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# Operant Conditioning of H-Reflex in Spinal Cord-Injured Rats

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## ABSTRACT

**Operant conditioning of the spinal stretch reflex or its electrical analog, the H-reflex, is a new model for exploring the mechanisms of supraspinal control over spinal cord function. Both rats and primates can gradually increase (HRup conditioning mode) or decrease (HRdown conditioning mode) soleus H-reflex magnitude when exposed to an operant conditioning task. This study used H-reflex operant conditioning to assess and modify spinal cord function after injury. Soleus H-reflexes were elicited and recorded with chronically implanted electrodes from rats that had been subjected to calibrated contusion injuries to the spinal cord at T8. From 18 to 140 days after injury, background EMG, M response amplitude, and initial H-reflex amplitude were not significantly different from those of normal rats. HRdown conditioning was successful in some, but not all, spinal cord-injured rats. The H-reflex decrease achieved by conditioning was inversely correlated with the severity of the injury as assessed histologically or by time to return of bladder function. It was not correlated with the length of time between injury and the beginning of conditioning. The results confirm the importance of descending control from supraspinal structures in mediating operantly conditioned change in H-reflex amplitude. In conjunction with recent human studies, they suggest that H-reflex conditioning could provide a sensitive new means for assessing spinal cord function after injury, and might also provide a method for initiating and guiding functional rehabilitation.**

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

## INTRODUCTION

SPINAL CORD FUNCTION is normally controlled by descending input from supraspinal structures and by input from the periphery. When injury removes or distorts descending input, function changes, and spasticity and other disabling problems eventually appear (Kuhn, 1950; Liu and Chambers, 1958; Fujimori et al., 1966; Ashby et al., 1974; Ashby and Verrier, 1975; Davis, 1975; Nelson and Mendell, 1979; Henneman, 1980; Little and Halar, 1985; Dimitrijevic et al., 1988; Boorman et al., 1992; Shefner et al., 1992; Doyle et al., 1993; St. George, 1993; Stein et al., 1993). Understanding how descending input controls spinal cord function could lead to novel methods for inducing, guiding, and assessing recovery after injury. Operant conditioning of the spinal stretch reflex

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(i.e., the tendon jerk) or of its electrical analog, the H-reflex, is a new model for studying the long-term control that descending pathways exert over spinal cord reflex function (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; Wolf et al., 1995). Furthermore, a recent human study indicates that such conditioning can reduce stretch reflex amplitude after incomplete spinal cord injury (Segal and Wolf, 1994). Thus, it may provide a new means for alleviating spasticity in the spinal cord-injured patient.

The goal of the present study was to define the capacity for soleus H-reflex conditioning in rats with calibrated contusion injuries of the thoracic spinal cord. The results indicate that conditioning is still possible after injury and suggest that it provides a sensitive measure of the severity of injury.

## MATERIALS AND METHODS

Subjects were 17 female Sprague-Dawley rats weighing 245–290 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, 1994, 1995) and are summarized here. Procedures satisifed DHEW Publ. No. (NIH) 85-23, "Guide for the Care and Use of Laboratory Animals," 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 with The Ohio State University spinal cord injury device as previously described (Stokes, 1992; Stokes et al., 1992). Briefly, the animal was anesthetized with an i.p. injection of ketamine HCl (80 mg/kg) and xylazine (10 mg/kg). A partial dorsal laminectomy was performed at T8–T9 with minimal disturbance of the dural envelope. The laminectomy was just over 7.0 $mm^2$ (i.e., the size of the impactor tip). The animal was quickly mounted in a spinal frame with the nearby dorsal processes rigidly fixed. Over a 23-msec time period, a Ling shaker (Model V203/S, Ling Dynamics Systems Corp.) compressed the dorsal cord surface approximately 0.7 mm (mild contusion, 13 rats) or 0.9 mm (medium contusion, 4 rats). The inter-animal consistency of the biomechanical data and of the injury produced has been described (Stokes and Reier, 1991, 1992; Bresnahan et al., 1991; Stokes et al., 1992; Behrmann et al., 1992). After lesioning, the injury site was covered with Durafilm to minimize connective tissue adhesions to the dura. Muscle and skin wounds were sutured in layers, and the animal was placed on a heating pad and allowed to recover. It was subsequently given fluids to maintain hydration and allowed to eat and drink freely. The bladder was expressed at least two times daily until spontaneous voiding returned. Antibiotics (4.0 mg trimethoprim and 20 mg sulfadiazine p.o. daily) were administered from 2 days before injury until the return of bladder function. To maintain urine acidity and thereby help prevent infection, a vitamin C supplement (8–10 mg/kg/day) was given from the day just before injury until the return of bladder function. A high calorie dietary supplement (i.e., Nutri-Cal) was given after the injury until body weight regained its preinjury level. In addition, a piece of apple (>10 g) was given each day from before injury until the time of euthanasia and perfusion.

Electrode implantation and operant conditioning were performed at the Wadsworth Center as previously described (Chen and Wolpaw, 1994, 1995). Briefly, 11–60 days after the injury, each rat was implanted under general anesthesia (pentobarbital sodium, 60 mg/kg, i.p., or ketamine HCl, 80 mg/kg, i.p., and xylazine, 10 mg/kg, i.p.) with chronic stimulating and recording electrodes in the right leg. To elicit the H-reflex, a silicon rubber nerve cuff containing a pair of stainless steel multi-stranded fine-wire electrodes was placed on the posterior tibial nerve just above the triceps surae branches. To record soleus EMG activity, two fine-wire electrodes with the final 0.5 cm stripped were placed in the right soleus muscle. The Teflon-coated wires form the nerve cuff and the muscle passed subcutaneously to a connector plug mounted on the skull with stainless steel screws and dental cement. Seven to ten days after implantation surgery, each rat was tested with nerve-cuff stimulation to ensure that an H-reflex was present at M response (i.e., direct muscle response) threshold.

Data collection began 18–120 days after the injury and lasted 20–160 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 thence 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

*Female rats are used in these spinal cord injury studies because bladder dysfunction after injury is much easier to manage in females than in males. The initial studies of H-reflex conditioning in rats (Chen and Wolpaw, 1995) used only males. However, recent data indicate that normal males and females are comparable in initial H-reflex and M response amplitudes and background EMG, and in magnitude, rate, and consistency of HRdown conditioning.

task described below. Animal well-being was checked carefully several times each day, and body weight was measured weekly. Laboratory lights were dimmed from 2100 to 0600 hours each day.

A minicomputer system continuously monitored soleus EMG and controlled two outputs: the nerve-cuff stimulus and the reward (a 20-mg food pellet). If the absolute value of background (i.e., ongoing) EMG (equivalent to the full-wave rectified value) remained within a defined range for a randomly varying 2.3–2.7 sec period, a stimulus pulse (typically 0.5 msec in duration) was delivered by the nerve cuff. Pulse amplitude was kept at M-response threshold (Wolpaw and Herchenroder, 1990). Under the control mode, the computer digitized soleus EMG and stored its absolute value for 50 msec following the stimulus. Under the HRdown or HRup conditioning mode, it gave a reward 200 msec after nerve stimulation if EMG amplitude (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, 3000–9000 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 10–20 days to determine the animal's initial H-reflex amplitude. It was then exposed to the HRdown mode for up to 50 days. Some animals were then switched to the opposite (i.e., HRup) mode for another 50 days, and one was then switched back to the HRdown mode for 40 days.

To determine the final effect on H-reflex amplitude of HRdown mode exposure (and of sequential HRdown/HRup 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 (control-mode) H-reflex amplitude. In addition, for HRdown/HRup exposures, the final effect of the HRup exposure was determined by calculating average H-reflex amplitude for the final 10 days as percent of H-reflex amplitude at the end of the initial (i.e., HRdown) exposure (Chen and Wolpaw, 1996a).

At the end of study, each rat was killed with an i.p. 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 formalin.

The percent of white matter remaining at the lesion epicenter was measured at The Ohio State University using the following modification of methods described previously(Behrmann et al., 1992). Transverse sections (25 $\mu$m) 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 were digitized at $\sim$50$\times$ magnification with the MCID Imaging System (Imaging Research, St. Catherines, Ontario) and outlined manually. Remaining white matter was identified by the presence of normal luxol fast blue staining. It was outlined at 100$\times$ and its area was determined. In accord with a recent, carefully documented method for measuring the tissue remaining at the epicenter of a spinal cord lesion (Olby and Blakemore, 1996), the area of remaining white matter was 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. Thus, for example, a value of 30% indicated that, at the lesion epicenter, 70% of the white matter was damaged or absent.

## RESULTS

### *Animal Behavior and Well-Being*

Immediately after injury, rats usually showed a transient hindlimb paralysis. The hindlimbs were extended and dragged behind the animal. This deficit abated rapidly over 2–5 days, and all rats recovered some coordinated locomotion within 3 weeks (Basso et al., 1995). Bladder function, absent immediately after injury, returned over 3–9 days. After the immediate post-traumatic period, all animals were healthy and active throughout data collection. Weight increased from 245–290 g at the time of injury to 267–300 g at the time of implantation and 279–364 g at the time of perfusion. After perfusion, soleus muscles weights (measured as percent of body weight) were symmetrical and did not differ significantly from those of normal rats.

### *Spinal Cord Lesions*

The percent of white matter remaining at the lesion epicenter (assessed in 10 of the 11 animals) ranged from 15% to 47%. While less white matter remained in the 3 rats with medium contusions (21% $\pm$ 7% mean $\pm$ SD) than in the 7 rats with mild contusions (34% $\pm$ 12%), the two groups were not significantly different ($p = 0.16$ by $t$-test) because of the small numbers of animals. These results were comparable to those of earlier studies, which used a different method for assessing white matter loss (Bresnahan et al., 1991; Behrmann et al., 1992). Figure 1 shows photographs of spinal cord sections at the lesion epicenter from two rats. For the rat in Figure 1A, which had a mild contusion, 47% of the white matter remains.

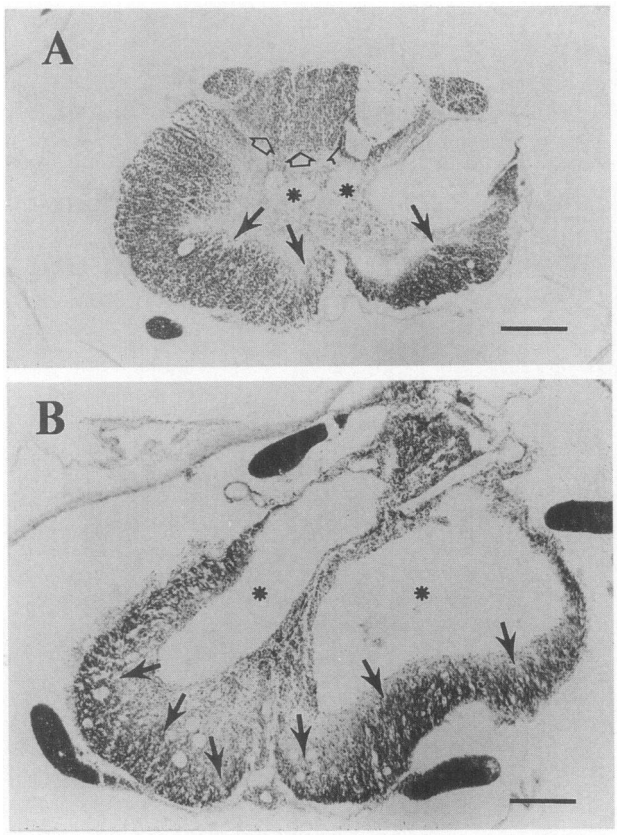


**FIG. 1.** Photomicrographs of 25-$\mu$m transverse sections through the epicenter of a mild (0.7-mm displacement (A) and a medium (0.9-mm displacement (B) contusion injury. The sections were stained with luxol fast blue (A) or luxol fast blue and cresyl violet (B). In both, the lesion center lacks healthy neuropil. It is filled instead with necrotic debris, cystic cavities (*) and fibrous septa (B). The limits of spared white matter are indicated by arrows. Note the spared dorsal column white matter in A (open arrows). Bars = 250 $\mu$m.

For the rat in Figure 1B, which had a medium contusion, only 20% remains.

As shown in Figure 2, the percent of remaining white matter showed a strong inverse correlation ($r = -0.89$, $p < 0.001$) with 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). Rats with more remaining white matter regained bladder function more quickly.

### *Control-Mode Data*

As a prerequisite for assessing the capacity for H-reflex conditioning in spinal cord-injured rats, we determine the effects of the injury itself on trials/day, background EMG, M response amplitude, and H-reflex amplitude. Control-mode data were collected from 17 spinal cord-injured rats (13 with mild and 4 with medium contusions) for 10–20 days starting 18–120 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 ($p > 0.32$ for all four measures by Pearson Product Moment Correlation test). Furthermore, these data did not differ from those of uninjured rats. Table 1 gives means ($\pm$SD) of trials/day, background EMG, M response amplitude, and H-reflex amplitude for these injured rats and for 34 normal rats studied to date (Chen and Wolpaw, 1994, 1995, and subsequent data). No significant differences between the two groups were detected ($p > 0.15$ by $t$-test for all four measures). The values of the two groups are very similar, except that H-reflex amplitude is slightly (i.e., 15.9%), but not significantly, higher for the injured rats. A comparable nonsignificant increase in H-reflex amplitude following spinal cord contusion in the rat was noted by Thompson et al. (1992).

### *HRdown Conditioning*

Eleven rats (7 with mild and 4 with medium contusions) were exposed to the HRdown conditioning mode. Figures 3 and 4 and Table 2 summarize the effects of this

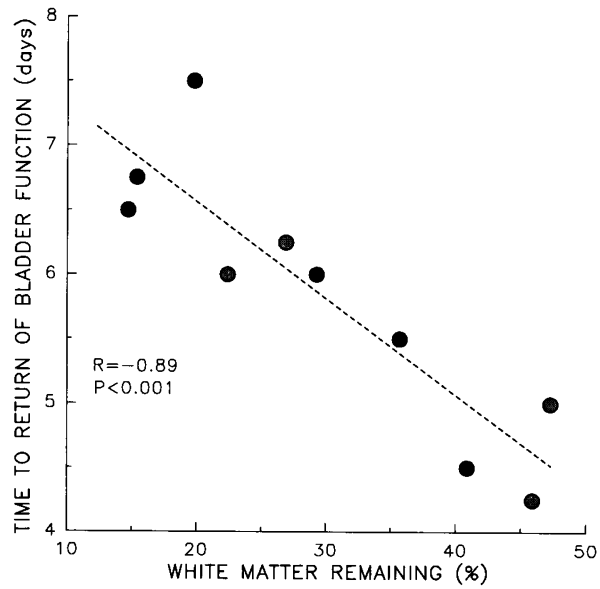


**FIG. 2.** Time to return of bladder function versus percent of white matter remaining at the lesion epicenter for rats with mild (shaded) or medium (black) spinal cord contusions. A strong inverse correlation is evident ($r = -0.89$, $p < 0.001$). Rats with more spared white matter regained bladder function more quickly.

TABLE 1. AVERAGE (±SD) CONTROL-MODE VALUES OF TRIALS/DAY, BACKGROUND EMG, M RESPONSE AMPLITUDE, AND H-REFLEX AMPLITUDE FOR SPINAL CORD-INJURED RATS AND NORMAL RATS

| | *Spinal cord-injured rats (n = 17)* | *Normal rats (n = 34)* |
|---|---|---|
| Trials/day | 5267 ± 1825 | 5797 ± 2419 |
| Background EMG ($\mu$V) | 103 ± 11 | 101 ± 29 |
| M response ($\mu$V) | 247 ± 39 | 233 ± 38 |
| H-reflex ($\mu$V) | 241 ± 80 | 208 ± 75 |

From Chen and Wolpaw, 1994, 1995, and subsequent data. Background EMG, M response, and H-reflex measurements are average absolute value in $\mu$V. The groups do not differ significantly (see text).

exposure in the injured animals and compare them with the effects observed in 14 normal animals studied to date. Figure 3 shows average values for H-reflex, M response, and background EMG before and during exposure to the HRdown mode. In both injured and normal groups, background EMG and M response amplitude remain stable throughout, whereas H-reflex amplitude falls gradually. The decrease is greater in the normal animals: their final values are significantly smaller than those of the injured animals ($p = 0.01$ for comparison of average values for final 10 days by $t$-test). Thus, spinal cord injury appeared to impair conditioning. Table 2 gives the average final values for the mild and medium contusion groups together and separately, and for the normal group. Figure 4 shows the distributions of final H-reflex amplitudes for the two groups. The dashed vertical lines indicate the standard criteria for successful conditioning: a change of at least 20% in the appropriate direction (Chen and Wolpaw, 1995). Whereas 12 of the 14 normal animals, or 86%, were successful, only 4 of the 11 injured animals, or 36%, were successful.† This difference was significant ($p < 0.02$, Fisher exact test). Success tended to be more common among animals with mild contusions (3 of 7) than among those with medium contusions (1 of 4).

Figure 5 addresses the question of whether success was correlated with time since injury. It shows final H-reflex amplitude as a function of the time after injury when HRdown exposure began. No significant correlation is evident ($r = 0.37$, $p > 0.25$).

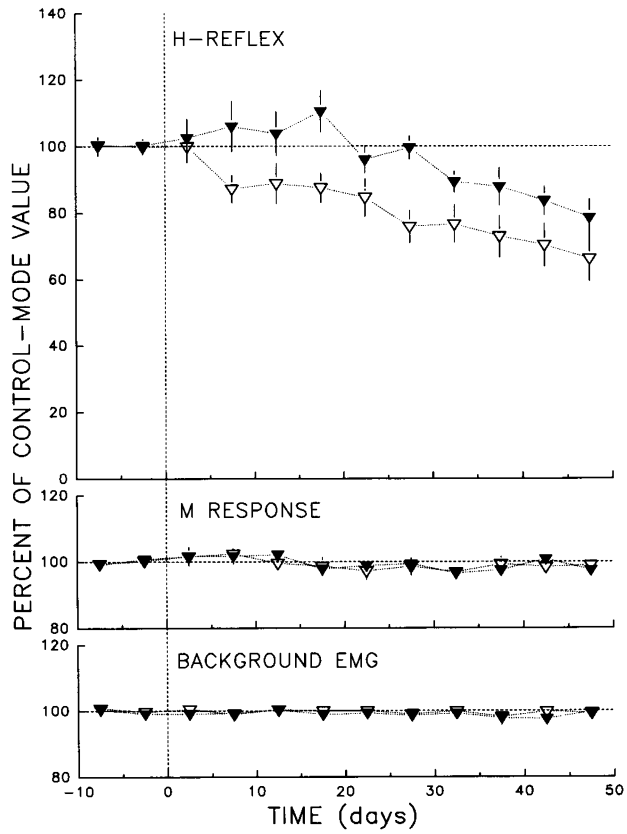


**FIG. 3.** Average H-reflex amplitude (top), M response amplitude (middle), and background EMG (bottom) for 11 spinal cord-injured rats (7 with mild and 4 with medium contusions, filled triangles) and 14 normal rats (open triangles) for each 5-day period during the control mode and the HRdown mode (in percent of control-mode value). Whereas H-reflex amplitude falls in both spinal cord-injured and normal rats, the decrease is much less dramatic in the spinal cord-injured rats than in the normal rats ($p = 0.01$ for comparison of average values for final 10 days by $t$-test). For both groups, background EMG and M response are stable throughout data collection.

†For two rats, HRdown exposure lasted only 30 and 36 days because of loss of the head plug. Their H-reflexes showed no decrease over these periods (final values were 101% and 98% of control, respectively), so that we are confident in classifying them as failures.

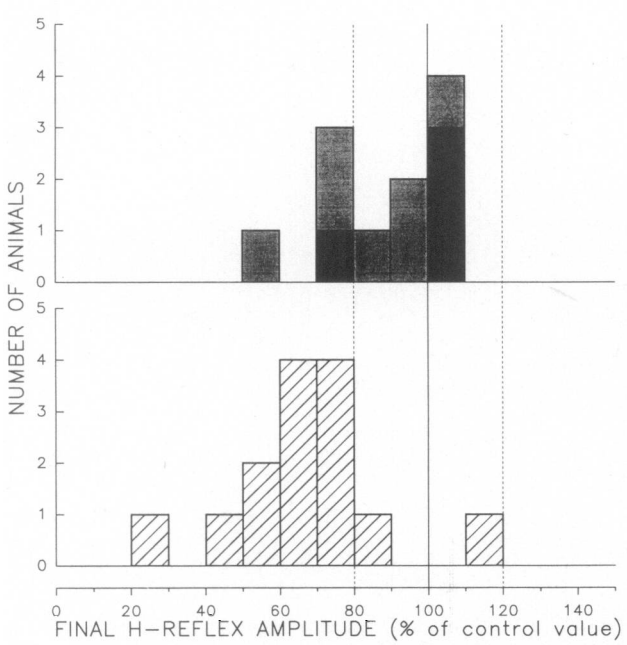


**FIG. 4.** Distributions of H-reflex amplitudes at end of HRdown conditioning for rats with mild (shaded, $n = 7$) or medium (black, $n = 4$) spinal cord contusions (top) and for normal rats (bottom, $n = 14$). Dashed line at 80% indicates criterion for successful HRdown conditioning (i.e., decrease ≥20%).

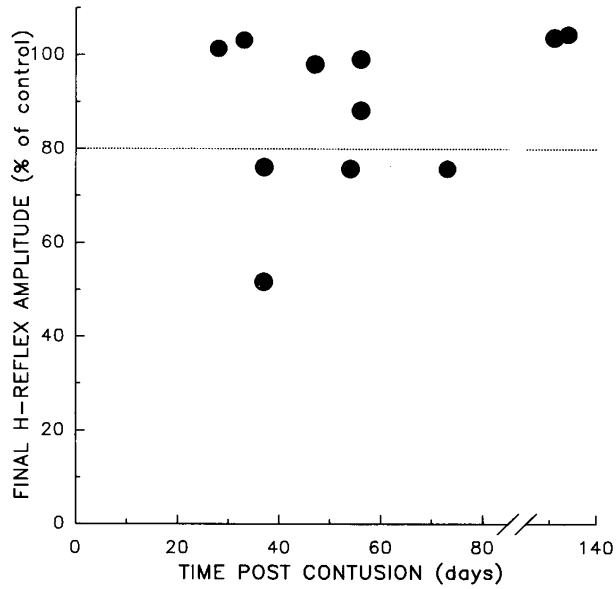


**FIG. 5.** H-reflex amplitudes at end of HRdown conditioning versus number of days after injury when conditioning began for rats with mild (shaded) and medium (black) spinal cord contusions. No significant correlation is evident ($r = 0.37$, $p > 0.25$).

### *HRdown Conditioning Versus Lesion Size*

Figure 6A shows final H-reflex amplitude as a function of the percentage of white matter remaining. A strong correlation is evident ($r = -0.86$, $p = 0.001$). Animals with more remaining white matter did better at HRdown conditioning.

### *HRdown Conditioning Versus Bladder Function*

Figure 6B shows final H-reflex amplitude as a function of the time that passed before bladder function returned. The two measures are clearly correlated ($r = 0.80$, $p < 0.005$). Animals in which bladder function returned more quickly did better at HRdown conditioning.

At the same time, H-reflex conditioning provided a more sensitive measure of injury, as it was still abnormal several months after the contusion (Fig. 5), long after bladder function had returned.

### *HRdown/HRup Conditioning*

After completing a 50-day exposure to the HRdown mode, four animals (1 with medium and 3 with mild contusions) were switched to the opposite (HRup) mode for another 50 days. Figure 7 shows final H-reflex amplitude at the ends of the HRdown and HRup exposures for each of these animals. The medium-contusion animal (with 20% of white matter remaining) showed no significant response to either mode exposure (HRdown exposure was associated with a change of only +2% from the control value, and HRup exposure was associated with a

**TABLE 2. HRDOWN CONDITIONING RESULTS FOR SPINAL CORD-INJURED RATS AND FOR NORMAL RATS**

| | *Final H-reflex amplitude (mean ± SD in % of control)* | *Number successful (%) (H-reflex decrease ≥20%)* |
|---|---|---|
| Spinal cord-injured rats ($n = 11$) | 89 ± 17 | 4 (36%) |
| Mild contusion ($n = 7$) | 85 ± 19 | 3 (43%) |
| Medium contusion ($n = 4$) | 96 ± 14 | 1 (25%) |
| Normal rats ($n = 14$) | 68 ± 22 | 12 (86%) |

From Chen and Wolpaw, 1994, 1995, and subsequent data. The two groups are significantly different in final H-reflex amplitude and in number of rats that were successful (see text).

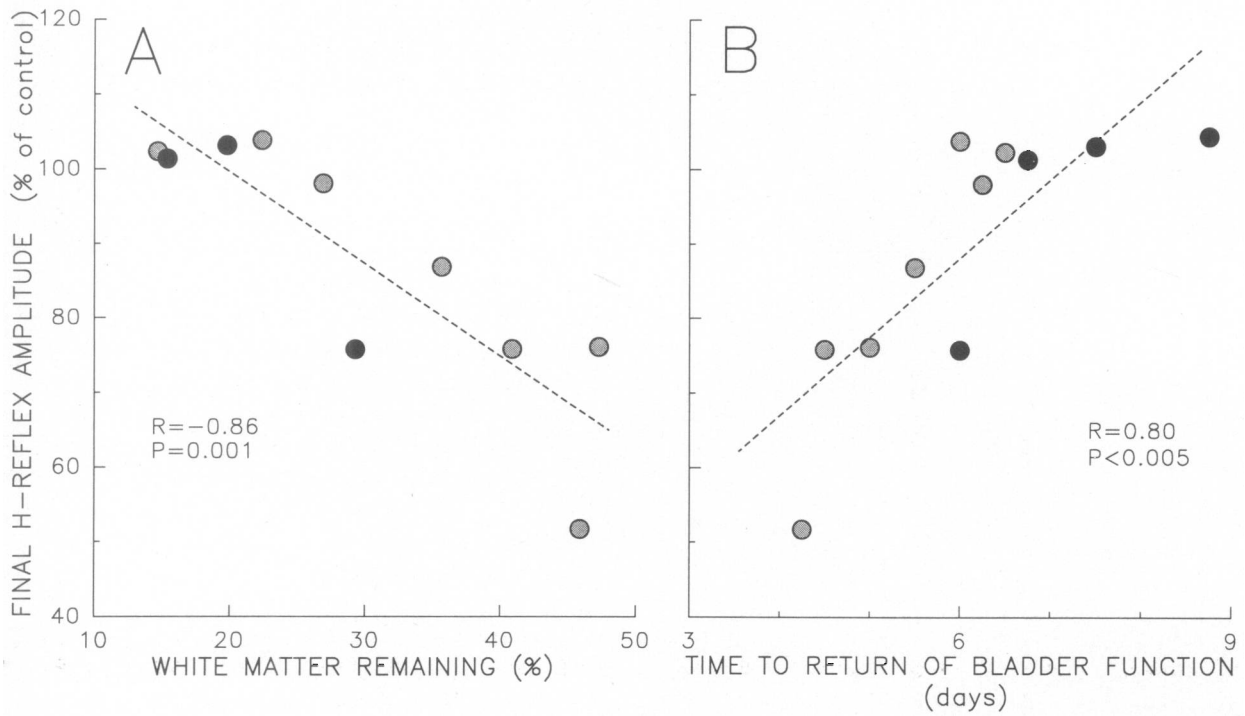


**FIG. 6.** (A) H-reflex amplitudes for rats with mild (shaded) and medium (black) spinal cord contusions at end of HRdown conditioning versus white matter remaining (in percent of white matter in reference section; see text. A strong correlation is evident ($r = -0.86$, $p = 0.001$). Rats with more spared white matter decreased the H-reflex more. (B) H-reflex amplitudes for rats with mild (shaded) and medium (black) spinal cord contusions at end of HRdown conditioning versus duration of impaired bladder function. A strong correlation is evident ($r = 0.80$, $p < 0.005$). Rats with shorter periods of impaired bladder function decreased the H-reflex more.

change of only +10% from the value at the end of HRdown exposure). Of the 3 mild-contusion animals, only 1 (with 46% of white matter remaining) displayed successful HRdown conditioning (decrease to 52% of control), although another (with 36% of white matter remaining) showed a decrease to 87%. The third (with 15% of white matter remaining) was 102% of control at the end of HRdown conditioning. In contrast, all three displayed successful HRup conditioning. They increased to 216%, 200%, and 124% of their final HRdown values, respectively (i.e., to 112%, 173%, and 127% of their control-mode values). These responses of the 3 mild-contusion animals to the HRup mode are better than or equal to those of normal animals exposed to the HRup mode following an initial HRdown exposure (Chen and Wolpaw, 1996a).

Figure 8 shows H-reflex amplitude throughout the course of study from the single mild-contusion animal (with 46% of white matter remaining) that was exposed in succession to the control mode, the HRdown mode, the HRup mode, and the HRdown mode. This animal clearly retained the capacity for H-reflex conditioning. The H-reflex decreases with the first HRdown exposure, increases with the HRup exposure, and then decreases again with reexposure to the HRdown mode.

## DISCUSSION

### *H-Reflex Conditioning and Supraspinal Control*

The H-reflex, the electrical analog of the spinal stretch reflex (SSR, or tendon jerk), is the simplest spinal cord behavior. It is produced by 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 influenced by descending activity from supraspinal regions (Burke and Rudomin, 1978; Baldissera et al., 1981), this simple behavior can be operantly conditioned. Operant conditioning of the SSR or of its electrical analog, the H-reflex, 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 of these studies operantly

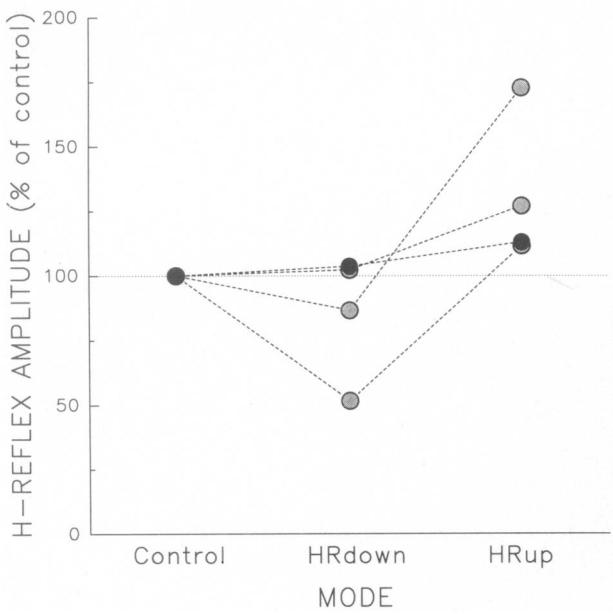


**FIG. 7.** H-reflex amplitudes at the end of HRdown conditioning and at the end of HRup conditioning for 3 rats with mild contusions (shaded) and 1 with a medium contusion (black) that were exposed to the HRdown mode for 50 days and then to the HRup mode for 50 days. HRdown conditioning is successful (change of at least 20% from initial value) in only 1 animal, whereas HRup conditioning is successful in 3 of the 4.

conditions the descending activity that controls the spinal reflex pathway. For example, the HRdown mode rewards descending activity that decreases H-reflex amplitude, and thereby presumably increases the prevalence of this activity. Because the H-reflex is elicited at an unpredictable time (see Materials and Methods) and occurs before any other CNS response, the animals can increase reward frequency only by continuously controlling the descending activity so as to maintain the spinal reflex arc in the proper state. Because this mode-appropriate descending activity is present for a prolonged period each day as the animal performs the task over the many days of conditioning, it gradually produces activity-driven plasticity in the spinal cord (Wolpaw and Lee, 1989; Carp and Wolpaw, 1994; Feng-Chen and Wolpaw, 1996).

H-reflex conditioning is a model for the normal changes in descending influences that modify spinal cord function during development (e.g., Myklebust et al., 1986) and for the pathologic changes in these influences that alter spinal cord function after injury. Current studies in animals after H-reflex conditioning are providing insight into the spinal cord plasticity produced by long-term changes in descending activity. In monkeys, H-reflex conditioning changes spinal motoneuron intrinsic properties and synaptic coverage and also causes changes elsewhere in the spinal cord (Wolpaw and Lee, 1989; Carp and Wolpaw, 1994; Feng-Chen and Wolpaw, 1996).

### *Effects of Spinal Cord Contusion on H-Reflex Conditioning*

The present study shows that after thoracic spinal cord contusion destroys up to 70% of the spinal cord white matter, some animals retain the capacity to undergo operantly conditioned decrease or increase or both in the soleus H-reflex. Thus, in the successful animals, mode-appropriate change in activity in surviving descending axons reaching the lumbrosacral spinal cord was able to support HRdown or HRup conditioning or both. These results are consistent with those reported by Segal and Wolf (1994) for humans with partial spinal cord injury. Five of eight individuals with injuries of cervical cord were able to reduce the biceps brachii SSR at least 20%.

The response to HRdown conditioning depended on the severity of injury as assessed by white matter destruction or by duration of impaired bladder function. As Figure 6 illustrates, animals that had more spared white matter, or regained bladder function more quickly, decreased the H-reflex more. Thus, failure of HRdown conditioning probably resulted from damage to those pathways conveying the descending control normally altered by conditioning. At the same time, the diffuse nature of the lesion produced by contusion prevents conclusions about which descending pathways are essential for conditioning. Concurrent studies of the effects on conditioning of dorsal or lateral column transection suggest that the corticospinal tract, located in the dorsal column (Tracey 1995), is essential for HRdown conditioning, whereas the rubrospinal tract and other pathways located in the lateral column are not essential (Chen and Wolpaw, 1996b,c). This evidence that lateral column lesions do not impair HRdown conditioning is also important for the present study in another way. It indicates that the laminectomy alone was not responsible for the impairment of HRdown conditioning noted here.

It is interesting that the limited data from animals exposed to both HRdown and HRup modes (Fig. 7) suggest that HRup conditioning may be more resistant to the spinal cord contusion injury than is HRdown conditioning. Only 1 of 4 HRdown/HRup animals displayed successful HRdown conditioning, while 3 displayed successful HRup conditioning. That the two modes may have different sensitivities to injury is not surprising. Physiologic data (Carp and Wolpaw, 1994, 1995) imply that HRup and HRdown conditioning have different spinal cord mechanisms and, thus, may also be controlled

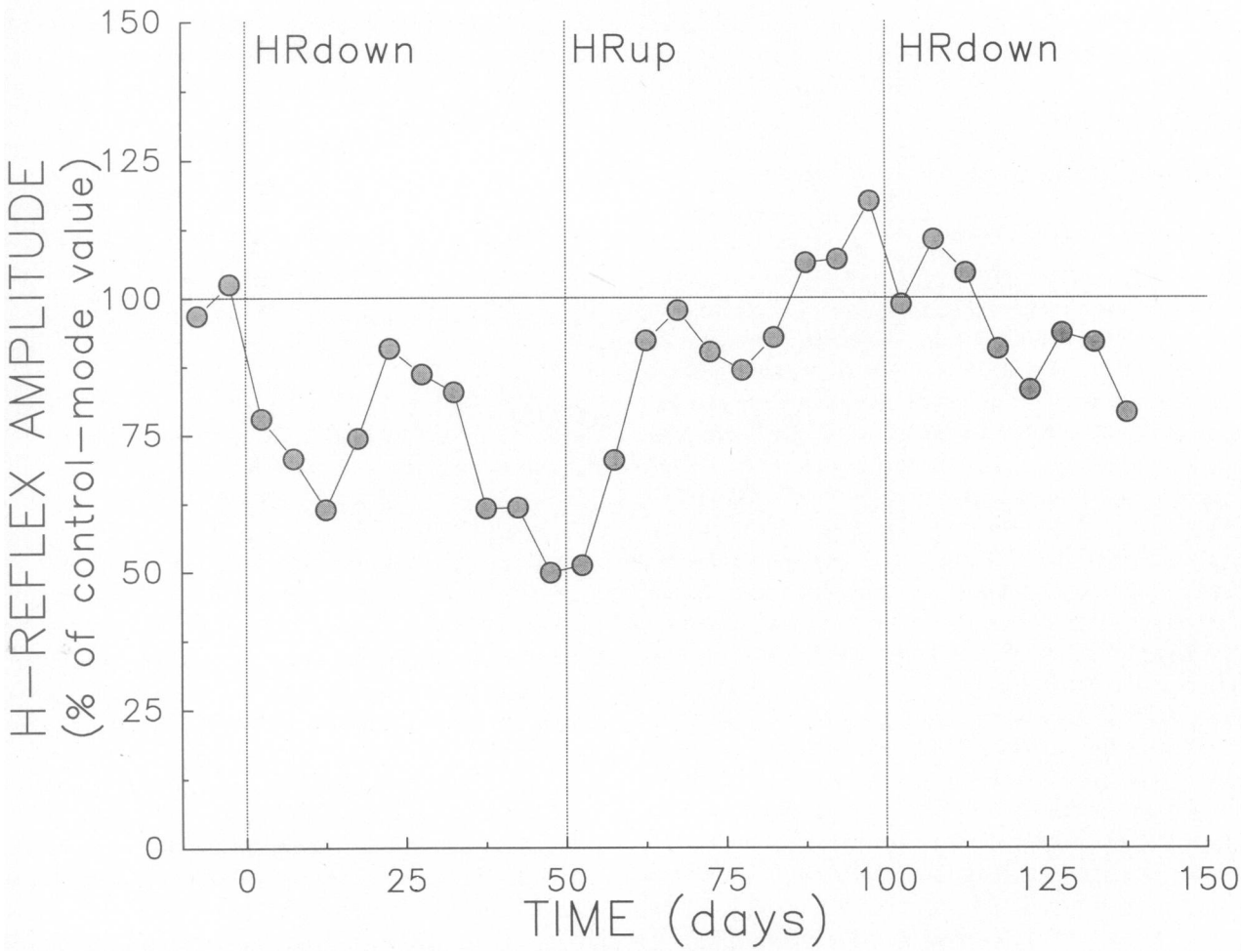


**FIG. 8.** Average H-reflex amplitude from a rat with a mild contusion for each 5-day period during the control-mode, first exposure to the HRdown mode, subsequent exposure to the HRup mode, and reexposure to the HRdown mode. H-reflex amplitude falls with the HRdown mode, rises with the HRup mode, and falls again with reexposure to the HRdown mode.

by different descending pathways. Resolution of this issue awaits further studies of pathway-specific lesions.

### *Other Effects of Spinal Cord Contusion on the H-Reflex*

Injury was associated with a small non-significant increase in the control-mode H-reflex (Table 1). This minimal effect may be attributable in part to the fact that the H-reflex was always measured in the presence of the same level of background EMG and, thus, presumably the same overall level of motoneuron tone. Most studies of injury effects on the H-reflex or the SSR itself have not controlled and/or assessed motoneuron background tone, so that injury-associated changes in motoneuron tone may account for much of any change reported. On the other hand, a recent detailed study of soleus H-reflexes for up to 60 days after contusion injury (Thompson et al., 1992), while noting substantial effects on H-reflex threshold and recruitment, also found only a modest increase in H-reflex amplitude.‡

Although it is conceivable that, in some animals, operantly conditioned decrease in the H-reflex was obscured by gradual post-traumatic H-reflex increases, this seems unlikely. First, the injury had no significant effect on H-reflex amplitude, M response amplitude, or background EMG from 18 to 140 days after the injury (Table 1), and control-mode H-reflex amplitude was not related to time post-injury. Furthermore as Figure 5 indicates, failure was not correlated with time postinjury, as might be expected if injury triggered a specific period of grad-

‡Thompson et al. (1992) noted an effect of injury on H-reflex rate dependence. However, the stimulus rate of the present study was too slow (i.e., ≤0.3 Hz) for this rate dependence to be a significant factor.

ual H-reflex increase. At the same 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 or the SSR or both may be of significant value for the study and/or treatment of spinal cord injury. It could provide a valuable complement to motor and somatosensory evoked potentials (i.e., MEPs and SEPs) in evaluating spinal cord capacities after injury. MEPs and SEPs are measures of conduction in descending and ascending spinal cord pathways. They can be used to assess the integrity of spinal cord pathways after injury (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, although requiring appropriate activity in descending pathways, focuses on the segmental response to that activity. It assesses the integrity of the segmental circuitry. Thus, H-reflex conditioning can supplement the MEP and SEP assessments of spinal cord long tracts with an assessment of segmental function.

The strong correlation between impairment of H-reflex conditioning and impairment of bladder function (Fig. 6B), 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 spinal cord capacity after injury. The high sensitivity of H-reflex conditioning is further indicated by comparing its impairment by injury to the effects of injury on locomotion. Rats and cats can walk when only about 10% of white matter remains (Blight 1983; Bresnahan et al., 1987), whereas HRdown conditioning in the rat requires about 30% (Fig. 6A). This high sensitivity, combined with the developing insight into the pathways essential for conditioning (Chen and Wolpaw 1996b,c), might help define the descending pathways necessary for a variety of functions.

The fact that the H-reflex and the SSR can still be operantly conditioned after spinal cord injury suggests that operant conditioning could be used to modify and improve spinal cord function after injury. This possibility was the primary impetus for the Segal and Wolf (1994) study, which demonstrated SSR conditioning in patients with spinal cord injuries, and for their investigation of associated changes (Segal and Wolf, 1992; Wolf et al., 1995). Finally, H-reflex conditioning may provide a sensitive and convenient means for assessing the efficacy of drugs or other interventions in restoring function after spinal cord injury. This use would be particularly important if retention of the capacity for H-reflex conditioning is found to be closely correlated with retention of other important aspects of spinal cord function.

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