JOURNAL OF NEUROTRAUMA
Volume 16, Number 2, 1999
Mary Ann Liebert, Inc.

Operant Conditioning of H-Reflex Increase in Spinal Cord–Injured Rats

XIANG YANG CHEN,[1] JONATHAN R. WOLPAW,[1] LYN B. JAKEMAN,[2] and BRADFORD T. STOKES[2]

ABSTRACT

Operant conditioning of the spinal stretch reflex or its electrical analog, the H-reflex, is a new model for exploring the mechanisms of long-term supraspinal control over spinal cord function. Primates and rats can gradually increase (HRup conditioning mode) or decrease (HRdown conditioning mode) the H-reflex when reward is based on H-reflex amplitude. An earlier study indicated that HRdown conditioning of the soleus H-reflex in rats is impaired following contusion injury to thoracic spinal cord. The extent of impairment was correlated with the percent of white matter lost at the injury site. The present study investigated the effects of spinal cord injury on HRup conditioning. Soleus H-reflexes were elicited and recorded with chronically implanted electrodes from 14 rats that had been subjected to calibrated contusion injuries to the spinal cord at T8. At the lesion epicenter, 12–39% of the white matter remained. After control-mode data were collected, each rat was exposed to the HRup conditioning mode for 50 days. Final H-reflex amplitudes after HRup conditioning averaged 112% (±22% SD) of control. This value was significantly smaller than that for 13 normal rats exposed to HRup conditioning, in which final amplitude averaged 153% (±51%) SD of control. As previously reported for HRdown conditioning after spinal cord injury, success was inversely correlated with the severity of the injury as assessed by white matter preservation and by time to return of bladder function. HRup and HRdown conditioning are similarly sensitive to injury. These results further demonstrate that H-reflex conditioning is a sensitive measure of the long-term effects of injury on supraspinal control over spinal cord functions and could prove a valuable measure of therapeutic efficacy.

Key words: H-reflex, operant conditioning, plasticity, rat, soleus muscle, spinal cord injury

INTRODUCTION

DESCENDING SPINAL CORD PATHWAYS regulate segmental reflexes. This regulation is both short-term—rapidly altering reflexes as needed for different motor behaviors—and long-term—gradually modifying reflex pathways during development and in the course of skill acquisition (Myklebust et al., 1986; Casabona et al., 1990; Wolf and Segal, 1990, 1996; Wolpaw, 1987, 1997; O'Sullivan et al., 1991; Nielsen et al., 1993). When spinal

[1]Wadsworth Center, New York State Department of Health and State University of New York, Albany, New York.
[2]Department of Physiology, Ohio State University, Columbus, Ohio.

cord injury impairs this long-term control, spasticity and other reflex abnormalities gradually develop (Taylor et al., 1984; Dimitrijevic et al., 1988; Boorman et al., 1992; Shefner et al., 1992; Calancie et al., 1993; Doyle et al., 1993; St. George, 1993; Stein et al., 1993; Hechman, 1994; Nozaki et al., 1996). These reflex abnormalities may be disabling in themselves and may also interfere with remaining voluntary motor control. Better understanding of the long-term control that descending pathways exercise over spinal reflexes could lead to new methods for assessing the effects of injury and for restoring function.

Operant conditioning of the spinal stretch reflex (SSR) or its electrical analog, the H-reflex, provides a model for exploring long-term descending regulation of reflex function in the normal spinal cord, after spinal cord injury, and after therapeutic interventions (Wolpaw et al., 1983; Wolpaw, 1987; Evatt et al., 1989; Wolpaw and Lee, 1989; Wolf and Segal, 1990, 1996; Wolpaw and Carp, 1993; Segal and Wolf, 1994; Chen and Wolpaw, 1995, 1997; Wolf et al., 1995; Chen et al., 1996). Both primates and rats can gradually increase (HRup conditioning mode) or decrease (HRdown conditioning mode) the H-reflex when reward is based on H-reflex amplitude. This is accomplished by a mode-appropriate change in descending regulation of the spinal arc of the H-reflex, and is accompanied by plasticity at several sites in the spinal cord (for review, see Wolpaw, 1997). It appears to be related to processes that underlie the development of motor skills throughout life (Myklebust et al., 1986; Casabona et al., 1990; Wolf and Segal, 1990, 1996; Wolpaw, 1997; O'Sullivan et al., 1991; Nielsen et al., 1993).

Our primary goal is to define the potential value of H-reflex conditioning as a measure of spinal cord function after injury, during recovery, and after therapeutic interventions. To accomplish this, we are studying the effects on H-reflex conditioning of calibrated spinal cord contusions and of pathway-specific lesions. An initial study showed that thoracic spinal cord contusion in rats impairs the capacity for HRdown conditioning of the soleus H-reflex (Chen et al., 1996). Impairment was closely correlated with the amount of white matter lost. Conditioning was highly sensitive to white matter loss: about 30% had to remain for successful HRdown conditioning. A subsequent study (Chen and Wolpaw, 1997) showed that HRdown conditioning was prevented by destruction of the dorsal column, which in rats contains the main corticospinal tracts. It was not prevented, however, by destruction of the ipsilateral lateral column, containing rubrospinal, vestibulospinal, and reticulospinal tracts (Holstege and Kuypers, 1987; Kennedy, 1990; Tracey, 1995). These results suggest that HRdown conditioning is dependent on the corticospinal tract. Consistent with this hypothesis are studies in human populations indicating that down conditioning of the SSR itself can occur after spinal cord injury and appears to be impaired by damage to sensorimotor cortex (Segal and Wolf, 1994; Segal, 1997).

Behavioral and physiological data suggest that HRup and HRdown conditioning have different mechanisms (Carp and Wolpaw, 1994, 1995), and thus might differ in their dependence on descending spinal cord pathways. Different pathways might be involved and/or sensitivity to pathway cord damage might differ. To address this issue, we explored the effects on HRup conditioning of calibrated thoracic spinal cord contusions like those used in the initial study of HRdown conditioning (Chen et al., 1996), and compared the results of the two studies.

METHODS

Subjects were 14 female Sprague-Dawley rats weighing 240–281 g at the beginning of study. Animal preparation and data collection methods have been described in detail previously (Bresnahan et al., 1991; Stokes and Reier, 1992; Chen and Wolpaw, 1995, 1996; Chen et al., 1996) and are summarized here. 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, D.C., 1996), and had been reviewed and approved by the Institutional Animal Care and Use Committees of the Wadsworth Center and the Ohio State University.

Each animal received a calibrated contusion injury to the thoracic spinal cord at the Ohio State University as previously described (Stokes, 1992; Stokes et al., 1992; Chen et al., 1996). Briefly, the animal was anesthetized with an intraperitoneal injection of ketamine HCl (80 mg/kg) and xylazine (10 mg/kg). A partial dorsal laminectomy was performed at the T8–T9 vertebral junction, and over a 23-msec period the dorsal surface of the cord was compressed either 0.7 mm (mild contusion, 10 rats) or 0.9 mm (medium contusion, four rats). The wound site was closed, and postoperative care was administered as previously described (Chen et al., 1996).

Electrode implantation and operant conditioning were performed at the Wadsworth Center (Chen and Wolpaw, 1995; Chen et al., 1996). Briefly, 13–46 days after the injury, each rat was implanted under general anesthesia (ketamine HCl, 80 mg/kg; xylazine, 10 mg/kg, intraperitoneal) with nerve cuff stimulating electrodes on the right posterior tibial nerve and EMG recording electrodes 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.

Data collection began 26–259 days after the injury and lasted 55–123 days. Throughout this period, the 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 electronic swivel above the cage and from there to an EMG amplifier and a nerve-cuff stimulation unit. All animals had free access to water. During H-reflex conditioning, they ate mainly by performing the task described below. Animal well-being was checked carefully several times per day, and body weight was measured every week. Laboratory lights were dimmed from 2100 to 0600 daily.

A computer system continuously monitored soleus EMG and controlled the nerve cuff stimulus and the reward (a 20-mg food pellet). If the absolute value of background (i.e., ongoing) soleus EMG stayed in a specified range for a randomly varying 2.3–2.7-sec period, a stimulus pulse (i.e., typically 0.5 msec in duration) was delivered by the nerve cuff. Pulse amplitude was automatically adjusted to maintain a stable M response amplitude (i.e., just above threshold) throughout the months of data collection. Under the control-mode, the computer simply digitized soleus EMG and stored its absolute value for 50 msec following the stimulus. Under the HRup or HRdown conditioning mode, it also gave a reward 200 msec after nerve stimulation if EMG amplitude (i.e., average absolute value) in the H-reflex interval (typically 5.5–9.0 msec after stimulation) was above (HRup-mode) or below (HRdown-mode) a criterion value. In the course of its daily activity, the animal normally satisfied the background EMG requirement, and thus received nerve cuff stimulation, 2,900–9,300 times per day. H-reflex amplitude was calculated as average EMG amplitude in the H-reflex interval minus average background EMG amplitude, and was expressed in units of average background EMG amplitude. For each rat, data were collected under the control-mode for 10–32 days to determine the animal's initial H-reflex amplitude. It was then exposed to the HRup-mode for up to 50 days. Some animals were then switched to the opposite (i.e., HRdown) mode for another 50 days.

To determine the final effect on H-reflex amplitude of HRup exposure (and of subsequent HRdown exposure in those animals exposed to both), average H-reflex amplitude for the final 10 days of the exposure was calculated as percent of initial H-reflex amplitude (i.e., average for final 10 control-mode days). In addition, for HRup/HRdown exposures, the final effect of HRdown exposure was also assessed by calculating average H-reflex amplitude for the final 10 days as percent of H-reflex amplitude at the end of the HRup exposure (Chen and Wolpaw, 1996).

At the end of study, each rat was sacrificed with an intraperitoneal overdose of pentobarbital sodium and perfused through the heart with saline, followed by 4% buffered formaldehyde solution. The soleus muscles were removed and weighed, and the spinal cord was removed and stored in the 4% formaldehyde solution.

The percent of white matter remaining at the lesion epicenter was measured as described previously (Chen et al., 1996). Transverse sections (10–25 μm) encompassing the lesion were cut from paraffin-embedded blocks, and stained with luxol fast blue for myelin or luxol fast blue and 0.1% cresyl violet for myelin and Nissl substance. Images through two sections with the greatest damage were digitized at approximately $\times 50$ magnification with the MCID Imaging System (Imaging Research, St. Catherines, Ontario) and outlined manually. Remaining white matter was defined at $\times 200$ by the presence of normal luxol fast blue staining. The area of remaining white matter was calculated using the MCID program, and expressed as percent of the white matter area of a reference section from thoracic spinal cord 3.5–4.0 mm rostral to the injury epicenter (Olby and Blakemore, 1996).

RESULTS

Animal Behavior and Well-Being

Following injury, rats showed transient hindlimb paralysis. This deficit waned rapidly over 2–5 days, and all rats recovered coordinated locomotion within 3 weeks. Bladder function, absent immediately after injury, returned over 2–7 days. The time to return of bladder function (defined as the time between injury and a point halfway between the last time at which the bladder was found to be distended and the next time when expression was attempted) was significantly longer for rats with medium (0.9-mm displacement) contusions than for those with mild (0.7-mm displacement) contusions (5.9 ± 0.6 SD and 3.8 ± 1.5 SD days, respectively; $p < 0.05$ by t test). After the immediate posttraumatic period, all animals remained healthy and active until perfusion. Weight increased from 240–281 g at injury, to 266–320 g at implantation and 308–442 g at perfusion. After perfusion, soleus muscle weights (measured as percent of body weight) were symmetrical and did not differ significantly from those of normal rats.

Control-Mode Data

As a necessary prerequisite for evaluating the effects of injury on H-reflex conditioning, we assessed the ef-

fects of injury on trials/day, background EMG, M response amplitude, and H-reflex amplitude under the control-mode (i.e., prior to H-reflex conditioning). For this assessment, we combined data from the 14 rats of this HRup conditioning study with that of the 15 rats of the previous HRdown conditioning study (Chen et al., 1996). Thus, we had control-mode data from each of 29 spinal cord–injured rats (i.e., 21 with mild and eight with medium contusions) for 10–20 days, starting 18–259 days after the injury. Control-mode values for trials/day, background EMG, M response amplitude, and H-reflex amplitude from the injured rats were not correlated with time since injury ($p > 0.4$ for all four measures by Pearson product moment correlation test), and did not differ be-

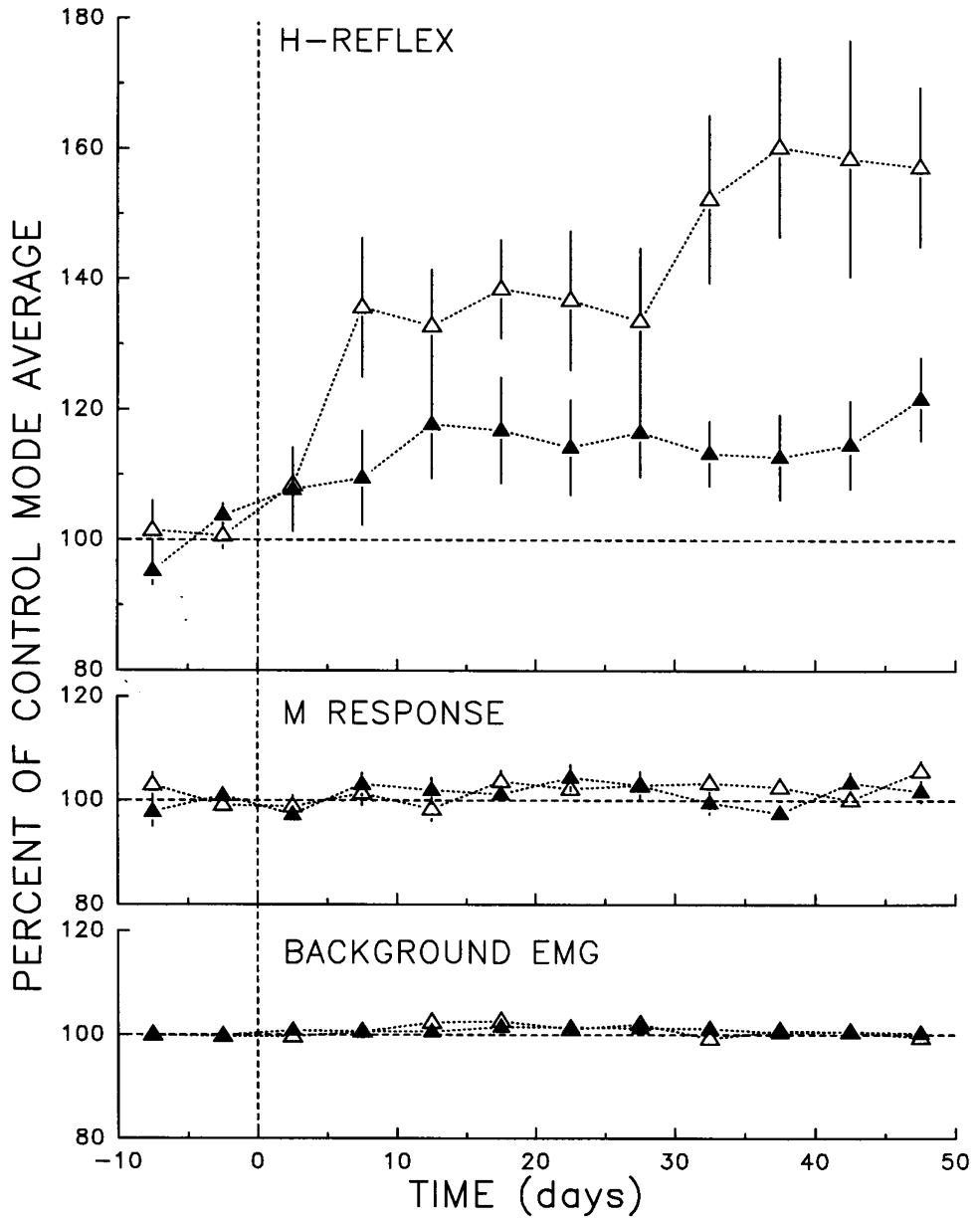

FIG. 1. Average (±SE) H-reflex amplitude, M response amplitude, and background EMG for 14 spinal cord–injured rats (10 with mild and four with medium contusions, filled triangles) and 13 normal rats (from Chen and Wolpaw, 1995, and subsequent data, empty triangles) for each 5-day period under the control-mode and the HRup-mode (in percent of control-mode value). While H-reflex amplitude rises in both spinal cord-injured and normal rats, the increase is much smaller in the injured rats ($p < 0.02$, ANOVA on group difference in linear trend). For both groups, background EMG and M response are stable throughout data collection.

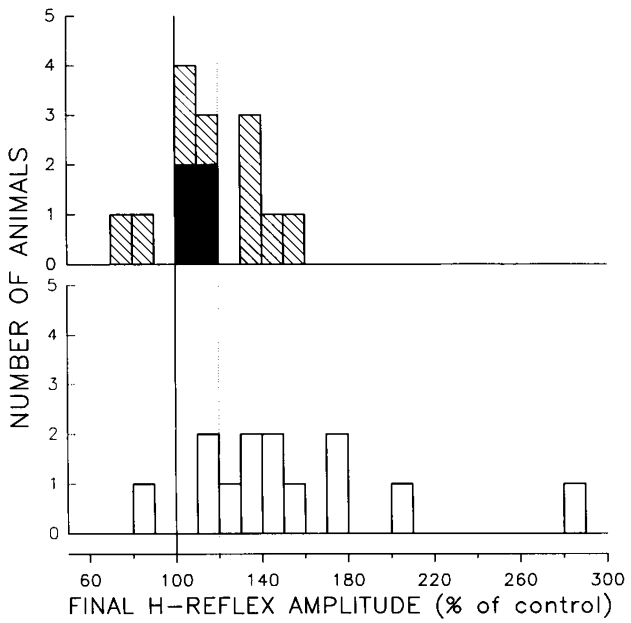

FIG. 2. Distributions of H-reflex amplitudes at end of HRup conditioning for rats with mild (hatched, $n = 10$) or medium (solid, $n = 4$) spinal cord contusions (top) and for normal rats (bottom, $n = 13$). Dotted line at 120% indicates criterion for successful HRup conditioning (i.e., increase ≥20%).

tween the HRup and HRdown studies ($p > 0.5$ for all four measures by t test). Furthermore, these data did not differ ($p > 0.1$ by t test for all four measures) from those of 44 normal rats studied to date (Chen and Wolpaw, 1994, 1995, and subsequent data). The values of the spinal cord-injured rats and the normal rats were very similar, except that mean H-reflex amplitude was substantially (i.e., 21.0%), but not significantly, higher in the injured rats.

HRup Conditioning

After control-mode data collection, all 14 injured rats were exposed to the HRup conditioning mode. Figures 1 and 2 and Table 1 summarize the effects of this exposure in the injured animals, and compares them with the effects observed in 13 normal HRup animals studied to date (Chen and Wolpaw, 1995, and subsequent data). Figure 1 shows average values for H-reflex, M response, and background EMG before and during exposure to the HRup-mode. In both injured and normal groups, background EMG and M response amplitude remain stable throughout, while H-reflex amplitude rises gradually. The H-reflex amplitude was evaluated with an analysis of variance with group as a between-subjects factor and days as a within-subjects factor. The effects of group, days, and the group-by-days interaction were significant (i.e., $p < 0.01$, $p < 0.001$, and $p < 0.001$, respectively). The interaction was then evaluated by a trend analysis of variance. The linear trend was greater in normal than in lesioned animals ($p < 0.02$). Furthermore, the final H-reflex values of the normal rats are significantly greater than those of the injured animals ($p < 0.05$ for comparison of average values for final 10 days by t test). The injured and normal groups do not differ in background EMG ($p > 0.8$) or M response ($p > 0.3$). Thus, HRup conditioning was impaired in the spinal cord–injured rats.

Table 1 gives the average final values for HRup conditioning for the mild and medium contusion groups together and separately, and for the normal group. Figure 2 shows the distributions of final H-reflex amplitudes for the normal and injured HRup animals. The dashed vertical line indicates the standard criterion for successful HRup conditioning: an increase of at least 20% (Chen and Wolpaw, 1995). While 10 of the 13 normal animals, or 77%, were successful, only 5 of the 14 injured animals, or 36%, were successful ($p = 0.05$, for intergroup difference in percent successful by Fisher exact test). Success was more common among animals with mild contusions (i.e., 5/10) than among those with medium contusions (i.e., 0/4) (though, with the small numbers of animals, the difference did not reach significance ($p > 0.2$ by Fisher exact test)).

Operant conditioning began 45–270 days after injury. Figure 3 addresses the question of whether success was correlated with time since injury. It shows final H-reflex

TABLE 1. HRUP CONDITIONING RESULTS FOR SPINAL CORD–INJURED RATS AND FOR NORMAL RATS

	Final H-reflex amplitude (mean ± SD in percentage of control)	*Number successful (%) (H-reflex increase ≥20%)*
Spinal cord–injured rats ($n = 14$)	117 ± 22	5 (36%)
Mild contusion ($n = 10$)	120 ± 26	5 (50%)
Medium contusion ($n = 4$)	110 ± 7	0 (0%)
Normal rats[a] ($n = 13$)	153 ± 51	10 (77%)

The injured and normal groups differ significantly in final H-reflex amplitude (see text).
[a]From Chen and Wolpaw, 1995, and subsequent data.

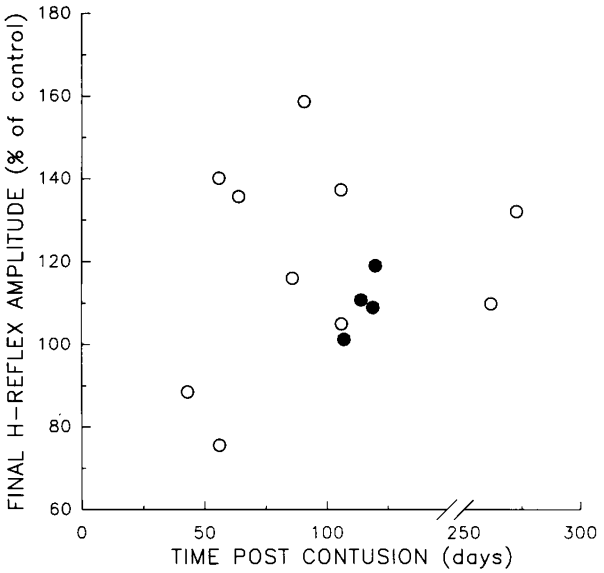

FIG. 3. H-reflex amplitudes at end of HRup conditioning versus number of days after injury when conditioning began for rats with mild (open circles) and medium (solid circles) spinal cord contusions. A significant correlation is not present ($r = 0.13$, $p > 0.6$).

amplitude as a function of the time after injury when HRup exposure began. No significant correlation is evident ($r = 0.13$, $p > 0.6$).

HRup Conditioning Versus Lesion Size and Versus Bladder Function

The percent of white matter remaining at the lesion epicenter ranged from 12% to 39%. This range was similar to that in the HRdown conditioning study (Chen et al., 1996). The values for the four rats with medium contusions averaged 16 ± 4% SD, and were significantly different ($p < 0.02$ by t-test) from those for the 10 rats with mild contusions, which averaged 29 ± 8%). Figure 4 shows photomicrographs of spinal cord sections at the lesion epicenter from two rats with mild injuries (Fig. 4A,B) and two rats with medium injuries (Fig. 4C,D).

Figure 5A shows final H-reflex amplitude as a function of white matter remaining. Animals with more remaining white matter were more successful at HRup conditioning ($r = 0.60$, $p < 0.05$). Figure 5B shows final H-reflex amplitude as a function of the time that passed before bladder function returned. The two measures are inversely correlated ($r = -0.62$, $p < 0.02$). Animals in which bladder function returned more quickly did better at HRup conditioning.

HRup/HRdown Conditioning

In order to evaluate the reversibility of H-reflex conditioning (Chen and Wolpaw, 1996) in spinal cord injured rats and to further compare the effects of injury on HRup and HRdown conditioning, three rats with mild contusions were switched to the HRdown-mode after completing a 50-day exposure to the HRup-mode. Two animals completed 50 days of HRdown conditioning. HRdown exposure in the third animal lasted only 31 days due to loss of EMG electrode function. Figure 6 shows for each of these animals final H-reflex amplitude at the ends of the HRup and HRdown exposures. Of the three animals, two displayed successful HRup conditioning (increase to 137%, and 136%, respectively, of their control values), and one showed no increase (101% of control at the end of HRup conditioning). After exposure to HRdown conditioning for 50 or 31 days, the H-reflex amplitude of the two HRup successful animals decreased to 77% and 81%, respectively, of their final HRup values (i.e., to 111% and 105% of their control-mode values). The other showed no decrease after 50 days of HRdown exposure. The H-reflex amplitude at the end of HRdown conditioning was 107% of its final HRup value (i.e., 109% of its control-mode value).

DISCUSSION

Long-Term Supraspinal Control of H-Reflex Amplitude

Spinal cord reflex pathways normally function under the control of descending pathways from supraspinal structures. When injury or disease eliminates or impairs this control, reflex pathways undergo acute and chronic changes that contribute to the development of spasticity and other functional abnormalities (Fujimori et al., 1966; Ashby and Verrier, 1975; Davis, 1975; Henneman, 1980; Little and Halar, 1985; Myklebust et al., 1986; Dimitrijevic et al., 1988; O'Sullivan et al., 1991; Boorman et al., 1992; Shefner et al., 1992; Doyle et al., 1993; St. George, 1993; Stein et al., 1993; Nielsen et al., 1993; Simpson et al., 1995). Better understanding of how descending pathways control spinal reflex function, and of the changes that occur after this control is impaired, could lead to novel methods for inducing, guiding, and assessing motor recovery.

Operant conditioning of the H-reflex is a product of alteration in the long-term descending control of spinal cord reflex function (Wolpaw, 1987, 1997; Chen and Wolpaw, 1995, 1996, 1997; Chen et al., 1996). Comparable changes occur during development and during ac-

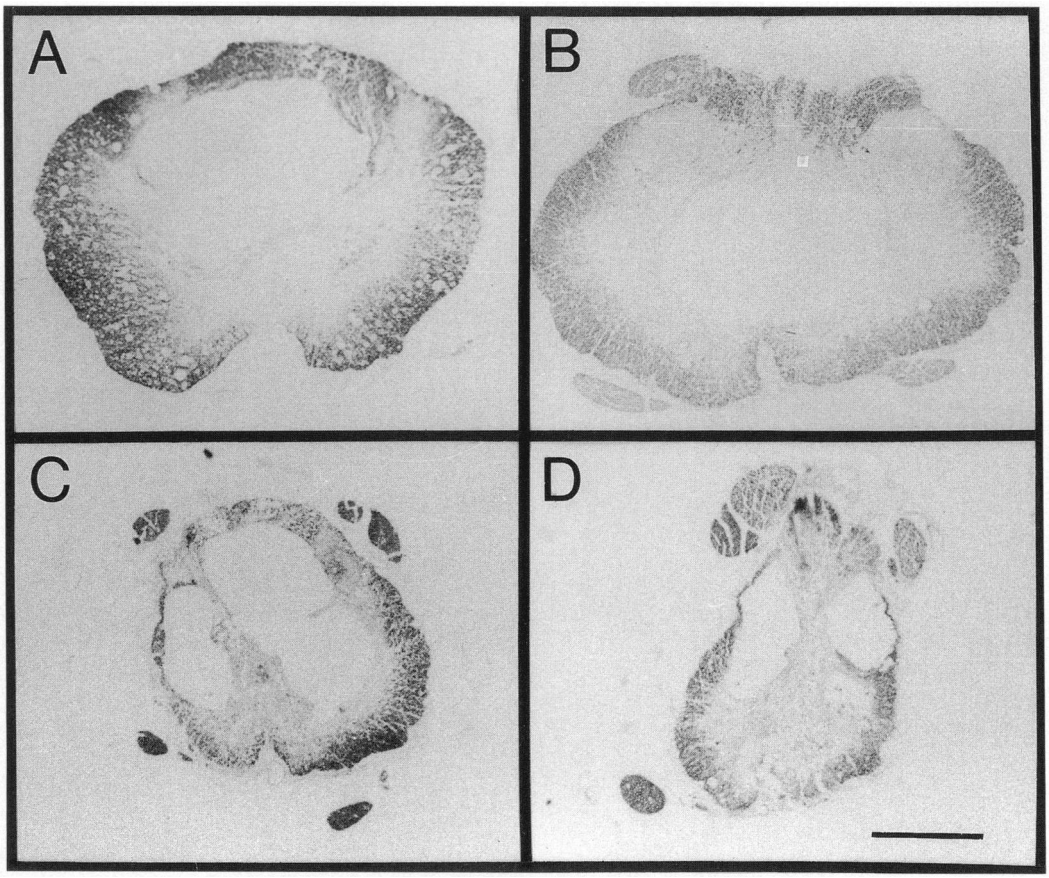

FIG. 4. Photomicrographs of 10-μm transverse sections through the epicenter of two mild (A,B) and two medium contusion injuries. Sections were stained with luxol fast blue. Animals illustrated in A and B successfully increased H-reflex amplitude (i.e., 136% and 140%, respectively); those in C and D did not. Amounts of white matter remaining for A–D are 31.4%, 35.5%, 20.7%, and 12.2%, respectively. Bar = 500 μm.

quisition of motor skills later in life (Myklebust et al., 1986; Casabona et al., 1990; Wolf and Segal, 1990, 1996; Wolpaw, 1987, 1997; O'Sullivan et al., 1991; Nielsen et al., 1993). The pathway of the H-reflex is a wholly spinal and largely monosynaptic pathway consisting of the Ia primary afferent neuron, its synapse on the spinal motoneuron, and the motoneuron itself (Matthews, 1972; Brown, 1984). Because the spinal components of the reflex arc are affected by descending activity from supraspinal regions (Burke and Rudomin, 1978; Baldissera et al., 1981; Nozaki et al., 1996), this simple behavior can be operantly conditioned. H-reflex and/or SSR conditioning has been demonstrated in monkeys (Wolpaw et al., 1983; Wolpaw, 1987), humans (Evatt et al., 1989; Wolf and Segal, 1990, 1996; Segal and Wolf, 1994; Wolf et al., 1995), and rats (Chen and Wolpaw, 1995). The experimental protocol used in these studies operantly conditions the descending activity that controls the spinal reflex pathway. This altered descending activity is present for a prolonged period each day over the days of exposure to the protocol. As a result, like the reflex changes that develop after spinal cord injury, H-reflex change develops over days and weeks.

H-reflex conditioning is associated with activity-driven plasticity at several sites in the spinal cord (Wolpaw and Lee, 1989; Carp and Wolpaw, 1994, 1995; Feng-Chen and Wolpaw, 1996; Wolpaw, 1997). These sites include the motoneuron itself, and probably several different populations of interneurons as well. Recent studies in the rat suggest that the main corticospinal tract is essential for HRdown conditioning, while other major descending tracts, including the reticulospinal, rubrospinal, and vestibulospinal tracts, are not essential (Chen and Wolpaw, 1997). Whether HRup conditioning is similarly dependent remains to be determined.

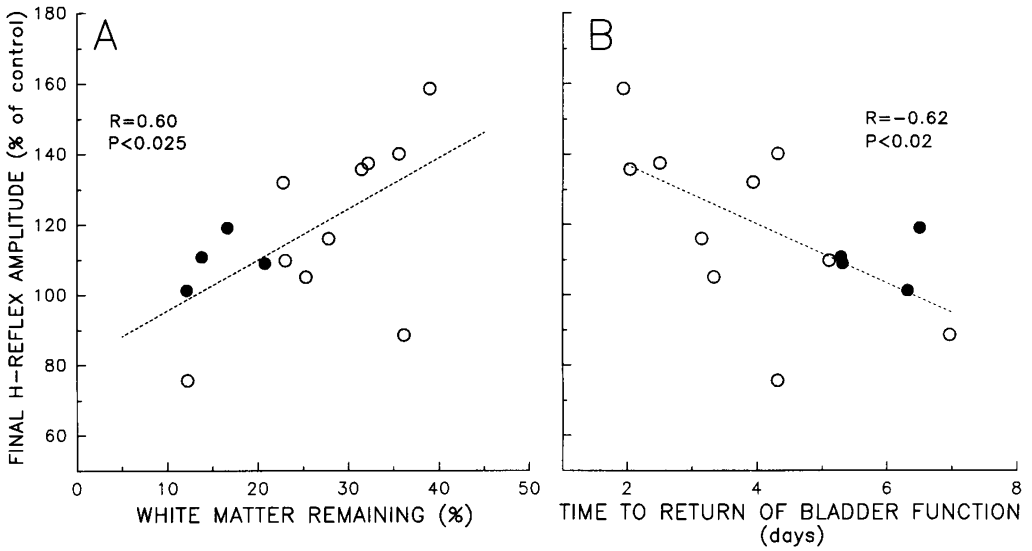

FIG. 5. (A) H-reflex amplitudes for rats with mild (open circles) and medium (solid circles) spinal cord contusions at end of HRup conditioning versus white matter remaining (in percent of white matter in reference section). Rats with more spared white matter increase the H-reflex more. (B) H-reflex amplitudes for rats with mild (open circles) and medium (solid circles) contusions at end of HRup conditioning versus duration of impaired bladder function. Rats with shorter periods of impaired bladder function increase the H-reflex more.

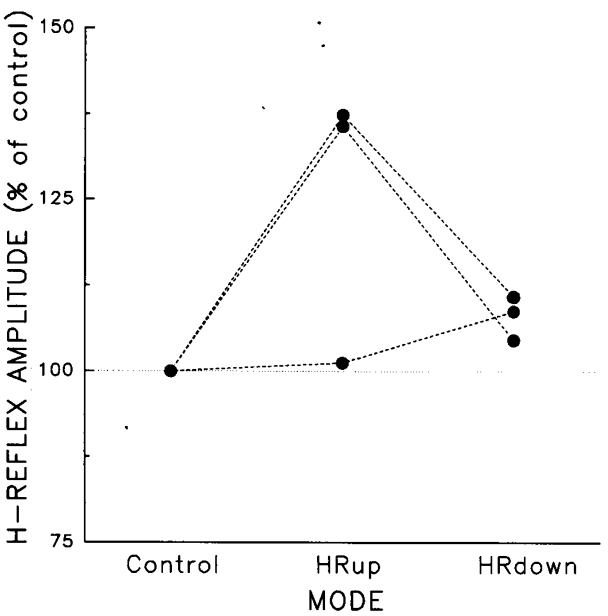

FIG. 6. H-reflex amplitudes at the end of HRup conditioning and at the end of HRdown conditioning for three rats with mild spinal cord contusions that were exposed to the HRup-mode and then to the HRdown-mode. HRup and HRdown conditioning are both successful (i.e., change of at least 20% in correct direction from initial value) in two animals and unsuccessful in one.

Effects of Spinal Cord Contusion on H-Reflex Conditioning

As the present study and the previous complementary study indicate (Chen et al., 1996), H-reflex conditioning, either HRup or HRdown, is sensitive to spinal cord injury. Furthermore, impairment of conditioning is proportional to white matter loss at the lesion epicenter. For HRup conditioning as for HRdown conditioning, success was correlated with severity of injury as assessed by white matter destruction or by duration of impaired bladder function. As Fig. 5 illustrates, animals that had more spared white matter and/or regained bladder function more quickly, increased the H-reflex more. Presumably, failure of HRup conditioning resulted from damage to those pathways conveying the descending control normally altered by conditioning. The correlation with white matter loss is not as strong for HRup conditioning as for HRdown conditioning (Chen et al., 1996). This difference is largely accounted for by the one animal that failed to increase the H-reflex even though 36% of white matter remained. Since failure occurs in about 20% of uninjured animals, it is possible that this animal's failure was not related to its spinal cord injury and would have occurred even if the animal had not been injured.

The two modes display very similar relationships to

the extent of injury. HRup conditioning, like HRdown conditioning (Chen et al., 1996), appears to be impaired in proportion to white matter damage. Figure 7 combines the present HRup conditioning data (i.e., from Fig. 5A) with previous data for HRdown conditioning—i.e., Fig. 6A from Chen et al. (1996)—and shows the regression line for each set of data and for the two sets combined. The close similarity is clear. It is consistent with the results from the three animals exposed to HRup and then HRdown conditioning: two succeeded at both, while one succeeded at neither (Fig. 6).

At the same time, physiological and anatomical data (Carp and Wolpaw, 1994, 1995; Feng-Chen and Wolpaw, 1996) suggest that HRup and HRdown conditioning have different spinal cord mechanisms. Present evidence suggests that HRdown conditioning is largely attributable to a change in motoneuron firing threshold. The mechanism of HRup conditioning is less clear, but may involve change in interneurons conveying disynaptic homonymous group 1 inhibition to the motoneuron.

Our recent pathway lesion studies indicate that HRdown conditioning is abolished by spinal cord dorsal column transection, suggesting that the main corticospinal tract is essential for HRdown conditioning (Chen and Wolpaw, 1997). If this is the case, the correlation evident in Figs. 5 and 7 between conditioned change in the H-reflex and the amount of white matter remaining may simply reflect a correlation between overall white matter damage and corticospinal tract damage. The data of the present study and Chen et al. (1996) cannot address this issue because the profound distortion of normal spinal cord histology associated with the contusion injury (e.g., Fig. 4) renders it difficult to determine with confidence the condition of the main corticospinal tract. Thus, while HRup and HRdown conditioning appear to be similarly sensitive to the particular contusion injury created in these studies, it remains to be determined whether they are also similarly sensitive to lesions of the dorsal column or the main corticospinal tract, and whether their dependence on white matter remaining is actually dependence on corticospinal tract remaining.

Other Effects of Spinal Cord Contusion on the H-Reflex

A nonsignificant increase in the control-mode H-reflex was seen in the spinal cord–injured animals (i.e., 136 ± 8.5 μV for spinal cord–injured rats and 112 ± 62 μV for normal rats). This increase was obtained when the H-reflex was measured in the presence of similar levels of background EMG (i.e., 102 ± 11 μV for spinal cord–injured rats and 101 ± 26 μV for normal rats) and at stimulus intensities that elicited similar M responses (i.e., 136 ± 36 μV for spinal cord injured rats and 138 ± 45 μV for normal rats). Thompson et al. (1992) also found a modest increase in plantar H-reflex amplitude in rats after contusion injuries.

While it is conceivable that, in some animals, operantly conditioned increase in the H-reflex was contaminated by gradual post-traumatic H-reflex increases, this seems unlikely. First, if gradual post-traumatic H-reflex increase occurs during the period of conditioning, more success would be expected for HRup conditioning than for HRdown conditioning. This was not the case. HRup and HRdown conditioning (i.e., Table 2 from Chen et al. (1996)) were similarly successful. Second, as in normal rats (Chen and Wolpaw, 1996), successful HRup conditioning in spinal cord–injured rats is reversible by exposure to HRdown-mode conditioning (Fig. 6). Third, in animals studied under the control-mode, contusion injury had no significant effect on H-reflex amplitude, M response amplitude, or background EMG from 18 to 259 days after the injury, and control-mode H-reflex amplitude was not related to time postinjury. Furthermore, as Fig. 3 and previous data for HRdown conditioning (i.e., Fig. 5 from Chen et al. (1996)) indicate, HRup or HRdown conditioning failure was not correlated with time postinjury, as might be expected if injury triggered a specific period of gradual H-reflex change. At the same

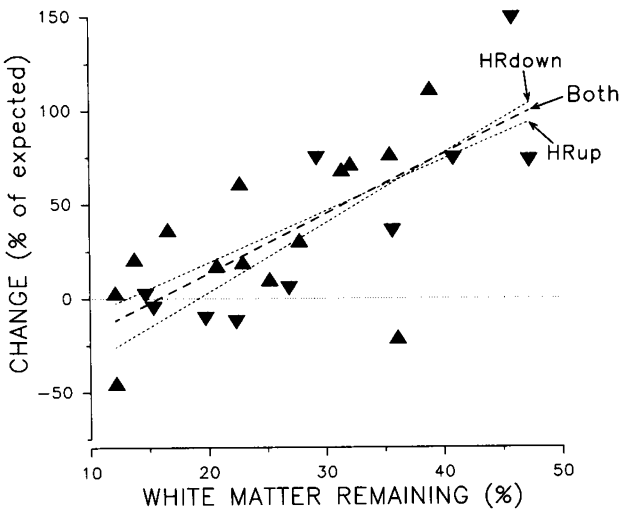

FIG. 7. Final H-reflex change (as percentage of expected change) versus white matter remaining for all spinal cord–injured rats exposed to HRup conditioning (up triangles; present data) or HRdown conditioning (down triangles; data from Chen et al., 1996). Regression lines are shown for HRup rats and HRdown rats separately (dotted lines) and together (dashed line). In all three cases, positive correlations are present: rats with more spared white matter changed the H-reflex more. The HRup and HRdown regressions are similar ($p > 0.5$).

time, it should be noted that data collection did not begin until at least 18 days after injury, so that transient early effects of the injury were not assessed.

Potential Usefulness of H-Reflex Conditioning

Operant conditioning of the H-reflex could complement motor and somatosensory evoked potentials (i.e., MEPs and SEPs) in evaluating spinal cord capacities after injury. MEPs and SEPs measure conduction in descending and ascending spinal cord pathways, and can thereby assess pathway integrity (Levy et al., 1986, 1987; Simpson and Baskin, 1987; Fehlings et al., 1987, 1989; Mutoh et al., 1991; Shiau et al., 1992). In contrast, H-reflex conditioning, while depending on appropriate activity in descending pathways, focuses on the segmental response to that activity. It reflects the integrity of the segmental circuitry and its responsiveness to descending influence. Furthermore, recent evidence that the corticospinal tract is essential for HRdown conditioning, and that other major descending tracts are not essential (Chen and Wolpaw, 1997), suggests that H-reflex conditioning provides a specific measure of corticospinal tract function.

The correlation between impairment of H-reflex conditioning and white matter loss (i.e., Fig. 7), combined with the fact that H-reflex conditioning remains impaired long after bladder function recovers, suggests that H-reflex conditioning is a sensitive and functionally relevant method for evaluating certain elements of spinal cord function after injury and for documenting the effects of therapeutic intervention on those functions. The high sensitivity of H-reflex conditioning is further indicated by comparing its sensitivity to injury to that of locomotion. Rats and cats can still produce effective locomotion when only about 10% of white matter remains (Blight, 1983; Bresnahan et al., 1987), while H-reflex conditioning in the rat is usually impaired when <30% remains (i.e., Fig. 7). This sensitivity, in combination with new insight into the pathways essential for conditioning (Chen and Wolpaw, 1997) could help define the descending pathways necessary for a variety of functions.

The fact that the H-reflex and the SSR can, in some instances, still be operantly conditioned after spinal cord injury suggests that operant conditioning could be used to modify and improve function after injury. This possibility inspired the Segal and Wolf (1994) study, which demonstrated SSR conditioning in patients with spinal cord injuries, and their investigation of associated changes (e.g., Segal and Wolf, 1992; Wolf et al., 1995). Finally, H-reflex conditioning might be a sensitive and convenient means for measuring the efficacy of drugs, implants, or other interventions in restoring function after injury. It might be particularly useful for assessing corticospinal tract function (Chen and Wolpaw, 1997). This use would be especially important if preservation of the capacity for H-reflex conditioning is found to be closely correlated with preservation of other important aspects of spinal cord function.

ACKNOWLEDGMENTS

We thank Dr. Zhen Guan, Lu Chen, Ping Wei, Trish Herchenroder, and Dayna M. Stark for excellent technical assistance. This work was supported in part by grants from the American Paralysis Association (X.Y.C.), the Paralyzed Veterans of America Spinal Cord Research Foundation (X.Y.C.), and the National Institutes of Health [NS22189 (J.R.W.), HD36020 (X.Y.C.), and NS10165 and NS37321 (B.T.S.)].

REFERENCES

ASHBY, P., and VERRIER, M. (1975). Neurophysiological changes following spinal cord lesions in man. Can. J. Neurol. Sci. **2,** 91–100.

BALDISSERA, F., HULTBORN, H., and ILLERT, M. (1981). Integration in spinal neuronal systems, in: *Handbook of Physiology. The Nervous System. Motor Control. Section I, Vol. II, Part 1.* American Physiology Society: Bethesda, MD, pps. 509–595.

BLIGHT, A.R. (1983). Cellular morphology of chronic spinal cord injury in the cat: analysis of myelinated axons by line-sampling. Neuroscience **10,** 521–543.

BOORMAN, G., BECKER, W.J., MORRICE, B.-L., and LEE, R.G. (1992). Modulation of the soleus H-reflex during pedalling in normal humans and in patients with spinal spasticity. J. Neurol. Neurosurg. Psychiatry **55,** 1150–1156.

BRESNAHAN, J.C., BEATTIE, M.S., STOKES, B.T., and CONWAY, K.M. (1991). Three-dimensional computer-assisted analysis of graded contusion lesions in the spinal cord of the rat. J. Neurotrauma **8,** 91–101.

BRESNAHAN, J.C., BEATTIE, M.S., TODD, F.D., III, and NOYES, D.H. (1987). A behavioral and anatomical analysis of spinal cord injury produced by a feedback-controlled impaction device. Exp. Neurol. **95,** 548–570.

BROWN, W.F. (1984). *The Physiological and Technical Basis of Electromyography.* Butterworths: Boston, 144–148, 474–479.

BURKE, R.E., and RUDOMIN, P. (1978). Spinal neurons and synapses, in: *Handbook of Physiology. The Nervous System. Cellular Biology of Neurons. Section I, Vol. II, Part 2.* American Physiology Society: Bethesda, MD, pps. 877–944.

CALANCIE, B., BROTON, J.G., KLOSE, K.J., TRAAD, M., DIFINI, J., and AYYAR, D.R. (1993). Evidence that alterations in presynaptic inhibition contribute to segmental hypo-

and hyperexcitability after spinal cord injury in man. Electroencephalogr. Clin. Neurophysiol. **89,** 177–186.

CARP, J.S., and WOLPAW, J.R. (1994). Motoneuron plasticity underlying operantly conditioned decrease in primate H-reflex. J. Neurophysiol. **72,** 431–442.

CARP, J.S., and WOLPAW, J.R. (1995). Motoneuron properties after operantly conditioned increase in primate H-reflex. J. Neurophysiol. **73,** 1365–1373.

CASABONA, A.J., POLIZZI, M.C., and PERCIAVALLE, V. (1990). Differences in H-reflex between athletes trained for explosive contractions and non-trained subjects. Eur. J. Appl. Physiol. **61,** 26–32.

CHEN, X.Y., and WOLPAW, J.R. (1994). Circadian rhythm in rat H-reflex. Brain Res. **648,** 167–170.

CHEN, X.Y., and WOLPAW, J.R. (1995). Operant conditioning of H-reflex in freely moving rats. J. Neurophysiol. **73,** 411–415.

CHEN, X.Y., and WOLPAW, J.R. (1996). Reversal of H-reflex operant conditioning in the rat. Exp. Brain Res. **112,** 58–62.

CHEN, X.Y., and WOLPAW, J.R. (1997). Dorsal column but not lateral column transection prevents down conditioning of H-reflex in rats. J. Neurophysiol. **78,** 1730–1734.

CHEN, X.Y., WOLPAW, J.R., JAKEMAN, L.B., and STOKES, B.T. (1996). Operant conditioning of H-reflex in spinal cord–injured rats. J. Neurotrama **13,** 755–766.

DAVIS, R. (1975). Spasticity following spinal cord injury. Clin. Orthop. **112,** 66–75.

DIMITRIJEVIC, M.R., FAGANEL, J., LEHMKUHL, D., and SHERWOOD, A. (1988). Motor control in man after partial or complete spinal cord injury, in: *Advances in Neurology. Vol. 39. Motor Control Mechanisms in Health and Disease.* J.E. Desmedt (ed), Raven Press: New York, pps. 915–926.

DOYLE, W.K., WILMOT, C., HALL, K.M., COOPER, P.R., and FADEN, A.I. (1993). Trauma of the spine and spinal cord, in: *Clinical Neurology. Vol. 3.* Joynt, R.J. (ed), Lippincott: Philadelphia, pps. 1–58.

EVATT, M.L., WOLF, S.L., and SEGAL, R.L. (1989). Modification of human spinal stretch reflexes: preliminary studies. Neurosci. Lett. **105,** 350–355.

FEHLINGS, M.G., TATOR, C.H., and LINDEN, R.D. (1989). The relationships among the severity of spinal cord injury, motor and somatosensory evoked potentials and spinal cord blood flow. Electroencephalograph. Clin. Neurophysiol. **74,** 241–259.

FEHLINGS, M.G., TATOR, C.H., LINDEN, R.D., and PIPER, I.R. (1987). Motor evoked potentials recorded from normal and spinal cord-injured rats. Neurosurgery **20,** 125–130.

FENG-CHEN, K.C., and WOLPAW, J.R. (1996). Operant conditioning of H-reflex changes synaptic terminals on primate motoneurons. Proc. Natl. Acad. Sci. U.S.A. **93,** 9206–9211.

FUJIMORI, B., KATO, M., MATSUSHIMA, S., MORI, S., and SHIMAMURA, M. (1966). Studies on the mechanism of spasticity following spinal hemisection in the cat, in: *Muscular Afferents and Motor Control.* R. Granit (ed), Wiley: New York, pps. 397–413.

HECHMAN, C.J. (1994). Alterations in synaptic input to motoneurons during partial spinal cord injury. Med. Sci. Sports Exerc. **26,** 1480–1490.

HENNEMAN, E. (1980). Organization of the spinal cord and its reflexes, motor functions of the brain stem and basal ganglia, in: *Medical Physiology,* Vol 1. V.B. Mountcastle (ed), Mosby: St Louis, pps. 762–786, 787–812.

HOLSTEGE, J.C., and KUYPERS, H.G.J.M. (1987). Brainstem projections to spinal motoneurons: an update. Neuroscience **23,** 809–821.

KENNEDY, P.R. (1990). Corticospinal, rubrospinal and rubroolivary projections: a unifying hypothesis. Trends Neurosci. **3,** 474–479.

LEVY, W., McCAFFERY, M., and YORK, D. (1986). Motor evoked potential in cats with acute spinal cord injury. Neurosurgery **19,** 9–19.

LEVY, W., McCAFFERY, M., and HAGICHI, S. (1987). Motor evoked potential as a predictor of recovery in chronic spinal cord injury. Neurosurgery **20,** 138–142.

LITTLE, J.W., and HALAR, E.M. (1985). H-reflex changes following spinal cord injury. Arch. Phys. Med. Rehabil. **66,** 19–22.

MATTHEWS, P.B.C. (1972). *Mammalian Muscle Receptors and Their Central Actions.* Williams and Wilkins: Baltimore, pps. 319–409.

MUTOH, K., OKUNO, T., ITO, M., FUJII, T., MIKAWA, H., and ASATO, R. (1991). Somatosensory evoked potentials after posterior tibial nerve stimulation in focal spinal cord diseases. Pediatr. Neurol. **7,** 326–332.

MYKLEBUST, B.M., GOTTLIEB, G.L., and AGARWAL, G.C. (1986). Stretch reflexes of the normal human infant. Dev. Med. Child. Neurol. **28,** 440–449.

NIELSEN, J., PETERSEN, N., BALLEGAARD, M., BIERING-SORENSEN, F., and KIEHN, O. (1993). H-reflex are less depressed following muscle stretch in spastic spinal cord injured patients than in healthy subjects. Exp. Brain Res. **97,** 173–176.

NOZAKI, D., NAKAZAWA, K., and YAMAMOTO, Y. (1996). Supraspinal effects on the fractal correlation in human H-reflex. Exp. Brain Res. **112,** 112–118.

OLBY, N.J., and BLAKEMORE, W.F. (1996). A new method of quantifying the extent of tissue loss following spinal cord injury in the rat. Exp. Neurol. **138,** 82–92.

O'SULLIVAN, M.C., EYRE, J.A., and MILLER, S. (1991). 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.

ST. GEORGE, C.L. (1993). Spasticity: mechanisms and nursing care. Neurosci. Nurs. **28,** 819–827.

SEGAL, R.L. (1997). Plasticity in the central nervous system: operant condition of the spinal stretch reflex. Topics Stroke Rehabil. **3,** 76–87.

SEGAL, R.L., and WOLF, S.L. (1992). Concurrent brachioradialis changes during training of biceps brachii spinal stretch reflexes in spinal cord injured patients. Soc. Neurosci. Abstr. **18,** 1402.

SEGAL, R.L., and WOLF, S.L. (1994). Operant conditioning of spinal stretch reflexes in patients with spinal cord injuries. Exp. Neurol. **130,** 202–213.

SHEFNER, J.M., BERMAN, S.A., SARKARATI, M., and YOUNG, R.R. (1992). Recurrent inhibition is increased in patients with spinal cord injury. Neurology **42,** 2162–2168.

SHIAU, J.S., ZAPPULLA, R.A., and NIEVES, J. (1992). The effect of graded spinal cord injury on the extrapyramidal and pyramidal motor evoked potentials of the rat. Neurosurgery **30,** 76–84.

SIMPSON, R.K., and BASKIN, D.S. (1987). Corticomotor evoked potentials in acute and chronic blunt spinal cord injury in the rat: correlation with neurological outcome and histological damage. Neurosurgery **20,** 131–137.

SIMPSON, R.K. JR., GONDO, M., ROBERTSON, C.S., and GOODMAN, J.C. (1995). The influence of glycine and related compounds on spinal cord injury–induced spasticity. Neurochem. Res. **20,** 1203–1210.

STEIN, R.B., YANG, J.F., BELANGER, M., and PEARSON, K.G. (1993). Modification of reflexes in normal and abnormal movements. Prog. Brain Res. **97,** 189–196.

STOKES, B.T. (1992). Experimental spinal cord injury: a dynamic and verifiable injury device. J. Neurotrauma **9,** 129–134.

STOKES, B.T., and REIER, P.J. (1992). Fetal grafts alter chronic behavioral outcome after contusion damage to the adult rat spinal cord. Exp. Neurol. **116,** 1–12.

STOKES, B.T., NOYES, D.H., and BEHRMANN, D.L. (1992). A electromagnetic spinal injury device with dynamic sensitivity. J. Neurotrauma **9,** 187–195.

TAYLOR, S., ASHBY, P., and VERRIER, M. (1984). Neurophysiological changes following spinal cord lesion in man. J. Neurol. Neurosurg. Psychiatry **47,** 1102–1108.

THOMPSON, F.J., REIER, P.J., LUCAS, C.C., and PARMER, R. (1992). Altered patterns of reflex excitability subsequent to contusion injury of the rat spinal cord. J. Neurophysiol. **68,** 1473–1486.

TRACEY, D.J. (1995). Ascending and descending pathways in the spinal cord, in: *The Rat Nervous System.* G. Paxinos (ed), Academic Press: San Diego, pps. 67–80.

WOLF, S.L., and SEGAL, R.L. (1990). Conditioning of the spinal stretch reflex: implications for rehabilitation. Phys. Ther. **70,** 652–656.

WOLF, S.L., and SEGAL, R.L. (1996). Reducing human biceps brachii spinal stretch reflex magnitude. J. Neurophysiol. **75,** 1637–1646.

WOLF, S.L., SEGAL, R.L., HETER, N.D., and CATLIN, P.A. (1995). Contralateral and long latency effects of human biceps brachii stretch reflex conditioning. Exp. Brain Res. **107,** 96–102.

WOLPAW, J.R. (1987). Operant conditioning of primate spinal reflexes: the H-reflex. J. Neurophysiol. **57,** 443–459.

WOLPAW, J.R. (1997). The complex structure of a simple memory. Trends Neurosci. **20,** 588–594.

WOLPAW, J.R., BRAITMAN, D.J., and SEEGAL, R.F. (1983). Adaptive plasticity in the primate spinal stretch reflex: initial development. J. Neurophysiol. **50,** 1296–1311.

WOLPAW, J.R., and CARP, J.S. (1993). Adaptive plasticity in spinal cord. Adv. Neurol. **59,** 163–173.

WOLPAW, J.R., and LEE, C.L. (1989). Memory traces in primate spinal cord produced by operant conditioning of H-reflex. J. Neurophysiol. **61,** 563–572.

Address reprint requests to:
Xiang Yang Chen, Ph.D.
Wadsworth Center
New York State Department of Health
P.O. Box 509
Albany, NY 12201-0509

e-mail: chenx@wadsworth.org

This article has been cited by:

1. Aiko K. Thompson, Jonathan R. WolpawOperant Conditioning of Spinal Reflexes for Motor Rehabilitation after CNS Damage 549-570. [CrossRef]
2. Richard B. Stein, Dirk G. Everaert, François D. Roy, SuLing Chong, Maryam Soleimani. 2013. Facilitation of Corticospinal Connections in Able-bodied People and People With Central Nervous System Disorders Using Eight Interventions. *Journal of Clinical Neurophysiology* **30**, 66-78. [CrossRef]
3. Mar Cortes, Gary W. Thickbroom, Josep Valls-Sole, Alvaro Pascual-Leone, Dylan J. Edwards. 2011. Spinal associative stimulation: A non-invasive stimulation paradigm to modulate spinal excitability. *Clinical Neurophysiology* . [CrossRef]
4. Richard B. Stein. 2008. The plasticity of the adult spinal cord continues to surprise. *The Journal of Physiology* **586**:10.1113/tjp.2008.586.issue-12, 2823-2823. [CrossRef]
5. David E. Poon, Francois D. Roy, Monica A. Gorassini, Richard B. Stein. 2008. Interaction of paired cortical and peripheral nerve stimulation on human motor neurons. *Experimental Brain Research* **188**, 13-21. [CrossRef]
6. Xiang Yang Chen, Yi Chen, Lu Chen, Ann M. Tennissen, Jonathan R. Wolpaw. 2006. Corticospinal Tract Transection Permanently Abolishes H-Reflex Down-Conditioning in Rats. *Journal of Neurotrauma* **23**:11, 1705-1712. [Abstract] [Full Text PDF] [Full Text PDF with Links]
7. X Chen. 2002. Corticospinal tract transection reduces H-reflex circadian rhythm in rats. *Brain Research* **942**, 101-108. [CrossRef]
8. Xiang Yang Chen, Kuo Ching Feng-Chen, Lu Chen, Dayna M. Stark, Jonathan R. Wolpaw. 2001. Short-Term and Medium-Term Effects of Spinal Cord Tract Transections on Soleus H-Reflex in Freely Moving Rats. *Journal of Neurotrauma* **18**:3, 313-327. [Abstract] [Full Text PDF] [Full Text PDF with Links]
9. Gillian D. Muir, Aubrey A. Webb. 2000. Assessment of behavioural recovery following spinal cord injury in rats. *European Journal of Neuroscience* **12**, 3079-3086. [CrossRef]
10. Paul Dcheney, Jennifer Hill-Karrer, Abderraouf Belhaj-Saïf, Brian J. McKiernan, Michael C. Park, Joanne K. MarcarioCortical motor areas and their properties: Implications for neuroprosthetics 135-160. [CrossRef]
11. Michael S. Beattie, Qun Li, Jacqueline C. BresnahanCell death and plasticity after experimental spinal cord injury 9-21. [CrossRef]