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H-Reflex Operant Conditioning in Mice

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Carp, Jonathan S., Ann M. Tennissen, Xiang Yang Chen, and Jonathan R. Wolpaw. H-reflex operant conditioning in mice. *J Neurophysiol* 96: 1718–1727, 2006. First published July 12, 2006. doi:10.1152/jn.00470.2006. Rats, monkeys, and humans can alter the size of their spinal stretch reflex and its electrically induced analog, the H-reflex (HR), when exposed to an operant conditioning paradigm. Because this conditioning induces plasticity in the spinal cord, it offers a unique opportunity to identify the neuronal sites and mechanisms that underlie a well-defined change in a simple behavior. To facilitate these studies, we developed an HR operant conditioning protocol in mice, which are better suited to genetic manipulation and electrophysiological spinal cord study in vitro than rats or primates. Eleven mice under deep surgical anesthesia were implanted with tibial nerve stimulating electrodes and soleus and gastrocnemius intramuscular electrodes for recording ongoing and stimulus-evoked EMG activity. During the 24-h/day computer-controlled experiment, mice received a liquid reward for either increasing (up-conditioning) or decreasing (down-conditioning) HR amplitude while maintaining target levels of ongoing EMG and directly evoked EMG (M-responses). After 3–7 wk of conditioning, the HR amplitude was 133 ± 7% (SE) of control for up-conditioning and 71 ± 8% of control for down-conditioning. HR conditioning was successful (i.e., ≥20% change in HR amplitude in the appropriate direction) in five of six up-conditioned animals (mean final HR amplitude = 139 ± 5% of control HR for successful mice) and in four of five down-conditioned animals (mean final HR amplitude = 63 ± 8% of control HR for successful mice). These effects were not attributable to differences in the net level of motoneuron pool excitation, stimulation strength, or distribution of HR trials throughout the day. Thus mice exhibit HR operant conditioning comparable with that observed in rats and monkeys.

INTRODUCTION

The spinal stretch reflex and its electrical analog the H-reflex (HR), which are wholly spinal and are mediated largely by monosynaptic pathways, are arguably the simplest motor behaviors of the mammalian nervous system. Operant conditioning of the HR has become a useful tool for the study of learning and memory in mammals (Wolpaw 2006; Wolpaw and Tennissen 2001). Exhibited by rats, monkeys, and humans, HR conditioning is associated with plasticity at multiple sites within the brain and spinal cord and requires the presence of an intact corticospinal tract for full expression (Chen and Wolpaw 2002; see Wolpaw and Tennissen 2001 for review).

In recent years, the availability and continuing development of mutant and genetically modified mice have enabled new approaches to the study of learning and memory (Elgersma et al. 2004; Morgan 2003; Powell 2006; Simonyi et al. 2005; Vaillend et al. 2002). To make these experimental tools available to the study of HR operant conditioning, our laboratory has recently developed methods for long-term continuous HR recordings in mice (Carp et al. 2005a). This study describes the establishment of HR conditioning in mice. Transfer of the HR conditioning paradigm to mice should facilitate exploration of the molecular mechanisms underlying this plasticity using mutant or transgenic mice. It will also permit assessment of the neuronal and synaptic mechanisms of this spinal cord plasticity using in vitro preparations of the adult mouse spinal cord (Carp et al. 2003; Hori et al. 2002).

A preliminary version of this work has been presented (Carp et al. 2005b).

METHODS

Animals and preparation

Eleven mice (10 Swiss-Webster and 1 C57BL/6, Taconic; male, 9–13 wk) were each implanted in the right leg with a pair of stimulating electrodes in a flexible cuff on the tibial nerve and pairs of recording electrodes in the soleus muscle (SOL) and in the medial and lateral gastrocnemius muscles (GAS) to record spontaneous EMG activity and evoked responses. All procedures in animals were 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), and Department of Health, Education and Welfare (DHEW).

Implant and cable construction, surgical procedures, and animal care have been described fully elsewhere (Carp et al. 2005a, 2006), and are summarized here. Mice were anesthetized with ketamine and xylazine (120 and 8 mg/kg, ip, respectively) and received additional doses as required. Just before surgery, the mice also received glycopyrrolate (0.02 mg/kg, ip) and penicillin G (10,000 units/kg, ip). Body temperature was maintained by a heating pad and a lamp.

The right SOL and GAS were each implanted with one pair of multistranded (7 × 50 gauge) stainless steel Teflon-insulated wires for recording EMG. To elicit the HR, the tibial nerve was encircled with a 3-mm-long silicone rubber cuff containing a pair of wires. The wires were routed subcutaneously from the leg to the back of the neck, where they were secured to a small circle of polyester mesh; they emerged through a small incision at the nape of the neck. The mesh was attached by sutures to an external nylon skin button. The wires traversed a 30-cm-long stainless steel spring, terminating in an electrical connector. After surgery, mice received an analgesic (meperidine, 3 mg/kg, ip). Each mouse received additional penicillin G on the second and fourth days after surgery and ibuprofen (16 mg/100 ml in drinking water) for 3–7 days after surgery.

Data collection

After recovering from surgical anesthesia, mice were transferred to custom-built octagonal cages (30.5 cm high by 16–17 cm between

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opposing sides) in which they resided for the duration of the experiment. The cable plug was connected through an electrical commutator atop the cage (SL-88-10; Dragonfly Research and Development) to amplifiers (gain = 1,000, band-pass filter = 10–3,000 Hz) and a stimulus isolation unit. Food (in an externally mounted hopper on the side of the cage) and water were available at all times. Room lights were on between 0700 and 1900 h.

Stimulus delivery and data collection were under computer control 24 h/day. The computer sampled ongoing EMG at 10 KHz and calculated the absolute value (equivalent to full-wave rectification) of EMG from both muscles. A trial began when the amplitude of the ongoing EMG remained within a specified range of values for a randomly varying period of 3.0–3.6 s. The lower ongoing EMG amplitude criterion required at least a minimal level of activity in both implanted muscles, so that the motoneuron pools would be sufficiently activated to be able to contribute to the HR. The upper ongoing EMG amplitude criterion was based on the size of the evoked response (selection of this criterion is specified after describing the evoked response below). When both ongoing EMG criteria were met, the computer saved the most recent 50 ms of ongoing EMG [defined as the background EMG (bEMG), with the 50-ms sampling period defined as the bEMG interval], delivered the tibial nerve cuff stimulus, and collected and saved EMG for another 100 ms. The pre- and poststimulus recording periods together comprised a single trial. The first 30 ms of poststimulus data were saved at the full 10-KHz resolution. Adjacent data points in the prestimulus period and the last 70 ms of the poststimulus period were averaged (to reduce storage requirements) and were saved at 1,000- and 200-Hz resolution, respectively. The ongoing EMG duration requirement and poststimulus reward delay ensured that the minimum intertrial interval was no $<$3.2 s.

During the experiment, on-line analysis focused on the bEMG and on EMG responses evoked during two poststimulus time intervals: the directly activated muscle response [i.e., the M-response (MR); typically 1–3 ms after the stimulus, defined as the MR interval] and the spinally mediated HR (typically 4–6 ms after the stimulus, defined as the HR interval). The bEMG was quantified as the average EMG amplitude within the bEMG interval. The MR and the HR were quantified as the average EMG amplitude within the MR interval and that within the HR interval, respectively, minus the level of the average bEMG amplitude.

At the start of recording, either SOL or GAS was selected as the target muscle (SOL was the standard target, except in cases where the SOL HR was much smaller than the GAS HR, or where the stimulus artifact encroached on the SOL MR interval). The MR amplitude at which the maximum HR amplitude was elicited was used as the MR amplitude target value during the computer-controlled experiment. After collecting each trial, the computer determined the average absolute value of the EMG within the MR interval and decreased or increased the stimulus amplitude by one D-A converter step (~5 mV) based on whether the average MR amplitude was above or below the target value, respectively. This computer-based negative feedback control of stimulus intensity adjusted for variations in stimulation efficacy in the freely moving animal so as to maintain the median (but not necessarily the mean) MR amplitude near the target value.

Because stimulus intensity was under negative feedback control, trials were only collected when the ongoing EMG amplitude did not exceed the MR amplitude target (see description of ongoing EMG amplitude criteria above). If this restriction had not been imposed, the computer could have inappropriately reduced the stimulus intensity below the firing threshold if bEMG consistently exceeded the MR target level.

Conditioning paradigm

Before conditioning, data were collected from the mice in the control mode. During the control-mode period, 8 of the 11 mice were presented with a droplet of 0.1% saccharin solution (4–8 μl/droplet) from a 27-gauge blunt stainless steel needle, 200 ms after nerve cuff stimulation for each trial, regardless of HR amplitude. The tip of the needle was located near the top of the food hopper, about 7 cm above the 1-cm-deep layer of wood fiber bedding on the cage floor. This location was chosen so as to minimize the risk of injury, impedance of movement within the cage, or contamination or plugging of the delivery needle with bedding or fur. The computer-triggered delivery of the droplets from a reservoir either by briefly opening a valve (Parker Hannifin) or by single-cycle actuation of a piezo-activated pump (thinXXS Microtechnology). The mice typically retrieved individual or accumulated droplets by stretching up their heads and/or rearing up on their hindlimbs. The other three mice received no saccharin in control mode (Table 1) to assess whether habituation to saccharin due to prolonged control-mode exposure affected the acquisition of the conditioned HR response.

The control-mode period continued for a total of 19–70 days (Table 1) until stable recordings (i.e., daily bEMG amplitude within ~15% of its overall mean and daily MR amplitude within ~25% of its overall mean) were acquired for 9–10 consecutive days. These data comprised the final control-mode data set. The mice were randomly assigned to either of two conditioning modes, in which they received the saccharin solution at the end of a trial only if the average HR

TABLE 1. *Summary of conditioning parameters and outcome*

Conditioning Mode	Strain	Target Muscle	Number of Control Mode Days	Number of Control Mode Days With Saccharin	Number of Conditioning Mode Days	Percentage of Trials Rewarded During Final Conditioning-Mode	Final Conditioning-Mode Days Average HR Amplitude (% of Final Control-Mode HR Amplitude)
	SW	SOL	36	5	27	25	40
	SW	GAS	34	20	50	31	65
Down	SW	GAS	70	0	50	26	71
	SW	SOL	67	3	28	22	79
	SW	SOL	46	17	24	24	107
	C57BL/6	SOL	24	10	50	34	111
	SW	SOL	22	0	27	26	125
	SW	SOL	22	0	50	36	132
Up	SW	GAS	24	23	50	40	143
	SW	GAS	19	12	24	33	145
	SW	SOL	32	26	50	37	152

Final control mode days, last 9 or 10 days under control mode; final conditioning-mode days, last 10 days under conditioning mode, except for mice with fewer than 30 conditioning-mode days, for which only days starting at day 21 of the conditioning mode were used. SW, Swiss-Webster; SOL, soleus; GAS, gastrocnemius.

amplitude exceeded (up-conditioning) or dropped below (down-conditioning) a criterion value. The initial criterion value was determined for each animal as the HR amplitude above which (up-conditioning) or below which (down-conditioning) rewards would be delivered for about one third of the trials based on the most recent 3–5 days of control-mode data. During the course of conditioning, the goal was to gradually increase or decrease the criterion value during the subsequent days in the up- or down-conditioning mode, respectively, thereby shaping the HR response in the appropriate direction, while maintaining the percentage of rewarded trials in the desired range. The distribution of HR amplitudes from the most recent 1–2 days of data were examined daily, and the criterion value was adjusted as needed. During the course of conditioning, successful down-conditioning (but not up-conditioning) tended to be facilitated through reduction of the probability of reward presentation below the initial level. By the end of the conditioning-mode period, the reward percentage was 35 ± 5 and 26 ± 4% (SD) in successful up-conditioned and down-conditioned mice, respectively (Table 1).

At the end of the experiment, mice were killed by pentobarbital sodium overdose. Electrode continuity and location were verified.

Data analysis

The data were used to calculate average daily amplitudes of bEMG, MR, and HR. Subsequent analyses based on daily average data only used data from days in which bEMG and MR amplitudes varied by no more than 15 and 25%, respectively, from the average daily values for the final control-mode days and all conditioning-mode days. These restrictions excluded ~10% of the days in which data were recorded, although <3% of the excluded days were final control-mode days (i.e., last 9–10 control-mode days) or final conditioning-mode days (i.e., the last 10 conditioning-mode days; for mice with <30 conditioning-mode days in total, only data from day 21 until the last conditioning-mode day were used; see Table 1). With these restrictions, the average SD of the daily bEMG, MR, and HR amplitudes in the control mode were 3 ± 1, 7 ± 2, and 12 ± 5% of the overall average for all final control-mode days. Data from the final conditioning-mode days were used to calculate the final conditioning-mode bEMG, MR, and HR amplitudes, which were expressed as percentages of the average final control-mode values. Five-day averages of the daily average data were calculated to evaluate the time-course of conditioning-induced effects on bEMG, MR, and HR amplitudes. In addition, 5-day averages were calculated using a subset of data in which the bEMG and MR amplitudes were closely matched between control-mode and conditioning-mode days (i.e., average bEMG and MR amplitudes of control-mode days and conditioning-mode days were within 1% of each other). This was accomplished by restricting the data set of each mouse to those trials in which the bEMG and MR amplitudes fell within the central 39–86% (median for all mice, 60%) of their distributions.

Additional analyses focused on the potential role of the circumstances of HR elicitation in the conditioning outcome. To address the contribution of the level of excitation of the motoneuron pool (as reflected by bEMG) and/or stimulus strength (as reflected by the MR), we compared data recorded during control and conditioning modes (as described above) using subsets of the entire data pool formed by grouping trials with similar values of average bEMG and MR amplitudes. bEMG, MR, and HR amplitudes were first normalized on the basis of their distributions in each animal. Normalized bEMG values were calculated for each trial through subtraction of the mean value of all trials, followed by division by the SD of all trials (i.e., z-score). Normalized MR and HR amplitudes were calculated by division of the MR and HR values of each trial by the SD calculated from all trials. Because the all-trial mean amplitude was not subtracted from the individual MR or HR amplitudes, MR and HR amplitudes of zero (i.e., where the EMG within the bEMG interval had the same average amplitude as that in the MR or HR) are the MR and HR thresholds, respectively, and thus are comparable among individual animals. All normalized data are in units of SD.

Data from all trials were pooled into subsets defined by three ranges of normalized bEMG (after exclusion of the trials with the lowest 0.5% and highest 0.5% of the entire distribution of bEMG values) having equal numbers of trials, and six ranges of normalized MR amplitudes (after exclusion of the trials with the lowest 2% and highest 8% of the entire distribution of MR values) having equal numbers of trials. Data from all trials in each of the 18 groups (reflecting all combinations of the three bEMG and six MR amplitude ranges) were averaged for each animal. The exclusion of the extreme values of bEMG and MR (especially the largest MR values) was important, enabling close matching of the bEMG and MR values between control-mode and conditioning-mode data. The distribution of MR amplitude values was typically skewed toward large values, having a small number of very large values that could introduce considerable variability among the small numbers of trials populating each of the bEMG–MR range groups.

To evaluate the possibility of interaction between HR conditioning and diurnal variation in the HR (Carp et al. 2006), we grouped data from individual evoked responses (i.e., mean absolute values of bEMG, MR, and HR amplitude) into eight 3-h periods for each day (i.e., 0100–0400, 0400–0700, 0700–1000, 1000–1300, 1300–1600, 1600–1900, 1900–2200, and 2200–0100 hours) and averaged them. The timing and magnitude of the daily minimum and maximum HR amplitudes were determined for each animal during the control-mode and conditioning-mode periods.

The portion of the time within the bEMG interval during which EMG was present was defined as the bEMG activity percentage. This was computed as the number of samples within the bEMG interval that had amplitudes >20 μV, divided by the total number of samples within the bEMG interval × 100.

The significance of effects was evaluated by Student's *t*-test between control- and conditioning-mode data in individual animals. Evaluations of the influence of bEMG and MR amplitudes on conditioning-induced HR change were performed by full factorial repeated-measure ANOVA, with bEMG amplitude, MR amplitude, and conditioning mode (i.e., the repeated measure) as the main effects. Post hoc comparisons were made by Tukey's honestly significant difference test. $P < 0.05$ was considered statistically significant.

RESULTS

All animals recovered rapidly from the implantation surgery and exhibited normal locomotion, feeding, and grooming within the first postoperative week. All animals gained weight throughout the course of the experiment (mean weight gain by end of experiment = 26 ± 16% (SD) of the presurgical body weight).

Control-mode data

EMG recordings were performed in control mode for 19–70 days (median, 36 days; see Table 1). The majority of trials were collected during bipedal or quadrupedal stance and not during periods of inactivity (e.g., sleep) or movement (e.g., locomotion). There was no indication that the mice attended to the stimulation or that the stimulation affected their normal behaviors. During the final control-mode days (i.e., the last 9–10 consecutive control-mode days), the mice performed an average of 2,861–7,244 trials/day (median, 5,704 trials/day). The MR interval extended from an average of 1.0 ± 0.1 to 2.8 ± 0.2 ms (SD) after the stimulus, and the HR interval extended from an average of 3.5 ± 0.2 to 6.0 ± 0.6 ms (SD) after the stimulus. The timing of the MR and the HR intervals for each animal remained constant and did not differ between

the group of four animals in which GAS was the target muscle and the group of seven animals in which SOL was the target muscle.

Conditioning-mode data

At the end of the control-mode period, the conditioning protocol was instituted by making reward (i.e., saccharin solution presentation) contingent on the average HR amplitude during each trial being above (up-conditioning) or below (down-conditioning) a criterion value. Mice continued in the same conditioning mode for the next 24–50 days (Table 1). During the conditioning-mode period, mice performed an average of 2,456–7,796 trials/day (median, 3,802 trials/day). The smaller number of trials collected during the conditioning-mode period than during the control-mode period reflected gradual shifts in the mean amplitude of the ongoing EMG in some animals. The bEMG amplitude criteria established during the control-mode period were sufficiently narrow that even small shifts in the distribution of ongoing EMG led to reductions in the number of trials collected.

Figure 1 shows examples of evoked responses from an up-conditioned mouse (*A*) and from a down-conditioned mouse (*B*). Evoked responses are averages of trials during the final control-mode days (solid lines) or during the final conditioning-mode days (dashed lines). Trials were restricted to those with similar bEMG and MR amplitudes, so that the average values for control- and conditioning-mode trials were closely matched (i.e., the mean up-conditioning-mode bEMG and MR amplitudes were 99.7 and 100.0% of the control-mode values, respectively; the mean down-conditioning-mode bEMG and MR amplitudes were 99.6 and 99.9% of the control-mode values, respectively). The HR is larger in the up-conditioning mode than in the corresponding control mode. The HR is smaller during the down-conditioning mode than in the corresponding control mode. In both cases, the bEMG and MR are similar in control and conditioning modes.

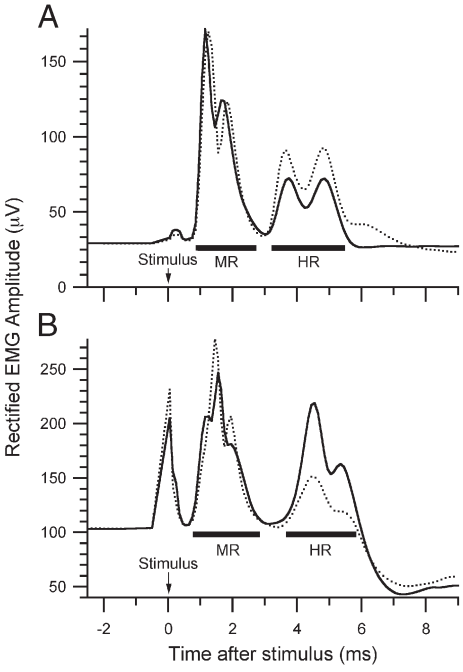

FIG. 1. Examples of average evoked responses recorded from 2 mice during the 10-day control period (solid line) and during the last 10 days of H-reflex (HR) conditioning (dashed line). Each mouse was rewarded with a droplet of 0.1% saccharin solution when the average amplitude of the evoked response within the HR interval (indicated by horizontal bar) above the average level of the background EMG (bEMG) was either above (up-conditioning, *A*) or below (down-conditioning, *B*) a criterion value. HR trials were restricted to those in which the average bEMG recorded during the 50-ms preceding nerve stimulation (stimulus at time = 0) and amplitudes of the evoked response within the M-responses (MR) interval (indicated by horizontal bar) were closely matched (average of 38,256 control-mode trials and 22,197 up-conditioning-mode trials in *A*; average of 24,607 control-mode trials and 26,333 down-conditioning-mode trials in *B*). Average HR amplitude was larger after up-conditioning (*A*) and smaller after down-conditioning (*B*) than during control mode, even though the bEMG and MR amplitudes were the same before and during conditioning. Note that time resolution is 0.1 ms/sample after stimulus (i.e., time-points to *right* of 0), and 1 ms/sample before stimulus (which accounts for stimulus artifact appearing to occur before time of stimulation).

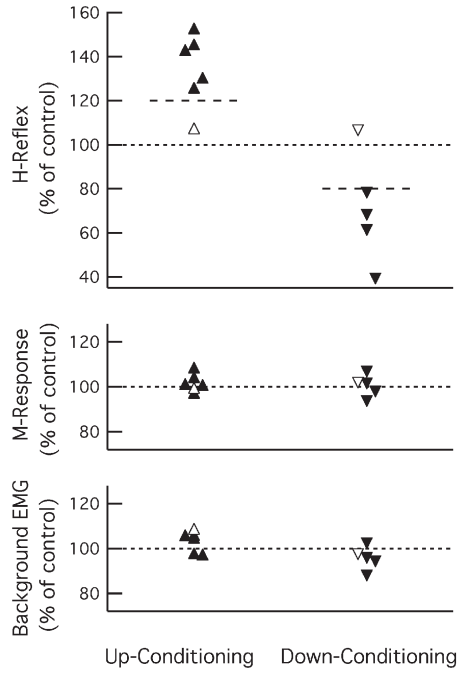

FIG. 2. HR (*top*), MR (*middle*), and bEMG (*bottom*) average amplitude during final up-conditioning days (*left*) or final down-conditioning days (*right*) expressed as a percentage of preconditioning control values (indicated by short dashed lines). Five of 6 animals exposed to up-conditioning mode and 4 of 5 animals exposed to down-conditioning mode were successful (i.e., HR amplitude changed by ≥20% in the appropriate direction; Chen and Wolpaw 1995; Wolpaw 1987) (filled symbols). The other 2 animals did not change their HR amplitudes very much (open symbols). In all animals, MR and bEMG amplitudes were little affected by either conditioning protocol.

Figure 2 shows the average daily HR amplitudes of each animal's final conditioning-mode days (i.e., last 10 days of the conditioning-mode period, except for the 5 mice with <30 conditioning-mode days, for which only data starting at day 21 of the conditioning mode were used). For all animals, average final HR increased during up-conditioning and decreased during down-conditioning, with only modest effects on bEMG or MR amplitude (Table 2). Average final HR amplitude changed in the appropriate direction by ≥20% (our standard definition of successful conditioning; Chen and Wolpaw 1995; Wolpaw 1987) in five of six mice exposed to the up-conditioning mode and in four of five mice exposed to the down-conditioning mode. Comparison of the final conditioning-mode data with the final control-mode data revealed a statistically significant

TABLE 2. *Effect of conditioning on the average of the final average values of H-reflex, M-response, and background EMG amplitudes*

Variable	Final Mean Value ± SE During Up-Conditioning (% of Control)			Final Mean Value ± SE During Down-Conditioning (% of Control)		
	All	Success only	Success only (matched bEMG and MR)	All	Success only	Success only (matched bEMG and MR)
HR Amplitude	133 ± 7	139 ± 5	141 ± 5	71 ± 11	63 ± 8	61 ± 9
MR Amplitude	101 ± 2	102 ± 2	100 ± 0	101 ± 2	101 ± 3	100 ± 0
bEMG Amplitude	102 ± 2	101 ± 2	100 ± 0	96 ± 2	96 ± 3	100 ± 1
Stimulus Amplitude	94 ± 3	94 ± 4	94 ± 4	105 ± 3	107 ± 4	107 ± 4

Final mean values are the average daily values recorded during the last 10 days under the conditioning mode, except for mice with fewer than 30 conditioning-mode days, for which days 21 until the end of the conditioning mode were used, expressed as a percentage of the mean value of the final 9–10 days during the control mode. Results are shown based on all animals (All), only those animals in which conditioning induced a ≥20% change in the appropriate direction (Success only), and a subset of the Success only group in which trials were restricted to those in which bEMG and MR amplitudes were closely matched (Success only, matched bEMG and MR). HR, H-reflex; MR, M-response; bEMG, background EMG.

difference in the HR amplitude in each successful (but not in either unsuccessful) mouse ($P < 0.0005$ for each successful down-conditioned mouse; $P < 0.0001$ for 3 and $P < 0.02$ for 2 successful up-conditioned mice).

Figure 3*A* shows the average HR, MR and bEMG amplitudes (*top, middle*, and *bottom*, respectively) for the successful up-conditioned (up-pointing triangles) and down-conditioned (down-pointing triangles) animals during the final control-mode days (i.e., before conditioning onset on day 0, indicated by the dashed vertical line) and during the course of conditioning. The average HR amplitude increased gradually in the up-conditioned mice and decreased gradually in the down-conditioned mice. Some day-to-day variability was evident in the bEMG and MR amplitudes during exposure to the conditioning mode. HR amplitude varied with the level of motoneuron pool activation (as reflected in the bEMG) and with the stimulus intensity (as reflected in the size of the MR; Carp et al. 2005a). However, off-line restriction of bEMG and MR amplitudes to fall within narrow ranges greatly reduced this variability, without affecting the time-course of acquisition of up- or down-conditioning (Fig. 3*B*). For successful animals with restricted bEMG and MR amplitudes, the average final HR increased during up-conditioning and decreased during down-conditioning, with no effects on bEMG or MR amplitude (Table 2). This indicates that the conditioning-induced change in the HR is not dependent on day-to-day variation in the level of motoneuron pool activation or on the size of the peripheral nerve volley arriving at the spinal cord.

Influence of conditioning parameters on conditioning-induced change in HR amplitude

There are many factors that could potentially contribute to the magnitude of the effect of HR conditioning. Table 3 shows

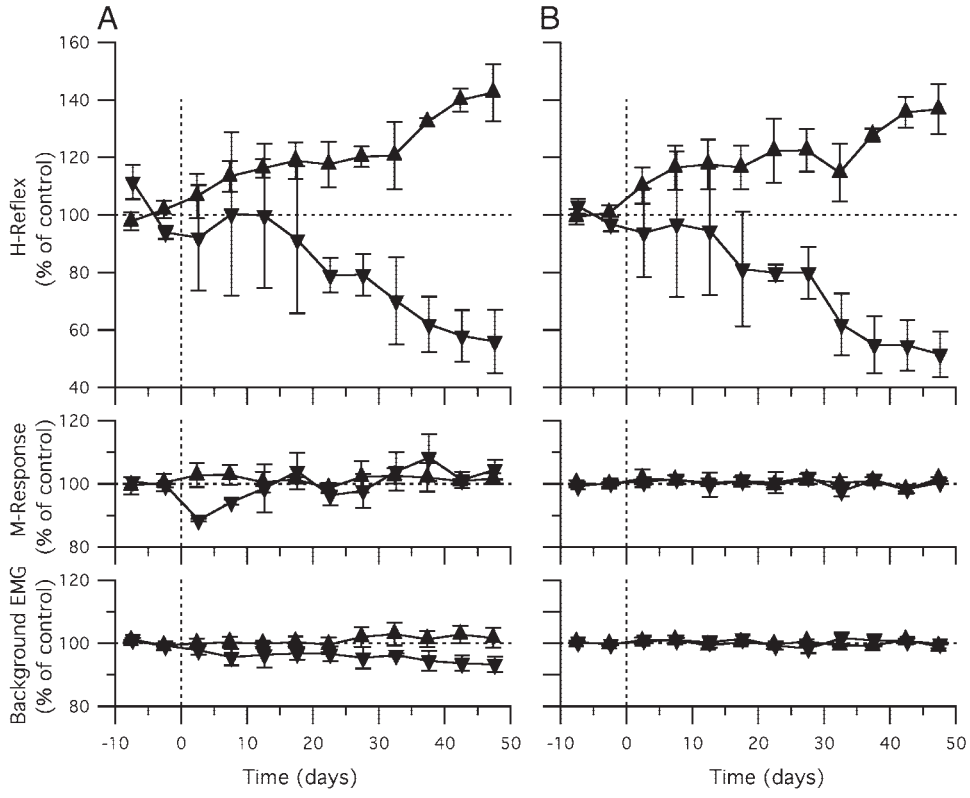

FIG. 3. Time-course of average HR (*top*), MR (*middle*), and bEMG (*bottom*) amplitudes during up-conditioning (up-pointing triangles) and down-conditioning (down-pointing triangles) expressed as a percentage of final control-mode average HR, MR, and bEMG amplitudes, respectively. *A*: each data point represents means ± SE of averages of 5 consecutive days of data from each of the 5 up-conditioned mice and 4 down-conditioned mice in which conditioning was successful (i.e., ≥20% change in final conditioning-mode HR amplitude from control-mode HR amplitude). Average HR amplitude increases gradually during up-conditioning or decreases during down-conditioning, whereas bEMG and MR amplitudes vary only modestly. *B*: for the same animals, comparable averages were calculated as for data in *A*, but only using trials with bEMG and MR amplitudes that fell within a restricted range of values. These data show a similar time-course of conditioning-induced change in HR amplitude, even when variability in bEMG and MR is greatly reduced.

TABLE 3. *Relationships between final HR amplitude (% of final control HR amplitude) and parameters of conditioning*

Independent variable	Up-Conditioned Only		Down-Conditioned Only	
	%VAF	*P*	%VAF	*P*
Number of conditioning-mode days	0	0.94	67	0.09
Number of control-mode days	12	0.50	10	0.61
Number of control-mode days with saccharin	12	0.57	42	0.17
Final reward percentage	15	0.44	8	0.64
Final stimulus intensity	7	0.61	13	0.56
HR trials/day in conditioning mode	1	0.84	19	0.46
Percentage weight gain	48	0.13	8	0.65
Target muscle (GAS vs SOL)	23	0.20	3	0.73

%VAF, percent of variance accounted for by linear regression (or by Student's *t*-test, for Target muscle); *P*, level of statistical significance of linear regression (or Student's *t*-test, for Target muscle); Final reward percentage, average number of rewarded trials as percentage of total number of trials during final conditioning-mode days; Final stimulus intensity, stimulus amplitude during final conditioning-mode days as percentage of stimulus amplitude during final control-mode days; HR trials/day in conditioning mode, average number of trials per day during final conditioning-mode days; Percentage change in HR trials/day, average number of trials per day during final conditioning-mode days expressed as a percentage of the average number of trials per day during the control-mode days; Percentage weight gain, body weight at end of experiment as percentage of weight at time of surgery.

the results of tests of the dependence of up- or down-conditioning on the number of conditioning-mode days, the number of control-mode days, the final reward percentage (expressed as a percentage of the total number of trials), the final stimulus intensity (expressed as a percentage of the average control-mode value), mean number of trials per day during the conditioning mode, mean change in number of trials per day during the conditioning mode (expressed as a percentage of the mean number of trials per day during the control mode), weight gain (expressed as a percentage increase from the day of implantation surgery until the end of the conditioning mode), and the target muscle (GAS or SOL). No statistically significant relationships were found, although the magnitude of the effect of up-conditioning tended to be larger in mice that were exposed to the conditioning paradigm for a longer time.

Eight of the 11 mice studied received saccharin after every trial during the control-mode period. Six of these eight mice eventually exhibited successful up-conditioning (3 mice) or down-conditioning (3 mice), whereas the other two were not successful (1 up- and 1 down-conditioned animal). The other three mice received no saccharin during the control-mode period. All of these mice exhibited successful up-conditioning (2 mice) or down-conditioning (1 mouse). In addition, there was no significant linear relationship between the number of control-mode days with saccharin and the average HR amplitude during the final conditioning-mode days (expressed as a percentage of the average HR amplitude during control-mode days; see Table 3). Thus the presence or absence of reward during the control-mode period had little influence on the efficacy of the HR conditioning paradigm.

In the four successful down-conditioned mice, stimulus intensity decreased in two mice and increased in the other two. In the five successful up-conditioned mice, stimulus intensity decreased in four mice and increased in the fifth. Overall, there was a small net decrease in stimulus intensity during up-conditioning and a small net increase during down-conditioning (Table 2); however, the overall effect was not statistically significant ($P = 0.10$ and 0.19 for up-conditioning and down-conditioning, respectively, by paired *t*-test of the difference in stimulus intensity between the final conditioning-mode days and the final control-mode days). Thus change in stimulus intensity was clearly not a requirement for successful conditioning.

Influence of level of ongoing activity and afferent input on HR conditioning

The normal variation throughout the day in the level of ongoing excitation of the motoneuron pool (as reflected by the bEMG) and in the level of afferent input to the spinal cord (as reflected by the MR) provided us with the opportunity to assess the effect of HR conditioning elicited under a wide range of conditions. For each successful mouse, HR trials from the final control-mode days and from conditioning-mode days were each divided into 18 groups according to their normalized bEMG amplitude (3 levels) and their normalized MR amplitudes (6 levels; see METHODS for description of normalization procedure and group assignment). Average bEMG and MR amplitudes were calculated for each of the groups.

Figure 4 shows, for each group, the difference in the average normalized HR amplitude between conditioning mode and control mode (ordinate) as a function of the average normalized MR amplitude (abscissa) at the different levels of normalized bEMG [3 curves each for up-conditioning (open symbols) and down-conditioning (filled symbols)]. The MR value of zero on the abscissa indicates the motor threshold, just above which the stimulus activates the first motor axon. Differences between control and conditioning mode were assessed by repeated-measure ANOVA, and post hoc comparisons were made using Tukey's HSD test. In up-conditioned mice, the normalized HR amplitude was significantly larger during the final conditioning-mode days than during the final control-mode days ($P < 0.01$ for the lowest normalized bEMG amplitude group, $P < 0.0001$ for all other groups). In down-conditioned mice, the normalized HR amplitude was significantly smaller during the final conditioning-mode days than during the final control-mode days for all groups ($P < 0.0001$), except for the group having the lowest normalized bEMG and MR amplitudes ($P > 0.05$). These data show that successful HR conditioning is evident under a wide range of states of the motoneuron pool excitation and afferent input.

Further analysis of these data revealed an unexpected difference between up- and down-conditioning. In up-conditioned mice, the HR curves for the three levels of bEMG are relatively flat with respect to normalized MR (with the exception of the highest MR level), but the curves for the lower two bEMG levels are much lower than that for the upper bEMG level. Statistical analysis by repeated-measures ANOVA revealed that the effect of up-conditioning (i.e., conditioning-mode normalized HR amplitude minus control-mode normalized HR amplitude) varies significantly with the level of normalized bEMG amplitude ($P = 0.04$), but not with the level of normalized MR amplitude ($P = 0.53$). This pattern suggests that the net level of excitation to the motoneuron pool has more effect on the impact of up-conditioning on HR amplitude than does the level of stimulation of the peripheral nerve. On the other hand, in down-conditioned mice, the three families of HR

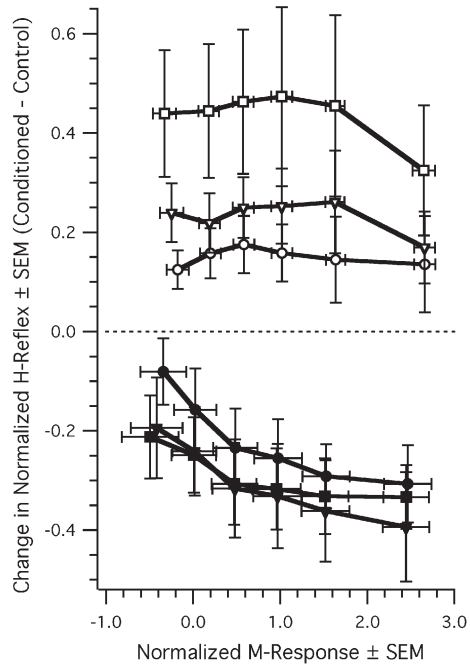

FIG. 4. Conditioning-induced change in HR elicited at various levels of bEMG and MR amplitude for all successful animals (i.e., animals in which final conditioning-mode HR amplitude changed by ≥20% from control-mode HR amplitude in mode-appropriate direction; Chen and Wolpaw 1995; Wolpaw 1987). Data were normalized according to each animal's SD of bEMG and MR amplitudes and were rank ordered to form 3 levels of bEMG (circle, triangle, and square symbols for the lowest, middle, and highest ranges, respectively) and 6 levels of MR amplitude. Mean difference in average HR amplitude ± SE between final conditioning-mode days and final control-mode days is shown on the ordinate as a function of average MR amplitude ± SE of final conditioning-mode and control-mode days for each of 18 combinations of bEMG and MR amplitude for down-conditioned animals (filled symbols) and for up-conditioned animals (open symbols). Dashed horizontal line at 0 on the ordinate indicates level at which there is no difference between conditioning-mode and control-mode HR amplitude. HR amplitude is larger after up-conditioning and smaller after down-conditioning than in control mode over a wide range of levels of bEMG and MR amplitude. Up- and down-conditioning have different dependencies on bEMG and MR. Effect of up-conditioning on HR amplitude varies little among different levels of MR, but it is sensitive to bEMG level at which the HR is elicited. On the other hand, effect of down-conditioning on HR amplitude is sensitive to MR level, but relatively insensitive to bEMG at which HR is elicited. Statistical analyses of these findings are described in text.

curves are characterized by marked variation with normalized MR amplitude, but differ little among the three levels of bEMG. Statistical analysis reveals that the effect of down-conditioning on the normalized HR amplitude varies significantly with the level of normalized MR amplitude ($P = 0.0001$) but not with the level of normalized bEMG amplitude ($P = 0.32$). Thus the efficacy of down-conditioning exhibits a sensitivity to bEMG and MR that is opposite to that of up-conditioning.

Diurnal HR variation and HR conditioning

As previously reported (Carp et al. 2006), diurnal variation in the HR was evident in control-mode recordings. For all 11 mice, the HR amplitude was largest in the dark (typically 2200–0400 hours) and smallest in the light (typically 1300–1900 hours). In the 6-h dark period encompassing the maximum HR amplitude, the average HR amplitude was 118 ± 2% (SE) of the daily average HR amplitude. In the 6-h light period encompassing the minimum HR amplitude, the average HR amplitude was 75 ± 3% of the daily average HR amplitude.

To evaluate the impact of the diurnal HR variation on HR conditioning, average final conditioning-mode HR amplitudes were calculated as a percentage of the average final control-mode values for each of the successful animals using the 6-h periods encompassing the within-day maximum and minimum modulations of the HR described above. Exposure to the up-conditioning mode increased the HR amplitude to the same extent during the diurnal HR amplitude maximum and minimum periods (mean final HR amplitude as percent of control HR amplitude was 146 ± 4 and 138 ± 5% (SE) during the diurnal maximum HR and minimum HR, respectively; $P = 0.27$ by paired t-test). Similarly, exposure to the down-conditioning mode decreased the HR amplitude to the same extent during the diurnal HR amplitude maximum and minimum periods (mean final HR amplitude as percentage of control HR amplitude was 60 ± 10 and 65 ± 10% during the diurnal maximum HR and minimum HR, respectively; $P = 0.62$ by paired t-test). Thus diurnal variation in the HR amplitude had no detectable influence on the magnitude of conditioning-induced change in the HR amplitude.

These results are consistent with our previous finding that the HR amplitude varies throughout the day in mice, being higher in the dark and lower in the light (Carp et al. 2006). It is theoretically possible that the mice could take advantage of their diurnal HR variation by increasing the proportion of trials that they perform during the night (for up-conditioning) or during the light (for down-conditioning), so as to increase their probability of receiving the reward. We assessed the distribution of trials by binning each animal's HR trials in eight 3-h time ranges and calculating the percentage of each day's trials that occurred in each time range (see METHODS). In control mode, all mice tended to perform more HR trials in the four time ranges during the dark than in those during the light. The mean percentage of daily dark-time trials was 58 ± 8% (SD) for all mice. The distribution of the number of trials performed in the dark did not change during HR conditioning. For successful down-conditioned mice, the average percentage of dark-time trials for the final control-mode and conditioning-mode days was 56.9 ± 2.7 and 55.3 ± 3.7% (SE), respectively; for successful up-conditioned mice, the average percentage of dark-time trials for the final control-mode and conditioning-mode days was 55.5 ± 4.7 and 56.1 ± 5.9% (SE), respectively ($P > 0.6$ for both up- and down-conditioned mice by paired t-test). In addition, the percentages of daily trials for each animal recorded during the eight 3-h daily time ranges were well correlated between the final control-mode and conditioning-mode recordings (correlation coefficients were between 0.77 and 0.95 for all successful animals; $P < 0.05$ for 2 mice, $P < 0.01$ for 5 mice, and $P < 0.001$ for 2 mice). These results suggest that differences in the proportions of trials occurring within different time periods did not confound our analysis of the effects of HR conditioning.

Contribution of the composition of ongoing EMG to the effect of HR conditioning

The pattern of activity produced within the bEMG interval was quantified by means of the bEMG activity percentage,

which reflects the portion of this interval that exhibits activity exceeding a minimal level (20 μV). The average daily bEMG activity percentage during the final control-mode days for the nine mice that showed successful HR conditioning was 74 ± 16% (SD; $P > 0.4$ by ANOVA). There was no significant difference in the bEMG percentage between control and conditioning modes for up-conditioned mice [average bEMG activity percentage = 67 ± 8 and 65 ± 7% (SE) for the final control- and conditioning-mode days, respectively; $P = 0.17$ by paired *t*-test] or down-conditioned mice [average bEMG activity percentage = 83 ± 5 and 82 ± 6% (SE) for the final control- and conditioning-mode days, respectively; $P = 0.71$ by paired *t*-test]. These data are consistent with the hypothesis that the motor unit firing patterns at the time of an HR trial are similar before and during HR conditioning. Thus the conditioning-induced changes in the HR are not likely to reflect changes in the number, duration, and/or frequency of firing of motor units that contribute to the ongoing EMG at the time of an HR trial.

DISCUSSION

HR conditioning in mice and other species

HR operant conditioning was performed in mice implanted with electrodes for continuous EMG studies over the course of 3–7 wk. Four of five mice exposed to the down-conditioning protocol successfully reduced the size of their HR. Five of six mice exposed to the up-conditioning protocol successfully increased the size of their HR. In the other two mice (1 up-conditioned, 1 down-conditioned), the HR remained close to the control level.

The HR conditioning evident in mice is generally similar to that seen in other species. Mice exhibit HR conditioning in a similar proportion of individual animals and following a similar time-course as has been seen for monkeys and rats (Chen and Wolpaw 1995; Wolpaw et al. 1983). In addition, mice, like rats and monkeys, exhibit a diurnal variation in the HR amplitude (although the phase relationships differ among species) that is independent of both the level of ongoing EMG and the level of afferent input (Carp et al. 2006; Chen and Wolpaw 1994; Dowman and Wolpaw 1989; Wolpaw and Seegal 1982), and the diurnal variation in the HR does not seem to cause or interact with HR conditioning.

HR conditioning in mice does differ from that in other species in certain respects. The magnitude of the decrease in the HR during down-conditioning in mice is similar to that in the other species, but the increase elicited by up-conditioning in mice is less pronounced. Given the tendency of the effect of up-conditioning to depend on the duration of the conditioning (Table 3), exposure of all of our mice to a full 50-day course of up-conditioning may have resulted in a greater up-conditioning-induced increase in the HR. In addition, there is a tendency in mice for the stimulus intensity to increase during the course of down-conditioning and to decrease during the course of up-conditioning (Table 2); this pattern has not been observed in other species. The differential effects of the two conditioning modes indicate that this phenomenon is not merely the result of deterioration of the efficacy of the implanted cuff electrode. Down-conditioning is associated with an increase in motoneuron firing threshold and a decrease in axonal conduction velocity, which have been suggested to reflect altered sodium channel properties (Carp and Wolpaw 1994; Carp et al. 2001; Halter et al. 1995). In theory, the increase in stimulus intensity seen in down-conditioned mice could reflect a conditioning-induced increase in the threshold of stimulation of the tibial nerve axons. However, the generality of this mechanism is unclear, given that substantial increases in the stimulus intensity were only observed in two of the four successful down-conditioned animals. Furthermore, because converse effects on motoneuron somatic and axonal excitability were not detected in up-conditioned animals (Carp and Wolpaw 1995; Carp et al. 2001), it is difficult for us to offer a unified hypothesis to explain these modest effects on stimulus intensity. In summary, although some differences exist between mice and other species, these results suggest that HR conditioning is generally similar among all species in which it has been thoroughly evaluated.

Different mechanisms for up- and down-conditioning

These results are also consistent with findings of previous studies that showed that down-conditioning is not simply the opposite of up-conditioning. The effects of down-conditioning, i.e., increased motoneuron firing threshold and decreased axonal conduction velocity, are not mirrored during up-conditioning (Carp and Wolpaw 1994, 1995; Carp et al. 2001). The enhanced oligosynaptic transmission to motoneurons that probably occurs with up-conditioning does not seem to be matched by a comparable reduction of oligosynaptic transmission after down-conditioning (Wolpaw and Chen 2001). Down-conditioning induces an increase in the number, size, and glutamic acid decarboxylase immunostaining of GABAergic terminals on motoneurons in down-conditioned rats (Wang et al. 2004, 2006). Opposite changes are not seen in up-conditioned rats (Wang et al. 2004). Finally, the corticospinal tract is needed for maintenance of down-conditioning but not for maintenance of up-conditioning (Chen and Wolpaw 2002; Chen et al. 2003).

This study revealed that the HR after up-conditioning and the HR after down-conditioning are affected differently by the bEMG and MR conditions under which the reflex is elicited. HR amplitude elicited during down-conditioning (but not up-conditioning) is sensitive to MR size (Fig. 4). This suggests that the effect of down-conditioning is affected by the degree of activation of afferent or efferent pathways. In theory, down-conditioning could increase the efficacy of presynaptic inhibition by low threshold afferents of the Ia afferent-mediated excitation of motoneurons that produces the HR. However, the weakness of presynaptic inhibition of extensor Ia afferents by homonymous or synergist Ia afferents (especially when elicited by a single stimulus) and its long latency to onset seem to rule it out as a potential contributor (Decandia et al. 1966; Provini et al. 1967; Schmidt 1971). Antidromically activated Renshaw cells monosynaptically inhibit motoneurons and could theoretically influence HR size in a stimulus intensity-dependent fashion. Thus a conditioning-induced enhancement of the recurrent inhibitory pathway could contribute to HR down-conditioning.

The corticospinal tract is necessary for acquisition of operantly conditioned plasticity in the HR in rats (Chen and Wolpaw 1997, 2002; Chen et al. 2002). Renshaw cells are

influenced by motor cortex (MacLean and Leffman 1967; Mazzocchio et al. 1994). Corticospinal influence over Renshaw cells could contribute to conditioning-induced change in the HR.

Significance of HR conditioning of mice in the study of learning and memory

The demonstration of HR conditioning in mice extends the generality of this model of the plasticity associated with acquisition of a simple skill. It does so not only because it shows HR conditioning in another species, but also because of the novel reward presentation. In previous studies, the reward for production of the appropriately sized HR was water (monkey: Wolpaw 1987; Wolpaw et al. 1983) or food (rat: Chen and Wolpaw 1995). Delivery of the reward was integrated into the particular species' routine method for obtaining food or water. In this study, mice were offered a nonnutritive appetitive reward in addition to unadulterated water and chow ad libitum. Use of this new reward modality showed that operantly conditioned HR plasticity can occur even when the powerful motivation of food or water availability is not used. Chen and Wolpaw (1995) showed a related phenomenon in rats by inducing successful HR conditioning with intracranial self-stimulation as the reward.

Extension of the HR conditioning paradigm to mice should open new avenues of research into neuronal plasticity beyond those afforded by HR conditioning in other species. The greatest potential advantage of performing HR operant conditioning in mice is the availability of animals with mutations in, or genetic modifications to, pathways that are relevant to learning and memory (Elgersma et al. 2004; Morgan 2003; Powell 2006; Simonyi et al. 2005; Vaillend et al. 2002). In addition, the availability of in vitro preparations of the lumbar spinal cord from adult mice offers the opportunity for electrophysiological studies into conditioning-induced changes in spinal neuronal properties (Biscoe and Duchen 1986; Chizh et al. 1997; Fulton 1986). We recently studied the use of a slice preparation of the adult mouse lumbar spinal cord for performing intracellular motoneuron recordings (Carp et al. 2003). In vitro preparations from rats of cervical and sacrocaudal spinal cord have been reported (Bennett et al. 2001; Hori et al. 2001; Long et al. 1988), but not preparations of lumbar spinal cord for intracellular recording of motoneurons (but see Kow and Pfaff 1996).

Although mice offer significant opportunities for the study of plasticity in the CNS, they also have several drawbacks. As in rats, there is considerable variation in the size of the MR and HR, so that large numbers of trials are needed (Chen and Wolpaw 1995). In addition, the small size of mice (particularly the inbred strains) makes surgery more difficult and places practical limits on the number of wires that can be implanted.

In summary, mice seem to exhibit adaptive spinal cord plasticity comparable with that seen in rats and monkeys. Despite certain practical limitations, the mouse offers several important experimental advantages over other species. Thus the mouse could provide an important new model for exploration of the normal spinal cord plasticity associated with skill acquisition and the abnormal plasticity associated with spinal cord injury and other neurological disorders.

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REFERENCES

Bennett DJ, Li Y, and Siu M. Plateau potentials in sacrocaudal motoneurons of chronic spinal rats, recorded in vitro. *J Neurophysiol* 86: 1955–1971, 2001.

Biscoe TJ and Duchen MR. Synaptic physiology of spinal motoneurones of normal and spastic mice: an in vitro study. *J Physiol* 379: 275–292, 1986.

Carp JS, Chen XY, Sheikh H, and Wolpaw JR. Operant conditioning of rat H-reflex affects motoneuron axonal conduction velocity. *Exp Brain Res* 136: 269–273, 2001.

Carp JS, Tennissen AM, Chen XY, Schalk G, and Wolpaw JR. Long-term spinal reflex studies in awake behaving mice. *J Neurosci Methods* 149: 134–143, 2005a.

Carp JS, Tennissen AM, Chen XY, and Wolpaw JR. Operant conditioning of H-reflex in mice. *Soc Neurosci Abstr* 2005b.

Carp JS, Tennissen AM, Chen XY, and Wolpaw JR. Diurnal H-reflex variation in mice. *Exp Brain Res* 168: 517–528, 2006.

Carp JS, Tennissen AM, and Wolpaw JR. Intracellular recording from adult mouse spinal motoneurons in vitro: methods development. *Soc Neurosci Abstr* 2003.

Carp JS and Wolpaw JR. Motoneuron plasticity underlying operantly conditioned decrease in primate H-reflex. *J Neurophysiol* 72: 431–442, 1994.

Carp JS and Wolpaw JR. Motoneuron properties after operantly conditioned increase in primate H-reflex. *J Neurophysiol* 73: 1365–1373, 1995.

Chen XY, Carp JS, Chen L, and Wolpaw JR. Corticospinal tract transection prevents operantly conditioned H-reflex increase in rats. *Exp Brain Res* 144: 88–94, 2002.

Chen XY, Chen L, and Wolpaw JR. Conditioned H-reflex increase persists after transection of the main corticospinal tract in rats. *J Neurophysiol* 90: 3572–3578, 2003.

Chen XY and Wolpaw JR. Circadian rhythm in rat H-reflex. *Brain Res* 648: 167–170, 1994.

Chen XY and Wolpaw JR. Operant conditioning of H-reflex in freely moving rats. *J Neurophysiol* 73: 411–415, 1995.

Chen XY and Wolpaw JR. Dorsal column but not lateral column transection prevents down-conditioning of H reflex in rats. *J Neurophysiol* 78: 1730–1734, 1997.

Chen XY and Wolpaw JR. Probable corticospinal tract control of spinal cord plasticity in the rat. *J Neurophysiol* 87: 645–652, 2002.

Chizh BA, Headley PM, and Paton JF. An arterially-perfused trunk-hindquarters preparation of adult mouse in vitro. *J Neurosci Methods* 76: 177–182, 1997.

Decandia M, Provini L, and Taborikova H. Excitability changes in the Ia extensor terminals induced by stimulation of agonist afferent fibres. *J Neurosci* 18: 402–404, 1966.

Dowman R and Wolpaw JR. Diurnal rhythms in primate spinal reflexes and accompanying cortical somatosensory evoked potentials. *Electroencephalogr Clin Neurophysiol* 72: 69–80, 1989.

Elgersma Y, Sweatt JD, and Giese KP. Mouse genetic approaches to investigating calcium/calmodulin-dependent protein kinase II function in plasticity and cognition. *J Neurosci* 24: 8410–8415, 2004.

Fulton BP. Motoneurone activity in an isolated spinal cord preparation from the adult mouse. *Neurosci Lett* 71: 175–180, 1986.

Halter JA, Carp JS, and Wolpaw JR. Operantly conditioned motoneuron plasticity: possible role of sodium channels. *J Neurophysiol* 73: 867–871, 1995.

Hori N, Tan Y, King M, Strominger NL, and Carpenter DO. Differential actions and excitotoxicity of glutamate agonists on motoneurons in adult mouse cervical spinal cord slices. *Brain Res* 958: 434–438, 2002.

Hori N, Tan Y, Strominger NL, and Carpenter DO. Intracellular activity of rat spinal cord motoneurons in slices. *J Neurosci Methods* 112: 185–191, 2001.

Kow LM and Pfaff DW. Thyrotropin-releasing hormone (TRH) has independent excitatory and modulatory actions on lamina IX neurons of lumbosacral spinal cord slices from adult rats. *Peptides* 17: 131–138, 1996.

Long SK, Evans RH, Cull L, Krijzer F, and Bevan P. An in vitro mature spinal cord preparation from the rat. *Neuropharmacology* 27: 541–546, 1988.

MacLean JB and Leffman H. Supraspinal control of Renshaw cells. *Exp Neurol* 18: 94–104, 1967.

Mazzocchio R, Rossi A, and Rothwell JC. Depression of Renshaw recurrent inhibition by activation of corticospinal fibres in human upper and lower limb. *J Physiol* 481: 487–498, 1994.

Morgan D. Learning and memory deficits in APP transgenic mouse models of amyloid deposition. *Neurochem Res* 28: 1029–1034, 2003.

Powell CM. Gene targeting of presynaptic proteins in synaptic plasticity and memory: across the great divide. *Neurobiol Learn Mem* 85: 2–15, 2006.

Provini L, Decandia M, and Marietti M. Presynaptic inhibition by gastrocnemius-soleus nerves. *Experientia* 23: 550–551, 1967.

Schmidt RF. Presynaptic inhibition in the vertebrate central nervous system. *Ergeb Physiol Biol Exp Pharmakol* 63: 20–101, 1971.

Simonyi A, Schachtman TR, and Christoffersen GR. The role of metabotropic glutamate receptor 5 in learning and memory processes. *Drug News Perspect* 18: 353–361, 2005.

Vaillend C, Rampon C, Davis S, and Laroche S. Gene control of synaptic plasticity and memory formation: implications for diseases and therapeutic strategies. *Curr Mol Med* 2: 613–628, 2002.

Wang Y, Pillai S, Chen XY, and Wolpaw JR. Effects of H-reflex up-conditioning on GABAergic terminals on rat soleus motoneurons. *Soc Neurosci Abstr* 2004.

Wang Y, Pillai S, Wolpaw JR, and Chen XY. Motor learning changes GABAergic terminals on spinal motoneurons in normal rats. *Eur J Neurosci* 23: 141–150, 2006.

Wolpaw JR. Operant conditioning of primate spinal reflexes: the H-reflex. *J Neurophysiol* 57: 443–459, 1987.

Wolpaw JR. Activity-dependent plasticity in the intact spinal cord. In: *Textbook of Neural Repair and Rehabilitation. Neural Repair and Plasticity*, edited by Selzer ME, Clarke S, Cohen LG, Duncan PW, and Gage FH. Cambridge: Cambridge University Press, 2006, vol. 1, p. 109–125.

Wolpaw JR, Braitman DJ, and Seegal RF. Adaptive plasticity in primate spinal stretch reflex: initial development. *J Neurophysiol* 50: 1296–1311, 1983.

Wolpaw JR and Chen XY. Operant conditioning of rat H-reflex: effects on mean latency and duration. *Exp Brain Res* 136: 274–279, 2001.

Wolpaw JR and Seegal RF. Diurnal rhythm in the spinal stretch reflex. *Brain Res* 244: 365–369, 1982.

Wolpaw JR and Tennissen AM. Activity-dependent spinal cord plasticity in health and disease. *Annu Rev Neurosci* 24: 807–843, 2001.