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Sensorimotor Cortex Ablation Prevents H-Reflex Up-Conditioning and Causes a Paradoxical Response to Down-Conditioning in Rats

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Chen, Xiang Yang, Jonathan S. Carp, Lu Chen, and Jonathan R. Wolpaw. Sensorimotor cortex ablation prevents H-reflex up-conditioning and causes a paradoxical response to down-conditioning in rats. *J Neurophysiol* 96: 119–127, 2006. First published April 5, 2006; doi:10.1152/jn.01271.2005. Operant conditioning of the H-reflex, a simple model for skill acquisition, requires the corticospinal tract (CST) and does not require other major descending pathways. To further explore its mechanisms, we assessed the effects of ablating contralateral sensorimotor cortex (cSMC). In 22 Sprague–Dawley rats, the hindlimb area of left cSMC was ablated. EMG electrodes were implanted in the right soleus muscle and a stimulating cuff was placed around the right posterior tibial nerve. When EMG remained in a specified range, nerve stimulation just above the M response threshold elicited the H-reflex. In control mode, no reward occurred. In conditioning mode, reward occurred if H-reflex size was above (HRup mode) or below (HRdown mode) a criterion value. After exposure to the control mode for ≥10 days, each rat was exposed for another 50 days to the control mode, the HRup mode, or the HRdown mode. In control and HRup rats, final H-reflex size was not significantly different from initial H-reflex size. In contrast, in HRdown rats, final H-reflex size was significantly increased to an average of 136% of initial size. Thus like recent CST transection, cSMC ablation greatly impaired up-conditioning. However, unlike recent CST transection, cSMC produced a paradoxical response to down-conditioning: the H-reflex actually increased. These results confirm the critical role of cSMC in H-reflex conditioning and suggest that this role extends beyond producing essential CST activity. Its interactions with ipsilateral SMC or other areas contribute to the complex pattern of spinal and supraspinal plasticity that underlies H-reflex conditioning.

INTRODUCTION

During development and skill acquisition, as well as after spinal cord injury or with other CNS disorders, descending input from the brain combines with peripheral input to change the spinal cord (for reviews see Casabona et al. 1990; Goode and Van Hoven 1982; Koceja et al. 1991; Levinsson et al. 1999; Myklebust et al. 1982, 1986; Nielsen et al. 1993; O'Sullivan et al. 1991; Straka and Dieringer 1995; Wolpaw and Tennissen 2001). The pathways and processes through which this descending input induces and maintains spinal cord plasticity remain largely unknown. The recently appreciated possibilities for CNS regeneration have raised questions regarding how regenerated neuronal tissue can become useful and how it can be shaped to provide normal, or at least acceptable, function (Wolpaw 2001, 2002). Thus they have drawn attention to the mechanisms by which the brain gradually shapes spinal cord pathways to function properly during movement (Bregman 1998; Fawcett 1998; Ramer et al. 2000; Tuszynski and Kordower 1999). Understanding these mechanisms could lead to novel methods for inducing, guiding, and assessing recovery after injury.

Operant conditioning of the spinal stretch reflex (SSR) or its electrical analog, the *H-reflex*, is a simple model for studying the brain's induction and maintenance of appropriate spinal cord function (Wolpaw 1997). Because the spinal pathway underlying these reflexes is influenced by descending activity from the brain, they can be operantly conditioned. When exposed to an operant conditioning protocol, monkeys, humans, and rats can gradually increase or decrease the SSR or the H-reflex (Chen and Wolpaw 1995; Evatt et al. 1989; Wolpaw 1987; Wolpaw et al. 1983; reviewed in Wolpaw 1997, 2001; Wolpaw and Tennissen 2001). In terms of a standard definition of "skill" as "an adaptive behavior acquired through practice" (Weiner and Simpson 1993), these operantly conditioned reflex changes are simple motor skills. They involve persistent anatomical and physiological changes in the spinal cord itself (Carp and Wolpaw 1994, 1995; Feng-Chen and Wolpaw 1996; Pillai et al. 2004; Wang et al. 2004, 2005; Wolpaw and Lee 1989). Thus this model provides an opportunity to define the activity-dependent plasticity that is associated with the acquisition of a simple motor skill (i.e., a larger or smaller SSR or H-reflex), the mechanisms that create and maintain this plasticity, and the manner in which it translates into behavior. In addition, it can help clarify the disordered motor function associated with spinal cord injury and may aid in development of new therapies (Chen et al. 2005b,c; Muir and Steeves 1997; Wolpaw and Tennissen 2001).

Recent studies explored the dependency of H-reflex conditioning on specific spinal cord pathways in rats (Chen and Wolpaw 1997, 2002; Chen et al. 2002, 2003). They showed that the main corticospinal tract (CST) is essential for both up-conditioning and down-conditioning and that other major pathways (i.e., the rubrospinal, vestibulospinal, and reticulospinal tracts, and the dorsal column ascending tract) are not essential (Chen and Wolpaw 1997, 2002). These results are consistent with human data indicating that operant conditioning of the biceps SSR is possible after partial spinal cord injury but is not possible after strokes that involve contralateral sensorimotor cortex, the main origin of the CST (Segal 1997; Segal and Wolf 1994).

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The rat CST comes mainly from contralateral sensorimotor cortex, but also has contributions from ipsilateral sensorimotor cortex and other cortical areas (Akinsegun and Buxton 1992; Amaral 2000; Clark 1984; Li et al. 1990; Paxinos and Watson 1986; Tracey 2004). To better define the dependency of H-reflex conditioning on the CST, this study evaluated the effects of ablation of contralateral sensorimotor cortex (cSMC). The results are at once both expected and surprising: they confirm the importance of cSMC implied by the effects of CST transection and they also indicate that its role in H-reflex conditioning extends beyond production of essential CST activity.

METHODS

Subjects were 22 male Sprague–Dawley rats weighing 300–500 g at the beginning of study. All procedures satisfied the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Academy Press, Washington, DC, 1996) and were previously reviewed and approved by the Institutional Animal Care and Use Committee of the Wadsworth Center. The protocol for monitoring and conditioning the H-reflex in freely moving rats, which is fully described elsewhere (Chen and Wolpaw 1994, 1995, 1997, 2002; Chen et al. 2001a,b, 2002; Wolpaw and Herchenroder 1990), is briefly summarized here. Other procedures are described in detail.

Ablation of cSMC and implantation of EMG and nerve cuff electrodes

Under general anesthesia [ketamine HCl; 80 mg/kg, administered intraperitoneally (ip)] and xylazine (10 mg/kg, ip), each rat underwent ablation of the contralateral (i.e., left) hindlimb area of sensorimotor cortex (cSMC) and was implanted with chronic stimulating and recording electrodes in the right leg. The rat was placed in a stereotaxic frame, with its head leveled and secured by ear bars and a tooth holder. The skull above the cSMC was opened and the cSMC area [0.5–4.0 mm caudal to bregma and 1.8–3.8 mm lateral to the midline (Paxinos and Watson 1986)] was carefully aspirated with a glass pipette (tip diameter 0.5 mm). After ablation, the opening in the skull was filled with bone wax, and the muscle and skin were sutured.

To elicit the H-reflex, a silicone rubber nerve cuff containing a pair of stainless steel multistranded fine-wire electrodes was placed on the right posterior tibial nerve just above the triceps surae branches. To record soleus EMG activity, a pair of fine-wire electrodes with the final 0.5 cm stripped was placed in the right soleus muscle. The Teflon-coated wires from the nerve cuff and the muscle passed subcutaneously to a connector plug mounted on the skull with stainless steel screws and dental cement.

Immediately after surgery, the rat was placed under a heating lamp and given an analgesic (Demerol, 0.2 mg, intramuscular). Once awake, it received a second dose of analgesic and was returned to its cage and provided with unrestricted access to food and water. Body weight was measured daily and a high-calorie dietary supplement (Nutri-Cal; 2–4 ml/day, per os) was given until body weight regained its presurgery level. Each rat also received a piece of apple (about 10 g) every day throughout the study.

H-reflex measurement and conditioning

Electrophysiological data collection began ≥25 days after surgery [43 ± 5 (mean ± SE; range 25–116)]. During data collection, each animal lived in a standard rat cage with a 40-cm flexible cable attached to the skull plug. The cable, which allowed the animal to move freely about the cage, carried the wires from the electrodes to an electrical swivel above the cage, and from there to an EMG amplifier (gain = 1,000, bandwidth 100–1,000 Hz) and a stimulus isolation unit. All animals had free access to water and food, except that during H-reflex conditioning they received food mainly by performing the task described below. Animal well-being was carefully checked several times each day and body weight was measured weekly. Laboratory lights were dimmed from 2100 to 0600 h each day.

A computer system continuously monitored ongoing soleus EMG, 24 h/day every day. Whenever the absolute value (equivalent to the full-wave rectified value) of background (i.e., ongoing) EMG remained within a defined range for a randomly varying 2.3- to 2.7-s period, the computer initiated a trial. In each trial, it stored the digitized EMG (absolute value digitized at 5,000 Hz) for the most recent 50 ms (i.e., the background EMG interval), delivered a stimulus pulse (typically 0.5 ms) to the nerve cuff, and stored the EMG for another 100 ms. The nerve cuff stimulus was initially set to produce a small M response (i.e., it was set just above the M response threshold), and then was automatically adjusted after each trial to maintain the amplitude of the EMG in the M response interval (typically 2.0–4.5 ms after stimulation) unchanged throughout the days and weeks of data collection.

Under the control mode, the computer simply digitized and stored the absolute value of soleus EMG for 100 ms after the stimulus. Under the up-conditioning (HRup) or down-conditioning (HRdown) mode, it gave a reward (i.e., a food pellet) 200 ms after nerve stimulation if EMG amplitude in the H-reflex interval (typically 6.0–10.0 ms after stimulation) was above (HRup mode) or below (HRdown mode) a criterion value. In the course of its normal activity, the animal usually satisfied the background EMG requirement, and thus received nerve cuff stimulation, 2,900–8,000 times per day.

For each rat, data were collected first under control mode for ≥10 days and then under HRup mode (HRup rats), HRdown mode (HRdown rats), or continued control mode (CTRL rats) for 50 more days. Under the HRup or HRdown mode, the criterion value was initially set on the basis of the control-mode data, and subsequently adjusted as needed each day, so that the rat received an adequate amount of food (e.g., about 1,000 reward pellets per day for a 500-g rat). Thus in the HRup mode, a typical rat was rewarded for the largest 1,000 H-reflexes of each day, whereas in the HRdown mode a typical rat was rewarded for the smallest 1,000 H-reflexes of each day. As noted below, all rats continued to gain weight throughout data collection.

Data analysis

Background EMG amplitude was calculated as average EMG amplitude during the 50 ms before nerve stimulation. M response size was calculated as average EMG amplitude in the M response interval minus average background EMG amplitude. H-reflex size, calculated as average EMG amplitude in the H-reflex interval minus average background EMG amplitude, was usually expressed in units of average background EMG amplitude. Daily averages of background EMG, M response, and H-reflex values were determined. Each rat's initial (i.e., control) values were the average values for the 10 days immediately before onset of the 50-day HRup, HRdown, or continued control-mode exposure, and its final values were the average values for the final 10 days (i.e., days 41–50) of this 50 days. (In one HRdown rat, the nerve stimulating cuff failed 31 days after conditioning, and days 22–31 provided the final values.)

In all rats, background EMG and M response size remained stable throughout data collection. To assess the effect of HRup, HRdown, or continued control-mode exposure on H-reflex size, a paired *t*-test was used to compare average final H-reflex sizes to initial H-reflex sizes. In addition, the average daily H-reflexes were used to calculate for each rat group the average course of H-reflex size throughout data collection.

Histology

At the end of study, each rat received an overdose of sodium pentobarbital (ip) and was perfused through the heart with saline followed by 3% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3). The EMG electrodes, nerve cuff, and posterior tibial nerve were examined and the soleus muscles of both sides were removed and weighed. Soleus weights (as percentage of body weight) were symmetrical and did not differ significantly from those of normal rats (Chen and Wolpaw 1995, 1997, 2002; Chen et al. 1996, 1999, 2001a,b, 2002, 2003; unpublished data). The brain was removed and examined to confirm the location and extent of the cSMC ablation. The dorsal aspect of the brain was photographed (Fig. 1*A*), the perimeters of the ablation and the entire left cerebral hemisphere were digitized, and the enclosed areas were determined. The area of the ablation was expressed as a percentage of the area of the hemisphere. The area encompassing the ablation was blocked and stored in 10% sucrose in 0.2 M phosphate buffer. Transverse 100-μm serial sections were cut with a vibrating microtome and stained with 1% neutral red (Fig. 1*B*).

This analysis indicated that the cSMC ablation was properly located and complete in all 22 rats. The ablation area averaged 3.5 ± 0.9 (SD)% of the total area of the hemisphere and did not differ significantly among CTRL, HRup, and HRdown rat groups ($P > 0.75$ by one-way ANOVA). In no rat was damage to underlying structures evident, and in no rat group was ablation size correlated with final H-reflex size ($P > 0.5$ by Pearson product-moment correlation). Figure 1 shows a brain from one cSMC rat. The left sensorimotor cortex hindlimb area (i.e., contralateral to the conditioned soleus H-reflex) is gone.

RESULTS

Ablation of cSMC

The cSMC ablation had no noticeable effects on animal well-being, locomotion, or other aspects of motor behavior. All rats recovered quickly from the ablation and electrode implantation surgery and remained healthy and active throughout the rest of the study. None of them showed any problem in bladder function. Body weight, which fell 0–12% in the first postsurgery week, recovered to its preoperative level in 1–4 wk. Every rat gained weight over the course of the study. Their average weight (±SD) increased from 422 ± 64 g (range 288–515 g) at the time of surgery to 555 ± 35 g (range 491–623 g) at the end of study.

As noted above, data collection under the control mode began ≥25 days after surgery [43 ± 5 (SE), range 25–116]. Onset of the HRup, HRdown, or continued control mode occurred at a comparable time in each rat group [i.e., 65 ± 9 (SE), range 38–107; 76 ± 12, range 38–147; and 68 ± 2, range 64–72 days after surgery for HRup, HRdown, and CTRL rats, respectively]. Baseline physiological data from the cSMC rats of this study were similar to those from normal rats. Table 1 compares initial control mode data from these cSMC rats to those from 135 normal (i.e., intact) rats of earlier studies (Chen and Wolpaw 1995, 1997, 2002; Chen et al. 1996, 1999, 2001a,b, 2002, 2003; unpublished data). The cSMC rats did not differ in background EMG level and H-reflex size ($P > 0.4$ and 0.9, respectively, by *t*-test). They had somewhat smaller M

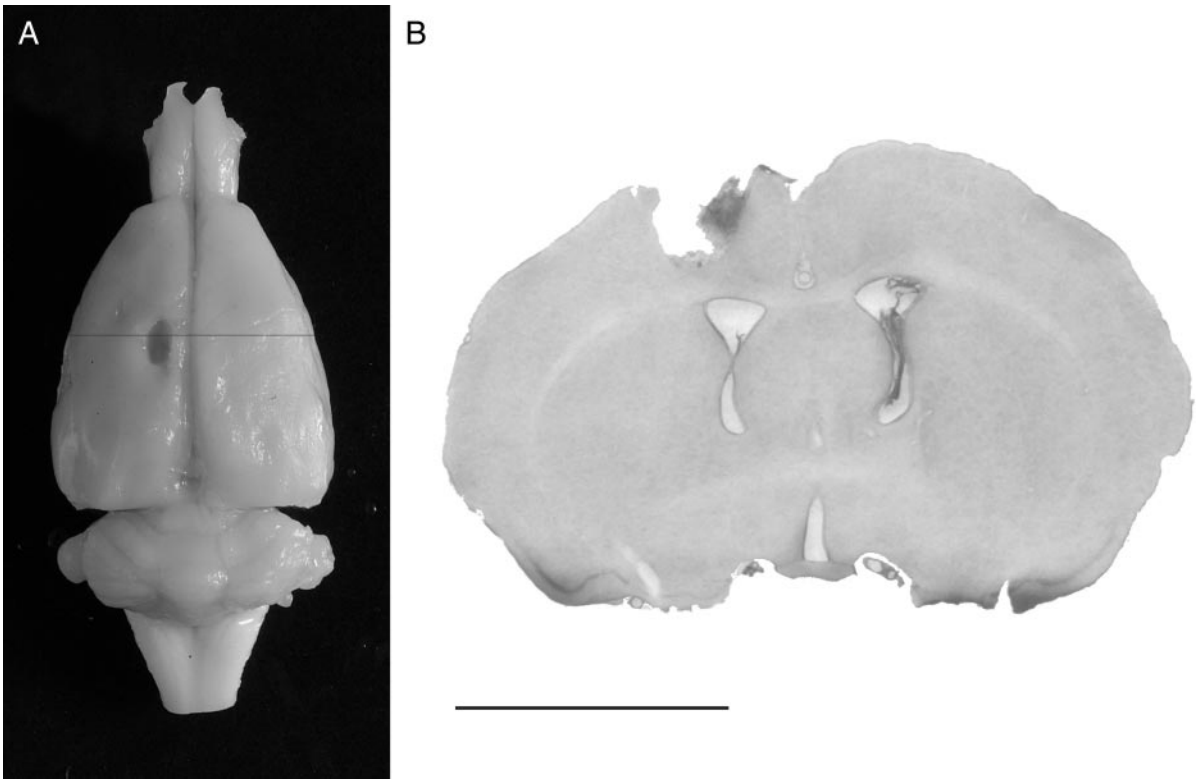

FIG. 1. Brain from a rat with a left (i.e., contralateral to the conditioned leg) sensorimotor cortex (cSMC) ablation. *A*: dorsal view. *B*: transverse section through the ablation (indicated by the line in *A*) showing the extent of ablated tissue. There is no apparent damage to underlying structures. Bars: 15 mm for *A* and 5 mm for *B*.

TABLE 1. *Measurements from cSMC rats and intact rats*

Parameter	Intact Rats (n = 135)	cSMC Rats (n = 22)
Trials/day	5,015 ± 1,784 (1,708–11,046)	5,869 ± 1,571* (2,871–8,040)
Background EMG, μV absolute value	101 ± 32 (19–330)	101 ± 19 (40–125)
M response, μV absolute value	124 ± 40 (53–382)	94 ± 22** (52–135)
H-reflex, μV absolute value	101 ± 55 (15–270)	112 ± 81 (23–412)

Values are average ± SD; values in parentheses represent range across rats. *$P < 0.05$ and ** $P < 0.001$ for intact rats versus cSMC rats by t-test. From Chen and Wolpaw (1995, 1997, 2002), Chen et al. (1996, 1999, 2001a,b, 2002, 2003), and unpublished data.

responses and performed slightly more trials/day ($P < 0.001$ and <0.05, respectively). In none of the three groups of cSMC rats did the average number of trials per day change significantly over the period of data collection.

Continued control mode exposure

In the CTRL group of five cSMC rats, H-reflex size was stable throughout data collection. Figure 2 shows their average (±SE) daily H-reflex, M response, and background EMG for 10 days before and 50 days after the beginning of the continued control-mode exposure (empty circles). It includes for comparison unpublished data from eight intact rats (filled circles) followed under the control mode for a comparable period of time. In both CTRL cSMC rats and intact rats, the average daily H-reflex stays close to its average initial amplitude (i.e., average amplitude for the first 10 days). In the cSMC rats, final H-reflex size (i.e., average amplitude for the last 10 days of continued control-mode exposure) averaged 110 ± 5% (SE) of the initial value and was not significantly different from it ($P > 0.14$, paired t-test). In the intact rats, the final H-reflex averaged 103 ± 2% of the initial value ($P > 0.19$, paired t-test). The cSMC and intact rats did not differ in their final H-reflex sizes ($P > 0.19$, t-test). Combined with the results in Table 1, these data indicate that any acute effect of cSMC ablation itself on H-reflex size or background EMG had disappeared, and that the small persistent effect on the M response had stabilized, well before H-reflex conditioning began.

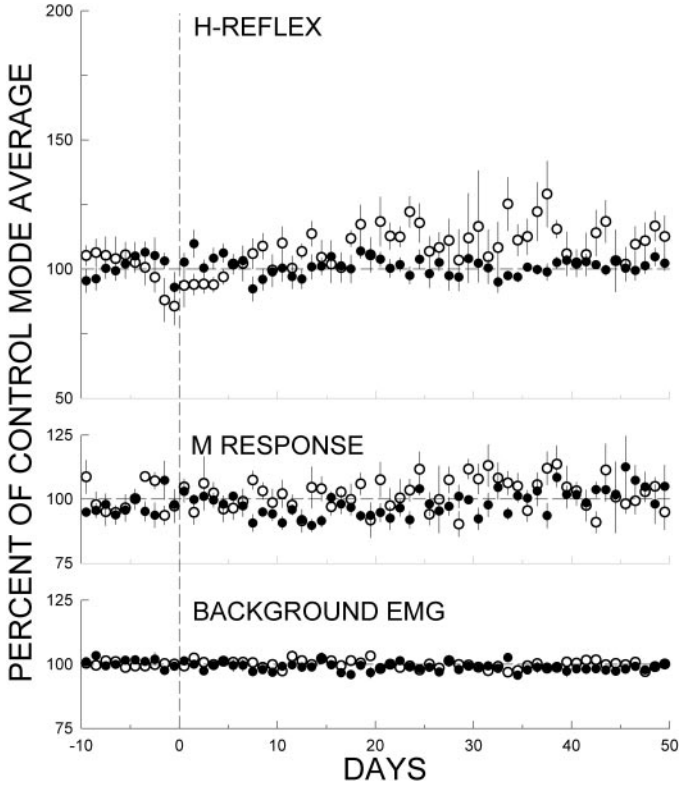

FIG. 2. Average (±SE) daily H-reflex, M response, and background EMG for 10 days before and 50 days after onset of the continued control-mode exposure for the 5 CTRL cSMC rats (open circles), and comparable unpublished data for 8 intact rats (solid circles) (as percentage of average value for the 10 days before). Neither intact nor cSMC rats show a significant change for any of the 3 measures.

HRup conditioning

Figure 3 shows, for the HRup group of eight cSMC rats, average (±SE) daily H-reflex, M response, and background EMG for the final 10 days in control mode and for 50 days after the up-conditioning mode began (open triangles), and includes for comparison data from 55 intact rats exposed to up-conditioning (solid triangles) (Chen and Wolpaw 1995, 1997, 2002; Chen et al. 1996, 1999, 2001a,b, 2002, 2003; unpublished

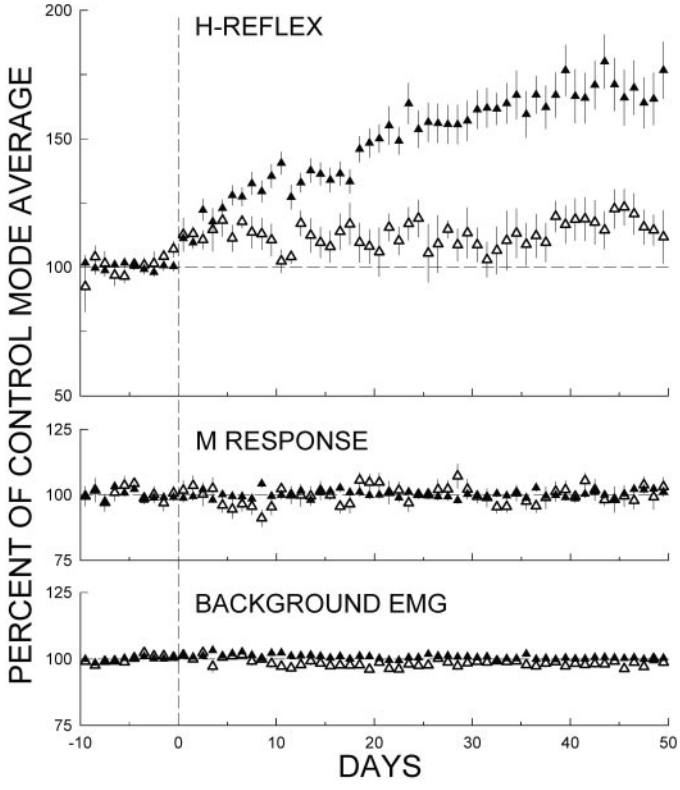

FIG. 3. Average (±SE) daily H-reflex, M response, and background EMG for 10 days before and 50 days after onset of exposure to the up-conditioning mode for the 8 cSMC rats (open up-triangles), and comparable data from 55 similarly exposed intact rats (solid up-triangles) (Chen and Wolpaw 1995, 1997, 2002; Chen et al. 1996, 1999, 2001a,b, 2002, 2003; unpublished data). H-reflex size markedly increases in intact rats, although it shows no significant change in cSMC rats. In both groups, M response and background EMG remain stable.

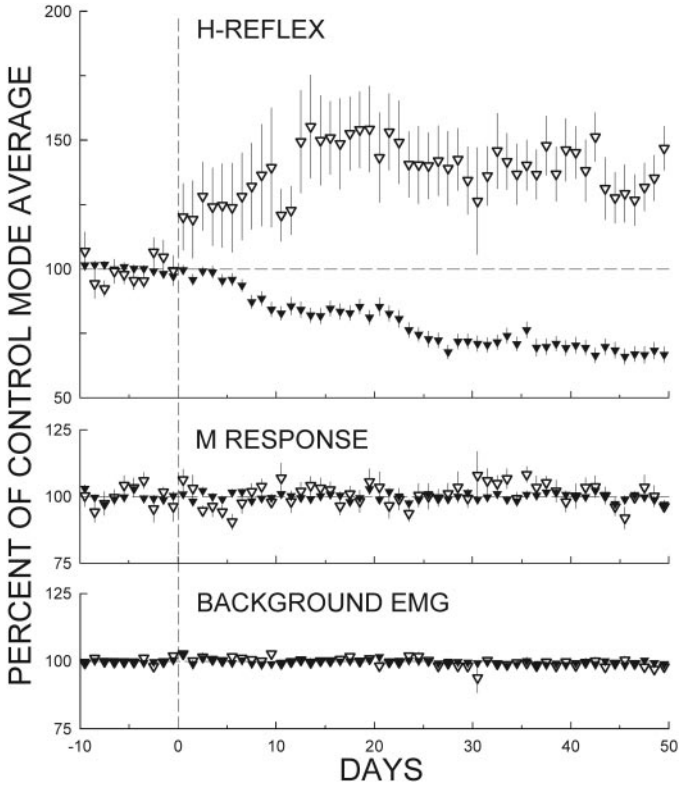

FIG. 4. Average (±SE) daily H-reflex, M response, and background EMG for 10 days before and 50 days after onset of exposure to the down-conditioning mode for the 9 cSMC rats (open down-triangles), and comparable data from 72 similarly exposed intact rats (solid down-triangles) (Chen and Wolpaw 1995, 1997, 2002; Chen et al. 1996, 1999, 2001a,b, 2002, 2003; unpublished data). H-reflex size markedly decreases in intact rats but increases in cSMC rats. This paradoxical increase with HRdown exposure occurs within 10 days. In both groups, M response and background EMG remain stable.

data). In both groups, M response and background EMG remain stable throughout data collection. In intact rats, H-reflex size increases steadily after HRup exposure begins and reaches a final (i.e., days 41–50) size of 166 ± 9% of initial value ($P < 0.001$, paired *t*-test). In contrast, HRup cSMC rats show an initial slight increase of 10–20% in the first few days of HRup exposure, and this remains until the end of data collection. The final H-reflex averages 115 ± 8% (SE), which is not significantly different from its initial size ($P > 0.10$, paired *t*-test), and was very similar to the final H-reflex (i.e., 110%) of the CTRL group. The final H-reflex of HRup cSMC rats is significantly smaller than that observed in the HRup intact rats ($P = 0.045$, *t*-test). Three of the eight (38%) HRup cSMC rats do show increases >20% (i.e., to 128, 129, and 150%), and thus satisfy the standard criterion for successful HRup-conditioning (Chen and Wolpaw 1995; Wolpaw et al. 1993). This success rate is significantly less than the success rate of 82% (45 of 55) for intact rats ($P = 0.015$, Fisher exact test). In sum, these results indicate that cSMC ablation greatly impaired HRup-conditioning.

HRdown conditioning

Figure 4 shows, for the HRdown group of nine cSMC rats, average (±SE) daily H-reflex, M response, and background EMG for the final 10 days in control mode and for 50 days after the down-conditioning mode began (open down-triangles), and includes for comparison data from 72 intact rats exposed to down-conditioning (solid down-triangles) (Chen and Wolpaw 1995, 1997, 2002; Chen et al. 1996, 1999, 2001a,b, 2002, 2003; unpublished data). In both groups, M response and background EMG remain stable throughout. In the intact rats, H-reflex size decreases steadily and reaches 67 ± 3% of its initial value ($P < 0.001$, paired *t*-test). In contrast, in the cSMC rats exposure to the HRdown mode leads to a paradoxical increase in H-reflex size that develops almost immediately after HRdown mode exposure begins and persists. Final H-reflex size averages 136 ± 9% (SE) of its initial value. It is significantly greater than its initial value ($P = 0.004$, paired *t*-test) and significantly greater than the final H-reflex size of the HRdown intact rats ($P < 0.001$, *t*-test). Successful down-conditioning [i.e., decrease of ≥20% (Chen and Wolpaw 1995; Wolpaw et al. 1993)] does not occur in any cSMC rat. Furthermore, six of the nine cSMC rats displayed increases >20% (i.e., 130, 146, 150, 156, 164, and 171%), which would have qualified as successful HRup-conditioning. In fact, the final H-reflex size and HRup success rate of these HRdown cSMC rats do not differ from those of HRup intact rats [$P = 0.215$ (*t*-test) and $P = 0.372$ (Fisher exact test), respectively].

Figure 5 shows average poststimulus EMG for representative days before (solid) and near the end (dashed) of HRdown mode exposure from an intact rat (*left*) and a cSMC rat (*right*).

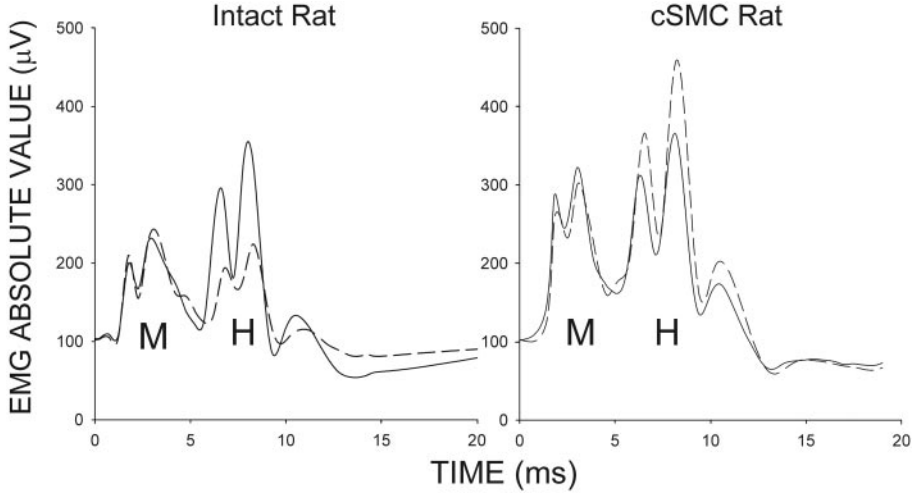

FIG. 5. Average poststimulus EMG for all the trials of representative days before (solid) and near the end (dashed) of HRdown mode exposure from an intact rat (*left*) and a cSMC rat (*right*). In both rats, background EMG (indicated by the value at 0 ms) and M response do not change. H-reflex of the intact rat is much smaller after down-conditioning, whereas that of the cSMC rat is larger.

In both rats, background EMG (indicated by the value at 0 ms) and M response do not change. The H-reflex of the intact rat is much smaller after down-conditioning, whereas that of the cSMC rat is larger.

As noted in METHODS, the reward criterion was continually adjusted during conditioning to ensure that each rat received a stable and adequate number of rewards/day. As a result of this adjustment, and the fact that the average number of trials/day remained stable, the percentage of trials in HRdown rats that were rewarded also remained stable [i.e., 25.9 ± 4 (SE) and 25.3 ± 4% for days 1–5 and days 41–50 of HRdown exposure, respectively]. Surprisingly, despite the fact that average H-reflex size in the HRdown rats increased to 136% of its initial value, the reward criterion itself remained stable [i.e., <87 ± 17 (SE) and <89 ± 14 μV for days 1–5 and days 41–50 of HRdown exposure, respectively]. This unexpected finding led us to examine the distributions of single-trial H-reflex interval amplitudes in the four HRdown rats for which we had stored all the individual trials as well as the daily averages. In these rats (as in the cSMC rats in general), the number of trials/day did not change during data collection. In addition, the diurnal distribution of their trials showed no significant change. However, in all four (three of which had substantially increased the H-reflex), the coefficient of variation (i.e., SD divided by mean) of the distribution of single-trial H-reflex interval amplitudes increased. As a result, the number of trials satisfying the reward criterion did not decrease, despite the fact that the average H-reflex increased.

Comparison to previous results

Figure 6 summarizes the final results for each cSMC group and compares them to final results obtained with the same H-reflex conditioning protocol from intact rats (Chen and Wolpaw 1995; Chen et al. 1996, 1999, 2001b, 2003; unpublished data) and from rats with recent corticospinal tract (CST) transection (i.e., rats conditioned within 2 mo after CST transection) (Chen and Wolpaw 2002; Chen et al. 2001a, 2002). It shows average (±SE) final H-reflex size (average size for final 10 days as percentage of initial size) for intact, CST, and cSMC rats after continued control, HRup, or HRdown mode exposure (Chen and Wolpaw 1995, 1997, 2002; Chen et al. 1996, 1999, 2001a,b, 2002, 2003; unpublished data). The continued control mode has no significant effect in any group. In intact rats, the HRup and HRdown modes have clear mode-appropriate effects. In CST rats, HRup and HRdown modes have no significant effects. In recent cSMC rats, the HRup mode has no significant effect; however, the HRdown mode produces a significant increase in H-reflex size.

DISCUSSION

Ablation of cSMC

Contralateral sensorimotor cortex (cSMC) ablation had no apparent effect on animal well-being, gross motor behavior, or activity level. The rats continued to gain weight and walked without any noticeable deficit. This preservation of normal motor function suggests that the effects of cSMC ablation on H-reflex conditioning did not arise from a nonspecific impairment of CNS function. Background EMG and H-reflex size of the cSMC rats were comparable to those of intact rats. The modest decrease in threshold M response size suggests that cSMC ablation may have had subtle effects on the excitation of motor axons by the nerve cuff stimulus (e.g., Halter et al. 1995) or on the firing patterns of the motoneurons providing background EMG (e.g., Dewald et al. 1995; Kamper and Rymer 2000; Suresh et al. 2005). Nevertheless, the results summarized in Table 1 and Fig. 2*A* indicate that cSMC ablation itself had no persistent or progressive effect on H-reflex size that might complicate the interpretation of its impact on H-reflex conditioning.

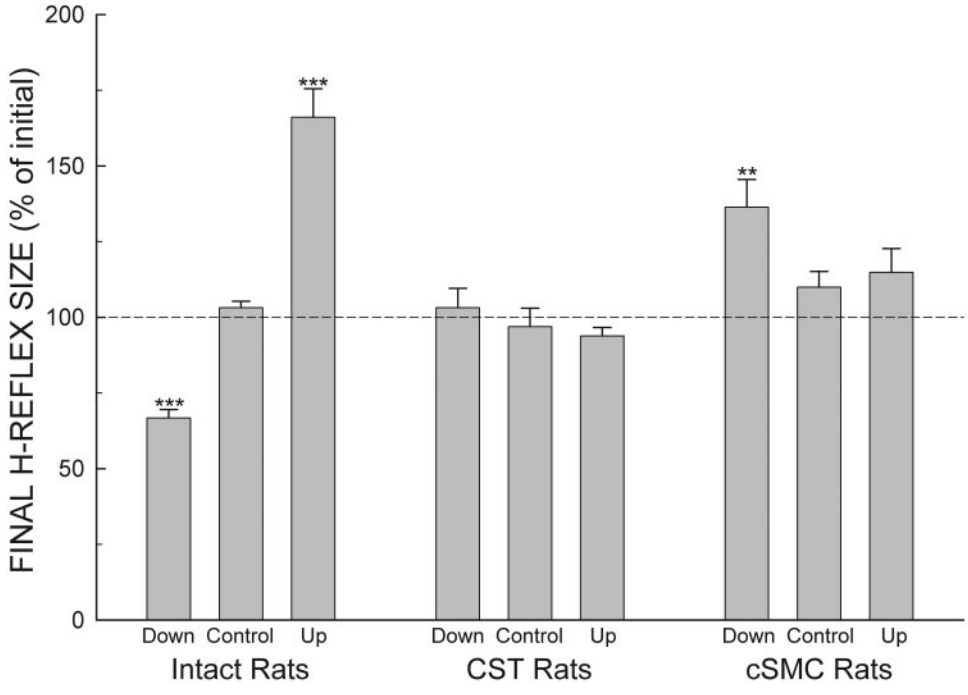

FIG. 6. Average (±SE) final H-reflex size (average size for final 10 days as percentage of initial size) for intact rats, CST-transected rats, and cSMC rats after continued-control, HRup, or HRdown mode exposure. Continued control-mode exposure has no significant effect in any group. In intact rats, the HRup and HRdown modes have mode-appropriate effects. In CST rats, the HRup and HRdown modes have no significant effect. In cSMC rats, the HRup mode has no significant effect; however, the HRdown mode increases H-reflex size. Asterisks indicate significant differences from initial size (***$P < 0.001$; **$P < 0.005$ by paired *t*-test).

The present study

The cSMC is the main origin of the CST (Akinsegun and Buxton 1992; Amaral 2000; Clark 1984; Li et al. 1990; Miller 1987; Paxinos and Watson 1986; Tracey 1995). Thus the present study is a logical extension of the earlier studies showing that the CST is essential for H-reflex conditioning and that other major descending pathways are not (Chen and Wolpaw 1997, 2002; Chen et al. 2002). By ablating cSMC, rather than transecting the entire (i.e., right and left) CST, the study addresses two issues. First, it focuses on cSMC alone, rather than on the sensorimotor cortices of both sides. Second, it explores the possibility that cSMC has a role in H-reflex conditioning beyond its production of appropriate CST influence, an additional role mediated through its interactions with ipsilateral sensorimotor cortex (iSMC) and/or other areas. The results, which are both expected (for up-conditioning) and surprising (for down-conditioning), provide important new information about both of these issues.

HRup conditioning in cSMC rats

The data for up-conditioning are consistent with the effects of CST transection (Chen and Wolpaw 1997, 2002; Chen et al. 2002). Ablation of cSMC largely prevents up-conditioning: final H-reflex size averaged only 115% of initial value. Furthermore, although the H-reflex increased ≥20% in three of the eight HRup cSMC rats, the fact that the H-reflex also increased slightly (to 110%) in the CTRL rats suggests that these three increases were in large part a nonspecific effect of the ablation. The up-conditioning results are also consistent with human studies indicating that strokes involving contralateral sensorimotor cortex abolish spinal stretch reflex conditioning (Segal 1997; Segal and Wolf 1994). These studies together indicate that activity arising in cSMC and descending in the CST is essential for up-conditioning.

HRdown conditioning in cSMC rats

The data for down-conditioning are also consistent with the effects of CST transection in rats and strokes in humans in that they show that cSMC ablation entirely prevents H-reflex decrease. At the same time, the data are surprising in showing that when cSMC rats are exposed to the HRdown mode the H-reflex actually increases. Indeed, final H-reflex size in the HRdown cSMC rats of this study is statistically indistinguishable from final H-reflex size in intact rats exposed to the HRup mode. This finding coupled with the fact that a similar increase with HRdown exposure does not occur after recent CST transection (Chen and Wolpaw 2002; Chen et al. 2002) imply that the role of cSMC in H-reflex down-conditioning extends beyond production of appropriate CST activity. Unlike recent CST transection, which does not directly damage cSMC, cSMC ablation also abolishes its interactions with other brain regions [i.e., ipsilateral sensorimotor cortex (iSMC), other cortical areas, and/or subcortical structures]. It appears that the loss of these interactions affects the impact of the HRdown mode on H-reflex size. This paradoxical effect is presumably mediated through descending axons other than those that arise in the cSMC and descend in the CST (such as axons that descend in lateral or ventral column pathways) (Hongo and Jankowska 1967; Jankowska and Tarnecki 1965; Lawrence and Kuypers 1968; Mitz and Humphrey 1986). The importance of these interactions is further supported by recent data indicating that, when rats are exposed to the HRdown mode 10–12 mo after CST transection (late CST rats), the H-reflex increases as it did in the HRdown cSMC rats of the present study (Tennissen et al. 2005). During the 10–12 mo between CST transection and HRdown exposure, the cSMC may undergo retrograde changes that mimic the effect of actual cSMC destruction on its interactions with other areas (Belhaj-Saif and Cheney 2000; Curt et al. 2002; Raineteau and Schwab 2001). Further study is needed to determine whether the loss of cSMC produces the paradoxical increase by altering function in other cortical areas and/or by directly affecting the subcortical areas that give rise to spinal cord descending pathways other than the CST.

Given that cSMC ablation prevented HRup-conditioning, it seems unlikely that the paradoxical increase seen with HRdown-conditioning resulted from mechanisms equivalent to those that underlie HRup-conditioning in normal rats. This paradoxical increase seen in cSMC rats exposed to the HRdown mode may be related to similarly unexpected increases found in previous studies of H-reflex conditioning. In intact rats that have decreased the H-reflex in response to the HRdown mode, either CST transection or ablation of cerebellar output nuclei leads to a similar increase: the H-reflex becomes larger than it was before down-conditioning (Chen and Wolpaw 2002; Wolpaw and Chen 2006). In down-conditioned monkeys, general anesthesia and spinal cord transection produce reflexes that are larger than expected on both the down-conditioned side and the other side (even though the reflex asymmetry created by down-conditioning remains evident) (Wolpaw and Lee 1989; Wolpaw et al. 1989). Finally, analysis of the time course of H-reflex down-conditioning in intact rats suggests that its beginning is associated with a small increase in H-reflex size that is obscured by the progressive development of the H-reflex decrease (Chen et al. 2001b).

These earlier observations and the increase found in this study are consistent with other data indicating that H-reflex conditioning is associated with a complex pattern of spinal and supraspinal plasticity (for reviews see Carp and Wolpaw 1994, 1995; Carp et al. 2001; Chen and Wolpaw 1997, 2002, 2005; Chen et al. 2002, 2003; Feng-Chen and Wolpaw 1996; Pillai et al. 2004; Wang et al. 2004, 2005; Wolpaw 1997; Wolpaw and Tennissen 2001). Although some of this plasticity appears responsible for the mode-appropriate H-reflex change (Belhaj-Saif and Cheney 2000; Carp and Wolpaw 1994; Curt et al. 2002; Feng-Chen and Wolpaw 1996; Pillai et al. 2004; Raineteau and Schwab 2001; Wang et al. 2006; Wolpaw 1987), the rest of it may ensure the preservation of older behaviors or may simply reflect reactive downstream effects caused by changes in activity associated with plasticity elsewhere (Carp and Wolpaw 1995; Chen et al. 2005a,b; Wang et al. 2004; Wolpaw and Lee 1989). The plasticity responsible for the paradoxical H-reflex increase seen in Fig. 2*C* may fit into one of these latter two categories. Finally, the paradoxical increase in HRdown cSMC rats, in combination with the simple failure to increase the H-reflex in HRup cSMC rats, is consistent with the growing evidence that up- and down-conditioning have different, rather than mirror-image, mechanisms (Carp and Wolpaw 1994, 1995; Carp et al. 2001; Pillai et al. 2004; Wang et al. 2004, 2005; Wolpaw and Chen 2001).

Finally, the increase in coefficient of variation that appears to occur in HRdown rats and to account for the fact that the percentage of rewarded trials does not decrease even though average H-reflex size increases and the reward criterion does not change is particularly intriguing. It suggests that the HR-down protocol is not entirely ineffective in cSMC rats, but rather induces an adaptive change in the descending influence on the H-reflex that is different from (and less effective than) that induced in intact rats. Rather than reducing the size of all H-reflexes, the rat widens the size distribution of the individual H-reflexes and thereby preserves the percentage of successful trials despite the overall increase in the average H-reflex. This effect contrasts with previous evidence that conditioning is associated with decrease rather than increase in the variability of reflex size (Wolpaw et al. 1985). Ablation of cSMC appears to prevent the rats from responding to the HRdown protocol in the same way that normal rats do. However, it does not prevent, and might even induce, another change (i.e., increase in the coefficient of variation) that maintains reward percentage despite an increase in the average H-reflex size. This effect illustrates the potential importance of the difference between the exact demand made by a conditioning protocol and the manner in which the result is assessed. Although the H-reflex protocol operantly conditions an increase in the number of trials satisfying the reward criterion, its results are usually measured by the average value of all the trials. In cSMC HRdown rats, however, these two measures may not correlate.

In conclusion, the present study strongly confirms previous studies suggesting that the cSMC produces output that descends by the CST to induce and guide the activity-dependent spinal cord plasticity that changes H-reflex size. In addition, it shows that cSMC involvement in H-reflex conditioning extends beyond its production of appropriate CST output to include aspects of its interaction with other brain areas. Thus it provides further evidence of and insight into the complex pattern of supraspinal and spinal plasticity that accompanies H-reflex conditioning.

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REFERENCES

Akinsegun A and Buxton DF. Differential sites of origin and collateralization of corticospinal neurons in the rat: a multiple fluorescent retrograde tracer study. *Brain Res* 575: 86–92, 1992.

Amaral DG. The functional organization of perception and movement. In: *Principles of Neural Science*, edited by Kandel ER, Schwartz JH, and Jessell TM. New York: McGraw-Hill, 2000, p. 337–348.

Baldissera F, Hultborn H, and Illert M. Integration in spinal neuronal systems. In: *Handbook of Physiology. The Nervous System. Motor Control*. Bethesda, MD: Am. Physiol. Soc., 1981, sect. 1, vol. II, pt. 1, p. 509–595.

Belhaj-Saif A and Cheney PD. Plasticity in the distribution of the red nucleus output to forearm muscles after unilateral lesions of the pyramidal tract. *J Neurophysiol* 83: 3147–3153, 2000.

Bregman BS. Regeneration in the spinal cord. *Curr Opin Neurobiol* 8: 800–807, 1998.

Brown WF. *The Physiological and Technical Basis of Electromyography*. Boston, MA: Butterworths, 1984.

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 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.

Casabona A, Polizzi MC, and Perciavalle V. Differences in H-reflex between athletes trained for explosive contraction and non-trained subjects. *Eur J Appl Physiol* 61: 26–32, 1990.

Chen L, Chen XY, Chen Y, Liu RL, and Wolpaw JR. Operant conditioning of reciprocal inhibition in freely moving rats. Program No. 175.5. *2005 Abstract Viewer/Itinerary Planner*. Washington, DC: Society for Neuroscience, 2005a, Online.

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. Time course of H-reflex conditioning in the rat. *Neurosci Lett* 302: 85–88, 2001b.

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, Feng-Chen KC, Chen L, Stark DM, and Wolpaw JR. Short-term and medium-term effects of spinal cord tract transections on soleus H-reflex in freely moving rats. *J Neurotrauma* 18: 313–327, 2001a.

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.

Chen XY and Wolpaw JR. Ablation of cerebellar nuclei prevents H-reflex down-conditioning in rats. *Learn Mem* 12: 248–254, 2005.

Chen XY, Wolpaw JR, Jakeman LB, and Stokes BT. Operant conditioning of H-reflex in spinal-cord injured rats. *J Neurotrauma* 13: 755–766, 1996.

Chen XY, Wolpaw JR, Jakeman LB, and Stokes BT. Operant conditioning of H-reflex increase in spinal-cord injured rats. *J Neurotrauma* 16: 175–186, 1999.

Chen Y, Chen XY, Jakeman LB, Chen L, Liu RL, Stokes BT, and Wolpaw JR. Re-educating the injured spinal cord by operant conditioning of a reflex pathway. Program No. 175.4. *2005 Abstract Viewer/Itinerary Planner*. Washington, DC: Society for Neuroscience, 2005c, Online.

Chen Y, Chen XY, Jakeman LB, Schalk G, Stokes BT, and Wolpaw JR. The interaction of a new motor skill and an old one: H-reflex conditioning and locomotion in rats. *J Neurosci* 25: 6898–6906, 2005b.

Clark RG. Anatomy of the mammalian cord. In: *Handbook of the Spinal Cord*, edited by Davidoff RA. New York: Marcel Dekker, 1984, p. 1–46.

Curt A, Bruehlmeier M, Leenders KL, Roelcke U, and Dietz V. Differential effect of spinal cord injury and functional impairment on human brain activation. *J Neurotrauma* 19: 43–51, 2002.

Dewald JP, Pope PS, Given JD, Buchanan TS, and Rymer WZ. Abnormal muscle coactivation during isometric torque generation at the elbow and shoulder in hemiparetic subjects. *Brain* 118: 495–510, 1995.

Evatt ML, Wolf SL, and Segal RL. Modification of human spinal stretch reflexes: preliminary studies. *Neurosci Lett* 105: 350–355, 1989.

Fawcett JW. Spinal cord repair: from experimental models to human application. *Spinal Cord* 36: 811–817, 1998.

Feng-Chen KC and Wolpaw JR. Changes in synaptic terminals of primate motoneurons after operant conditioning of H-reflex. *Proc Natl Acad Sci USA* 93: 9206–9211, 1996.

Goode DJ and Van Hoven J. Loss of patellar and Achilles tendon reflex in classical ballet dancers. *Arch Neurol* 39: 323, 1982.

Halter JA, Carp JS, and Wolpaw JR. Operantly conditioned motoneuron plasticity: possible role of sodium channels. *J Neurophysiol* 74: 867–871, 1995.

Henneman E and Mendell LM. Functional organization of motoneuron pool and inputs. In: *Handbook of Physiology. The Nervous System. Motor Control*. Bethesda, MD: Am. Physiol. Soc., 1981, sect. 1, vol. II, pt. 1, p. 423–507.

Hongo T and Jankowska E. Effects from the sensorimotor cortex on the spinal cord in cats with transected pyramids. *Exp Brain Res* 3: 117–134, 1967.

Jankowska E and Tarnecki R. Extrapyramidal activation of muscles from the sensori-motor cortex in cats. *Experientia* 21: 656–657, 1965.

Kamper DG and Rymer WZ. Quantitative features of the stretch response of extrinsic finger muscle in hemiparetic stroke. *Muscle Nerve* 23: 954–961, 2000.

Koceja DM, Burke JR, and Kamen G. Organization of segmental reflexes in trained dancers. *Int J Sports Med* 12: 285–289, 1991.

Lawrence DG and Kuypers HG. The functional organization of the motor system in the monkey. I. The effects of bilateral pyramidal lesions. *Brain* 91: 1–14, 1968.

Levinsson A, Luo XL, Holmberg H, and Schouenborg J. Developmental tuning in a spinal nociceptive system: effects of neonatal spinalization. *J Neurosci* 19: 10397–10403, 1999.

Li XG, Florence SL, and Kaas JH. Areal distributions of cortical neurons projecting to different levels of the caudal brain stem and spinal cord in rats. *Somatosens Mot Res* 7: 315–335, 1990.

Magladery JW, Porter WE, Park AM, and Teasdall RD. Electrophysiological studies of nerve and reflex activity in normal man. IV. The two-neuron reflex and identification of certain action potentials from spinal roots and cord. *Bull John Hopkins Hosp* 88: 499–519, 1951.

Matthews PBC. *Mammalian Muscle Receptors and Their Central Actions*. Baltimore, MD: Williams & Wilkins, 1972, p. 319–409.

Miller MW. The origin of corticospinal projection neurons in rat. *Exp Brain Res* 67: 339–351, 1987.

Mitz AR and Humphrey DR. Intracortical stimulation in pyramidotomized monkeys. *Neurosci Lett* 64: 59–64, 1986.

Muir GD and Steeves JD. Sensorimotor stimulation to improve locomotor recovery after spinal cord injury. *Trends Neurosci* 20: 72–77, 1997.

Myklebust BM, Gottlieb GL, and Agarwal GC. Stretch reflexes of the normal human infant. *Dev Med Child Neurol* 28: 440–449, 1986.

Myklebust BM, Gottlieb GL, Penn RD, and Agarwal GC. Reciprocal excitation of antagonistic muscles as a differentiating feature in spasticity. *Ann Neurol* 12: 367–374, 1982.

Nielsen J, Crone C, and Hultborn H. H-reflexes are smaller in dancers from the Royal Danish Ballet than in well-trained athletes. *Eur J Appl Physiol* 66: 116–121, 1993.

O'Sullivan MC, Eyre JA, and Miller SD. Radiation of phasic stretch reflex in biceps brachii to muscles of the arm in man and its restriction during development. *J Physiol* 439: 529–543, 1991.

Paxinos G and Watson C. *The Rat Brain in Stereotaxic Coordinates*. San Diego, CA: Academic Press, 1986.

Pillai S, Wang Y, Wolpaw JR, and Chen XY. H-reflex down-conditioning in rats increases number and size of GABAergic terminals on soleus motoneurons. *Soc Neurosci Abstr* 30: 417.2, 2004.

Raineteau O and Schwab ME. Plasticity of motor systems after incomplete spinal cord injury. *Nat Rev Neurosci* 2: 263–273, 2001.

Ramer MS, Harper GP, and Bradbury EJ. Progress in spinal cord research. *Spinal Cord* 38: 449–472, 2000.

Segal RL. Plasticity in the central nervous system: operant conditioning of the spinal stretch reflex. *Top Stroke Rehabil* 3: 76–87, 1997.

Segal RL and Wolf SL. Operant conditioning of spinal stretch reflex in patients with spinal cord injuries. *Exp Neurol* 130: 202–213, 1994.

Straka H and Dieringer N. Spinal plasticity after hemilabyrinthectomy and its relation to postural recovery in the frog. *J Neurophysiol* 73: 1617–1631, 1995.

Suresh NL, Ellis MD, and Rymer WZ. Impaired spatial control of motor units in hemiparetic stroke. Program No. 399.19. *2005 Abstract Viewer/Itinerary Planner*. Washington, DC: Society for Neuroscience, 2005, Online.

Tennissen AM, Chen XY, Chen Y, Chen L, Liu RL, and Wolpaw JR. Abolition of H-reflex down conditioning by corticospinal tract transection in rats is permanent. Program No. 175.3. *2005 Abstract Viewer/Itinerary Planner*. Washington, DC: Society for Neuroscience, 2005, Online.

Tracey DJ. Ascending and descending pathways in the spinal cord. In: *The Rat Nervous System*, edited by Paxinos G. San Diego, CA: Elsevier Academic Press, 2004, p. 149–164.

Tuszynski MH and Kordower JE. *CNS Regeneration—Basic Science Clinical Advances*. San Diego, CA: Academic Press, 1999.

Wang Y, Pillai S, Wolpaw JR, and Chen XY. Effects of H-reflex up-conditioning on GABAaergic terminals on rat soleus motoneurons. *Soc Neurosci Abstr* 30: 417.7, 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.

Weiner ESC and Simpson JA. (Editors). *Compact Oxford English Dictionary* (2nd ed.). Oxford, UK: Oxford Univ. Press, 1993, p. 1782.

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

Wolpaw JR. The complex structure of a simple memory. *Trends Neurosci* 20: 588–594, 1997.

Wolpaw JR. Spinal cord plasticity in the acquisition of a simple motor skill. In: *Spinal Cord Plasticity: Alterations in Reflex Function*, edited by Patterson MM and Grau JW. Boston, MA: Kluwer Academic, 2001, p. 101–126.

Wolpaw JR. Memory in neuroscience: rhetoric versus reality. *Behav Cogn Neurosci Rev* 1: 130–163, 2002.

Wolpaw JR, Braitman DJ, and Seegal RF. Adaptive plasticity in the 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 Chen XY. The cerebellum in maintenance of a motor skill: a hierarchy of brain and spinal cord plasticity underlies H-reflex conditioning. *Learn Mem* 13: 208–215, 2006.

Wolpaw JR and Herchenroder PA. Operant conditioning of H-reflex in freely moving monkeys. *J Neurosci Methods* 31: 145–152, 1990.

Wolpaw JR, Herchenroder PA, and Carp JS. Operant conditioning of the primate H-reflex: factors affecting the magnitude of change. *Exp Brain Res* 97: 31–39, 1993.

Wolpaw JR and Lee CL. Memory traces in primate spinal cord produced by operant conditioning of H-reflex. *J Neurophysiol* 61: 563–572, 1989.

Wolpaw JR, Lee CL, and Calaitges JG. Operant conditioning of primate triceps surae H-reflex produced reflex asymmetry. *Exp Brain Res* 75: 35–39, 1989.

Wolpaw JR, O'Keefe JA, Kieffer VA, and Sanders MG. Reduced day-to-day variation accompanies adaptive plasticity in the primate spinal stretch reflex. *Neurosci Lett* 54: 165–171, 1985.

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