Eccles et al. 1954b; Heckman and Binder 1988; Schomburg et al. 1998), and humans (Burke et al. 1984; Leong et al. 2009; Prasartwuth et al. 2008; Sang et al. 2003). Nerve stimulation at the strengths used in this paper would also activate motoneuron axons and their collaterals; orthodromically conducted action potentials would be expected to have no effect on intrafusal or extrafusal muscle fibers because of the paralytic drug, whereas the antidromic activity would be expected to induce antidromic action potentials in motoneurons and their collateral axons, likely activating Renshaw cells; none of these effects would be expected to affect afferent CVs acutely. Given a physiologically and mechanically stable preparation, intraaxonal recording from the adult decerebrate mouse preparations was quite productive, in 1 case yielding 25 intraaxonal recordings from afferents, with a range of CVs, over the course of 2 h during 1 experiment, similar to what could be recorded from adult rats or cats. Forty-five afferents were recorded intraaxionally from two preparations, with the durations of the recordings ranging from as little as 3–5 s up to several minutes before the electrode was moved to target other afferent axons.

The amplitudes of the action potentials recorded intraaxionally in the adult decerebrate mouse ranged from 5 to 40 mV, and resting potentials ranged from 30 to 40 mV (Fig. 8A). The CV of each afferent was calculated by measuring the conduction delay between the onset of the stimulus artifact to the onset of the action potential recorded intraaxonally and then dividing the conduction delay by the conduction distance between the stimulation electrode and recording site (Fig. 8A). The range of afferent CVs recorded in the adult decerebrate mouse (7–140 m/s; Fig. 8B) included the range seen in rats (1.2–63 m/s) and some faster conducting afferents (Haftel et al. 2005; Kagitani et al. 2005; Kim et al. 2007; Lewin and McMahon 1991) and was similar to ranges observed in cat preparations (21–125 m/s; Eccles and Krnjevic 1959; Schafer et al. 1999; Webb and Cope 1992). In rat and cat preparations, this range of afferent CVs corresponds to diverse sensory modalities, but the relationship among CVs, stimulus thresholds, and sensory modalities has not yet been examined in the adult mouse in vivo.

Group Ia afferents innervate muscle spindles, are extremely sensitive to changes in muscle length (Haftel et al. 2004; Matthews 1963), and, as a population, have fast CVs. In the adult decerebrate mouse, one afferent was recorded that exhibited characteristics consistent with being a putative Ia afferent; this afferent had a fast CV (~90 m/s), fired tonically while the muscle tendon was positioned at a length consistent with a 90° ankle angle, increased its firing rate when the muscle was additionally stretched approximately 1–2 mm, paused firing when the stretch was released, and then returned to tonic firing (Fig. 8C). Further confirmation of Ia afferent identity would include responses to high-frequency, small-amplitude muscle vibration, and anatomic confirmation of the afferent innervating the central position of a muscle spindle peripherally and monosynaptic innervations of α-motoneurons centrally, but here we provide the example as proof of principle.

Intracellular recording. In a subset of experiments using the paralyzed adult decerebrate mouse preparation, recordings...
were made from motoneurons, a putative Renshaw cell, and a spontaneously rhythmically active interneuron in the lumbar enlargement, from approximately levels L4 to L5. Intracellular recording in the adult decerebrate mouse was notably more challenging compared with cats and rats. One issue that makes mice more difficult to record from is that the heart and lungs are physically closer to the lumbar enlargement, where we aimed to record motoneurons and interneurons. Second, we found that removing pia mater from the surface of the spinal cord without damaging nearby blood vessels was difficult and in two cases resulted in rupturing blood vessels on the surface of the spinal cord, which led to terminating the experiment. Intracellular recording in paralyzed decerebrate adult mice is feasible, but the yield per animal was as few as four to six quality recordings of various cell types out of four preparations.

Antidromic action potentials in motoneurons were triggered by stimulating the tibial nerve with bipolar hook electrodes at 2.5–5 times threshold for initiating observable muscle contractions, determined before administering the paralytic drug (rocuronium bromide). Motoneurons were electrophysiologically identified as having a short latency between the stimulus artifact and the onset of an action potential recorded through the sharp glass microelectrode (Fig. 9A). Motoneuron conduction latencies ranged from 0.31–0.82 ms over a conduction distance from ~40 mm, which corresponds to calculated CVs that ranged from 49 to 129 m/s, similar to values seen in rats (40–101 m/s; Gardiner and Kernell 1990) and cats (40–125 m/s; Eccles 1955; Emonet-Denand et al. 1988). The resting potentials (40 to 50 mV) and action potential amplitudes (50–55 mV) of good quality adult mouse motoneuron recordings were also similar to those reported for adult rats and cats.

Renshaw cells are interneurons located in the ventral spinal cord that receive excitatory synaptic input from motoneuron axon collaterals (Eccles et al. 1954a; Renshaw 1946) and form inhibitory synaptic connections onto motoneurons (Alvarez and Fyffe 2007). Previous studies have shown that Renshaw cells respond to peripheral nerve stimulation with a slight delay (~1–2 ms; motoneuron axon conduction delay and motoneuron-to-Renshaw cell synaptic delay) and then a burst of five to seven action potentials in response to single peripheral nerve stimuli (Nishimaru et al. 2006). In the decerebrate adult mouse preparation, a neuron with Renshaw cell-like firing properties was recorded, with the char-

**Fig. 5.** EMG recordings of various muscle groups during treadmill locomotion at constant velocities. A: example of simultaneous raw recordings of the left tibialis anterior muscle (TA), left triceps, and right triceps muscles. B, top: cross-wavelet spectrogram showing the predominant alternating relationship between the left TA and left triceps surae muscles. The high-power band (red) at ~3 Hz shows a consistent frequency relationship between the ipsilateral TA and triceps surae muscle groups over the time course of the recording. Bottom: phase diagram of the left TA and left triceps surae muscles. C, top: cross-wavelet spectrogram showing the predominant alternating relationship between the left triceps and right triceps surae muscles. The high-power band (red) at ~3 Hz shows a consistent frequency relationship between the contralateral triceps muscle groups over the time course of the recording. Bottom: phase diagram of the left TA and left triceps surae muscles. D: auto-wavelet spectrogram of the left triceps muscle group during treadmill locomotion at a slow speed. E: auto-wavelet spectrogram of the left triceps surae muscle group during treadmill locomotion at a faster speed.
characteristic burst of action potentials consistently time-locked to the peripheral nerve stimulation (Fig. 9B).

The spinal cord contains multiple classes of interneurons that can be defined by electrophysiological and genetic markers including transcription factors, electrical properties, synaptic connectivity, and activity patterns. One randomly intracellularly recorded interneuron exhibited spontaneous rhythmic bursting activity (Fig. 9, C and D). Although the identity of this neuron was not further studied, the bursting pattern timing illustrates the potential for future recording of rhythmically active interneuronal populations.

DISCUSSION

The overall goal of this study was to describe the preparation of an adult decerebrate mouse preparation that could be useful for studying locomotion, sensory, and motor systems. To date, these kinds of studies have been accomplished using cat and rat...
preparations, but the potential opportunity to study transgenic animals using these methods provides the potential for many new experiments. The main difficulties with using smaller animals such as the mouse are blood loss and microelectrode stability; however, with appropriate care, these difficulties can be overcome.

The main focus of the work presented here was establishing feasibility for studying locomotion, sensory afferents, and motoneurons; but aside from these interests, the EKG data presented show that this preparation is potentially also useful for studying heart and lung functions as well. The data presented show changes in the EKG amplitude and rate in response to changes in the parameters of the ventilator. Although EKG activity was monitored in the present study mainly to maintain the health of the preparation, studies of various transgenic mouse models and their cardiovascular responses to various drugs and other treatments could be accomplished using the preparation presented here. For example, there are currently hundreds of commercially available strains of transgenic mice with abnormal cardiovascular systems (The Jackson Laboratory, Bar Harbor, ME), some of which could provide insight into human cardiovascular diseases including studying problems in the contractile properties of the cardiac muscle fibers and examining abnormal regulation of the blood pressure. That said, the EKG amplitude can vary as a function of many variables including CO₂ levels, and it would be useful to monitor CO₂ in future experiments.

Fig. 7. Split-treadmill locomotion. A: examples of EMG activity of the left and right triceps muscle groups during treadmill locomotion. B: auto-wavelet spectrograms of simultaneous left and right triceps activity divided into 3 epochs; during epoch 1, the treadmill is initially stopped (0–20 s) and then begins to move with both sides moving at the same velocity (20–45 s); in epoch 2, the left side maintains the same velocity as in epoch 1, whereas the right side velocity is slowed (45–70 s); finally, in epoch 3, the velocity of the left side maintains its velocity, whereas the right side of the treadmill returns to its original velocity (70–80 s). The example data shown in A were taken from epoch 2 in B.
The adult decerebrate mouse preparation is potentially a very useful preparation for studying nociception. Crossed-extension reflexes could be robustly elicited in the decerebrate mouse. The sensory afferents associated with crossed-extension reflexes include group II (Arya et al. 1991), cutaneous (Gauthier and Rossignol 1981), and nociceptive afferents (C and Aδ afferents; Lundberg 1979). Nociceptive afferents, which transmit information about noxious stimuli, send signals to the spinal cord that are processed by various interneurons in the dorsal horn, notably interneurons in Rexed lamina I and II. In chronic neuropathic pain, there are documented changes in both nociceptive afferents (Latremoliere and Woolf 2009) and synaptic transmission between the nociceptive afferents onto their interneuronal targets (Zimmermann 2001) as well as the processing of this information by the interneurons (Gwak and Hulsebosch 2011; Itoh et al. 2011; Zimmermann 2001). In addition, descending systems have been shown to modulate nociceptive processing within the spinal cord (Herz and Millan 1988; Millan 2002). The adult decerebrate mouse preparation offers the opportunity to study all of these areas, from the systemic response to nociceptive input in the form of crossed-extension reflexes, to changes in primary afferent physiology during intraaxonal recordings, to interneuronal excitability and synaptic transmission, all in adult animals, without the confounds of anesthesia or analgesic drugs, and to manipulate aspects of the system using transgenic techniques.

Fig. 8. Intraaxonal afferent recording. A: diagram of the afferent stimulation and recording experimental setup, including conduction distance measure. Inset shows an example recording and example of conduction delay measure. Stim, stimulation. B: histogram of various afferent conduction velocities. C: example recording of an afferent with a high conduction velocity and stretch sensitivity; arrows indicate onset of muscle stretches.
The treadmill locomotion experiments using the adult decerebrate mouse preparation are useful for studying how changes in the velocity of the treadmill movements can lead to changes in locomotor output and coordination. The decerebrate preparation described here has sufficient muscle tone to maintain weight support with minimal or no other assistance provided. The accelerating treadmill shows how sensory information leads to changes in the timing and position of the limbs can lead to coordinated changes in the motor output from the spinal cord. In the near future, these kinds of studies could aim at studying transitions in locomotor patterning at different speeds and gait transitions in both control, wild-type and transgenic lines. For example, awake, behaving EphA4 mutant mice show a “hopping” locomotor pattern at the same speeds that wild-type, control mice show alternating locomotor patterns (Kullander et al. 2003). The treadmill experiments described in the

Fig. 9. Intracellular recording. A: example of an antidromic action potential recorded in a motoneuron; arrow marks the stimulus artifact. B: putative Renshaw cell recordings; arrow marks the stimulus artifact. C: intracellular recording of a spontaneously rhythmically active interneuron. D: instantaneous firing rate of the record shown in C.
present study would provide an opportunity for further examination of the locomotor patterns of various transgenic mice. The split-treadmill locomotion patterns are potentially useful to probe and challenge the left-right alternation produced by the locomotor pattern generating circuitry in the spinal cord. Recent studies have shown that in human unilateral stroke patients, the reflex patterns that the paretic side exhibit are not matched to their walking speed (Achache et al. 2010), and split-treadmill training appears to be potentially useful for stroke rehabilitation (Reisman et al. 2010). Understanding how left-right coordination can be modulated in mice could offer some insight into controlling left-right alternation to help humans that have suffered a stroke regain some ability to walk.

Previous studies of the spinal locomotor CPG are consistent with the idea that the CPG consists of neurons distributed across multiple segments of the spinal cord (Cowley and Schmidt 1997; Grillner 2003; Kiehn and Kjaerulff 1996; Kjaerulff and Kiehn 1996). More specifically, lesion studies of neonatal mice spinal cord preparations have shown that potentially rhythmogenic components of the hindlimb locomotor CPG extend from approximately T11 to L6, across nine spinal segments (Cowley and Schmidt 1997; Kremer and Lev-Tov 1997). Given that there may be developmental changes in the distributed nature and connectivity of interneurons in the locomotor CPG between neonatal and adult mice, there is now an opportunity to test these ideas with the multiple adult-based in vitro slice (Husch et al. 2011) and in vivo recording techniques (Manuel et al. 2009; Meehan et al. 2010a,b).

The intraxaonal recordings from the decerebrate mouse, like related recordings from cats (Eccles et al. 1954b; Eccles and Kmjevic 1959) and rats (Haftel et al. 2004), provide data on the sensory encoding of peripheral stimuli and the transmission of this information to the central nervous system. As mentioned above, nociceptive afferent encoding of noxious stimuli in primary afferents can be recorded intraxaonally. Aside from nociceptive sensory information, proprioceptive information from the periphery can also be recorded in the adult decerebrate mouse. Recent studies have shown that after a peripheral nerve is damaged or axotomized, peripheral reinnervation of both motor and sensory fibers occurs over time (Bichler et al. 2007; Chang et al. 2009; Foehring and Munson 1990; Haftel et al. 2005; Nakanishi et al. 2005). However, both the peripheral sensory encoding and the central processing of this sensory information exhibit long-term changes; for example, in adult rats (Bichler et al. 2007; Haftel et al. 2005), the ability to visualize, record, and manipulate the activity of subsets of afferents may provide important tools for further research to better understand the effects of peripheral nerve injuries and potential novel treatments.

Finally, intracellular recordings from the spinal cord of adult decerebrate mice, from motoneurons and various interneurons in the spinal cord, are useful for studying the intrinsic properties and synaptic interactions between neurons in normal, healthy conditions as well as to study various disease models. In terms of motoneuron recordings, there have been notable recent advances in the procedures to produce spinal cord slices from adult mice, and the motoneuron recordings from these slices are stable (Carp et al. 2008). However, most spinal motoneurons have dendrites that extend for several tens of millimeters in all directions (Brownstone 2006; Burke 1981), which means that the dendritic trees of motoneurons recorded from thin slice preparations are likely compromised. One notable advantage of recording motoneurons in vivo is that the dendrites of motoneurons and other cells in the spinal cord are not directly damaged by slicing the tissue. On the other hand, applying various drugs, agonists, and antagonists is far simpler using a slice preparation in a superfused bath system. For the adult decerebrate in vivo preparation, drugs that are applied systemically may have various effects throughout the preparation that may compromise the neuronal target specificity of the drug effect; alternatively, previous studies have used multibarrelled glass micropipettes with the aim of performing local pharmacological experiments, although the localization of drug application and drug concentrations at distances away from the application point have to be considered in interpreting these data (Walmsley and Bolton 1994). Still, the intact motoneuron dendritic arborizations and intact synaptic connectivity of the decerebrate in vivo preparation will be well-suited to address a subset of experimental questions.

Although the results and discussion have focused on advantages and potential uses for the adult decerebrate mouse preparation, there are also some notable limitations. One limitation is that the sensory and motor functions to be studied must be based on the spinal cord and brain stem that remain intact after surgery; the decerebration procedure removes the cortex and thalamus, so motor behaviors involving these higher areas will not be accessible. A second limitation that is closely related to the removal of the higher structures is that the descending projections from the brain stem to the spinal cord, which are intact, likely have action potential activity and transmitter release patterns that are different from the awake, behaving condition. So although the motor activity seen in the decerebrate system probably uses the same neuronal components as in the awake, behaving condition, the descending control and modulation of the spinal circuits is likely different to some degree. Third, there is mortality during surgery; at best, approximately 60–70% of the decerebrate preparations provided data. The mortality rate may be an important consideration for long-term studies and those that might involve chronic treatments, which later would involve a decerebrate component. Previous studies examining decerebrate cats and rats have used a CO₂ monitor; although a monitor was not used in the studies described in this paper, adjustments to the animal’s ventilation based on CO₂ levels might decrease the mortality rate.

The array of locomotor, sensory, and motor recordings presented in this paper show that the decerebrate mouse preparation provides many opportunities to study adult mice in vivo. With the wide variety of transgenic mouse models that are available, the methods described here can be used to explore and understand better how genetic manipulations may lead to changes in the physiology of mammals that can be examined at cellular and systems levels, and we believe that bridging these levels of analysis will be a useful tool for research.

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