Research

Ablation of cerebellar nuclei prevents H-reflex down-conditioning in rats

Xiang Yang Chen[1] and Jonathan R. Wolpaw

Laboratory of Nervous System Disorders, Wadsworth Center, New York State Department of Health and State University of New York at Albany, Albany, New York 12201-0509, USA

While studies of cerebellar involvement in learning and memory have described plasticity within the cerebellum, its role in acquisition of plasticity elsewhere in the CNS is largely unexplored. This study set out to determine whether the cerebellum is needed for acquisition of the spinal cord plasticity that underlies operantly conditioned decrease in the H-reflex, the electrical analog of the spinal stretch reflex. Rats in which the cerebellar output nuclei dentate and interpositus (DIN) had been ablated were exposed for 50 d to the H-reflex down-conditioning protocol. DIN ablation, which in itself had no significant long-term effect on H-reflex size, entirely prevented acquisition of a smaller H-reflex. Since previous studies show that corticospinal tract (CST) transection also prevents down-conditioning while transection of the rubrospinal tract and other major descending tracts does not, this result implies that DIN output that affects cortex is essential for generation of the CST activity that induces the spinal cord plasticity, which is, in turn, directly responsible for the smaller H-reflex. The result extends the role of the cerebellum in learning and memory to include participation in induction of plasticity elsewhere in the CNS, specifically in the spinal cord. The cerebellum might simply support processes in sensorimotor cortex or elsewhere that change the spinal cord, or the cerebellum itself might undergo plasticity similar to that occurring with vestibulo-ocular reflex (VOR) or eyeblink conditioning.

Activity-dependent plasticity, which is now known to occur throughout the CNS and through a variety of mechanisms, is presumed to underlie learning. However, the mechanistic relationships between this plasticity and learned behavioral changes remain largely obscure. The complexity and limited accessibility of the CNS, combined with the fact that even simple learning involves plasticity at multiple sites (Wolpaw and Lee 1989; Carrier et al. 1997; Cohen et al. 1997; Lieb and Frost 1997; Thompson et al. 1997; Whalen and Pearson 1997; Lisberger 1998; Garcia et al. 1999; Medina et al. 2000, 2002; Hansel et al. 2001; King et al. 2001; Wolpaw and Tennissen 2001; Carey and Lisberger 2002; van Alphen and De Zeeuw 2002; Blazquez et al. 2003), makes it hard to trace the connections from specific experiences to specific occurrences of activity-dependent plasticity, and thence to specific behavioral changes (Wolpaw 2002). Efforts to do so use experimental models based on simple behaviors produced by defined and accessible neural circuitry.

Two of these models, vestibulo-ocular reflex (VOR) plasticity and eyelid conditioning, reveal plasticity in the cerebellum and associated brainstem nuclei that underlies relatively rapid behavioral change (Kim and Thompson 1997; Pellegrini and Evinger 1997; Ito 1998, 2000; Lisberger 1998; Strata and Rossi 1998; Yeo and Hesslow 1998; Garcia et al. 1999; Mauk et al. 2000; Medina et al. 2000, 2002; Raymond and Lisberger 2000; Hansel et al. 2001; King et al. 2001; Carey and Lisberger 2002; van Alphen and De Zeeuw 2002; Blazquez et al. 2003). A third model, operant conditioning of the H-reflex, reveals spinal cord plasticity that underlies gradual behavioral change (Wolpaw and Lee 1989; Wolpaw 2001; Wolpaw and Tennissen 2001). This study is the first step toward determining whether cerebellar plasticity of the kind described in the VOR and eyeblink conditioning models has a role in the induction and maintenance of the spinal cord plasticity described in the H-reflex conditioning model.

The H-reflex, the electrical analog of the spinal stretch reflex (SSR), is mediated largely by a monosynaptic pathway consisting of the primary afferent neuron from the muscle spindle, its synapse on the alpha motoneuron in the spinal cord, and the motoneuron itself (Magladery et al. 1951; Matthews 1972; Henneman and Mendell 1981; Brown 1984). The SSR and the H-reflex change during early development, during skill acquisition later in life, after spinal cord trauma, and in response to an operant conditioning protocol (for review, see Wolpaw and Tennissen 2001). With exposure to this protocol, monkeys, humans, and rats can gradually decrease (i.e., down-conditioning) or increase (i.e., up-conditioning) the SSR or the H-reflex (Wolpaw et al. 1983; Wolpaw 1987; Evatt et al. 1989; Chen and Wolpaw 1995; Wolf et al. 1995). This simple behavioral change has two distinct phases: a small phase I change that occurs within the first 1–2 d and a much larger phase II change that develops over 6–7 wk and is ultimately responsible for most of the final change in the reflex (Wolpaw and O'Keefe 1984; Chen et al. 2001a). The learning is associated with multisite spinal cord plasticity that includes changes in spinal cord motoneuron properties, in synaptic terminals on these motoneurons, and probably in spinal interneurons as well (Wolpaw and Lee 1989; Carp and Wolpaw 1994, 1995; Feng-Chen and Wolpaw 1996; Wolpaw 1997; Carp et al. 2001; Wang et al. 2003). The smaller H-reflex produced by down-conditioning is largely explained by a positive shift in motoneuron firing threshold, which may be due to a comparable shift in sodium channel activation voltage (Carp and Wolpaw 1994; Halter et al. 1995). In contrast, the larger H-reflex produced by up-conditioning may reflect a decrease in nonreciprocal oligosynaptic group I inhibition of the motoneuron (Carp and Wolpaw 1995; Wolpaw and Chen 2001).

Studies based on carefully defined transections of the main corticospinal tract (CST), the ipsilateral lateral column, and the dorsal column ascending tract in rats have indicated that down-

[1]**Corresponding author.**
E-mail chenx@wadsworth.org; fax (518) 486-4910.
Article and publication are at http://www.learnmem.org/cgi/doi/10.1101/lm.91305.

12:248–254 ©2005 by Cold Spring Harbor Laboratory Press ISSN 1072-0502/05; www.learnmem.org

conditioning of the H-reflex requires the CST and does not require other major descending pathways such as the rubrospinal tract (Chen and Wolpaw 1997, 2002; Chen et al. 2001b). In combination with the previous studies noted above, this finding implies that the conditioning protocol rapidly induces a change in CST activity that accounts for the small phase I change in the reflex, and that the continuation of this CST activity over weeks gradually produces the spinal cord plasticity (e.g., the positive shift in motoneuron firing threshold) that accounts for the large phase II change.

We hypothesize that this essential CST activity results from rapid cerebellar plasticity comparable to that described with VOR and eyeblink conditioning. To test this hypothesis, we must first determine whether the cerebellum is essential for H-reflex conditioning, and then, if this proves to be the case, determine whether cerebellar plasticity is essential for H-reflex conditioning. The present study takes the first step by assessing the effects of destroying the cerebellar output nuclei dentate and interpositus before exposing rats to the down-conditioning protocol.

Results

As a necessary prelude to evaluating the effects of ablating the dentate and interpositus nuclei (DIN) on H-reflex conditioning, we first assessed the effects of DIN ablation on the H-reflex in the absence of conditioning. In each of five rats, control H-reflex size was measured for 14–48 d, the DIN were ablated (Fig. 1A), and data collection continued for 60 more days. (Postmortem histological analysis showed that DIN ablation had been effective in all of these five rats; see Materials and Methods.) Figure 1B summarizes the results. H-reflex size increases in the first 2 d after DIN ablation, remains slightly elevated for ~2 wk, returns to its pre-ablation level, and for days 40–60 is slightly but not significantly elevated ($P > 0.18$; see Materials and Methods). The brief increase in the first 2 d, which is similar to that seen after cerebellar ablation in cats (Van Der Meulen and Gilman 1965; McLeod and Van Der Meulen 1967), also occurs after several different spinal cord pathway transections (Chen et al. 2001b; Chen and Wolpaw 2002). Thus, it may be a nonspecific short-term effect of the surgery and/or the accompanying general anesthesia. In the present context, the important finding is that DIN ablation itself has little or no long-term effect on H-reflex size. Furthermore, DIN ablation had no long-term effect on the number of trials per day (i.e., the number of H-reflex elicitations per day; see Materials and Methods), the distribution of trials throughout the day, or the average value of background EMG at the time of H-reflex elicitation.

Figure 2A shows the protocol used to assess the effect of DIN ablation on the acquisition of an H-reflex decrease. At least 30 d after DIN ablation, the control H-reflex size was measured for at least 10 d, and then the rat was exposed to down-conditioning for 50 d. During this down-conditioning period, the number of trials per day, their distribution throughout the day, and the average background EMG did not change from their values during the control period.

Figure 2B summarizes the results for the six rats in which the DIN were largely destroyed (i.e., DIN rats). It shows H-reflex size at the end of down-conditioning for these rats and includes, for comparison, data from normal rats (Chen and Wolpaw 1995, 1997, 2002; X.Y. Chen and J.R. Wolpaw, unpubl.) and from rats with lateral column (LC) or CST transections (Chen and Wolpaw 1997, 2002). These LC and CST transections were accomplished with methods comparable to those described here (see Materials and Methods), were fully defined in terms of completeness (i.e., percentage of the targeted pathway destroyed), and were carefully analyzed to ensure that collateral damage did not account

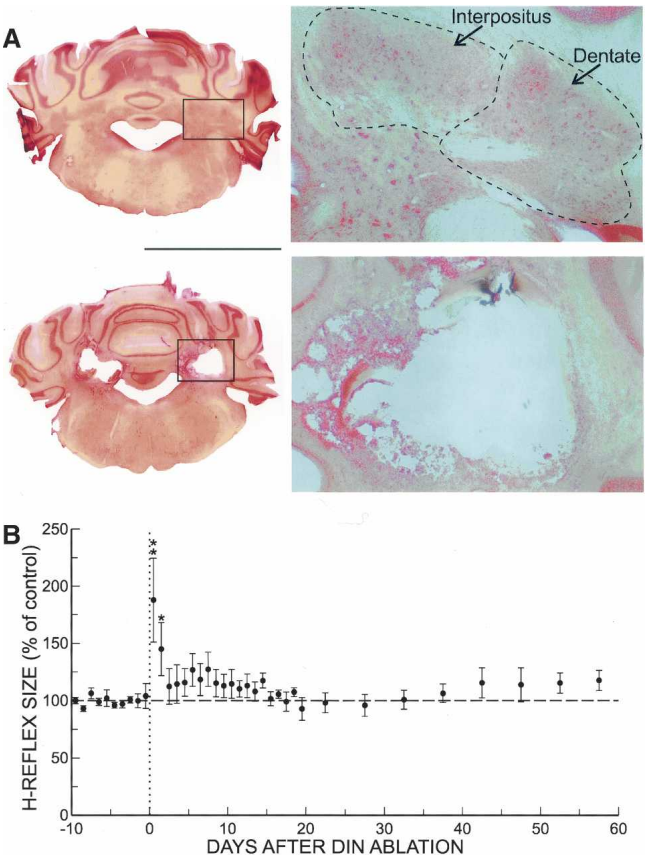

Figure 1. (*A*) DIN ablation. (*Left*) Photomicrographs of brain through the cerebellum from a normal rat (*top*) and a rat with DIN ablation showing the cavities left by bilateral ablation of dentate and interpositus nuclei (*bottom*). Scale bar, 6.0 mm. (*Right*) Higher-magnification photomicrographs of the right DIN area from the normal rat (*top*) and from the DIN rat (*bottom*). The nuclei are absent in the DIN rat. Scale bar, 1.0 mm. (*B*) Effects of DIN ablation on H-reflex size. Average H-reflex (in percent of pre-ablation average size) under the control mode for five rats for 10 d before and 60 d after DIN ablation. Background EMG amplitude and M-response size were stable throughout. The asterisks indicate values that are significantly different from pre-ablation values (** $P < 0.01$; * $P < 0.05$). DIN ablation has no significant long-term effect on H-reflex size ($P > 0.18$). The apparent lower variability prior to ablation reflects the fact that these pre-ablation data were used to define each rat's control reflex size (i.e., 100%). This largely eliminates inter-animal variability from the pre-ablation data.

for the effects on down-conditioning (Chen and Wolpaw 1997, 2002). Solid circles indicate successful down-conditioning (i.e., the H-reflex decreased to ≤80% of its initial value) (Wolpaw et al. 1993; Chen and Wolpaw 1995).

In the normal rats, 75% were successful and final H-reflex size averaged 66% (±25% SD). In the LC rats, 86% were successful and final H-reflex size averaged 71% (±20%). For both normal and LC rats, final H-reflex size was significantly smaller than control size ($P < 0.001$ and $P < 0.01$, respectively, by paired *t*-test). In contrast, none of the DIN or CST rats was successful, and final H-reflex size averaged 120% (±32%) in DIN rats and 103% (±14%) in CST rats. Furthermore, in both DIN and CST rats, final H-reflex size did not differ significantly from the control size ($P > 0.1$ and $P > 0.6$, respectively, by paired *t*-test).

The four groups of rats differed significantly by ANOVA ($P < 0.001$), and the DIN and CST rats differed from normal rats both in the number that were successful ($P < 0.001$ and $P < 0.01$ by Fisher exact test, respectively) and in final H-reflex size

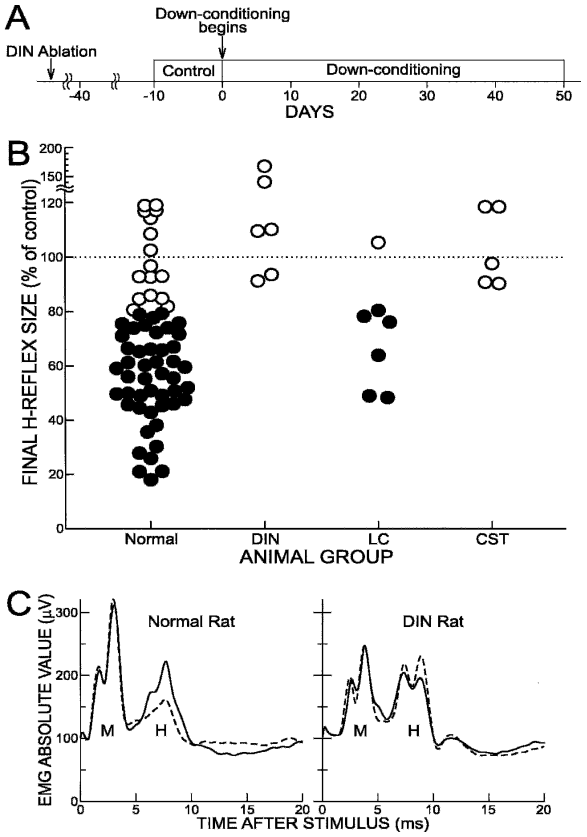

Figure 2. Effects of DIN ablation on acquisition of a smaller H-reflex. (*A*) Study protocol. At least 30 d after DIN ablation, control H-reflex size was measured over at least 10 d, and then the rats were exposed to down-conditioning for 50 d. Background EMG amplitude and M-response size were stable throughout. (*B*) Average H-reflex size for the final 10 d of down-conditioning (days 41–50) as percent of control H-reflex size for DIN rats, with comparable data from normal rats (Chen and Wolpaw 1995, 1997, 2002; X.Y. Chen and J.R. Wolpaw, unpubl.) and from CST and LC rats (Chen and Wolpaw 1997, 2002) included for comparison. Solid circles indicate that down-conditioning was successful (i.e., the H-reflex decreased to ≤80% of its initial value) (Wolpaw et al. 1993; Chen and Wolpaw 1995), while open circles indicate that it was unsuccessful. As detailed in the text, LC transection did not impair conditioning, while CST transection or DIN ablation prevented it entirely. (*C*) Average post-stimulus EMG for all the trials of representative days before (solid) and near the end (dashed) of down-conditioning from a normal rat (*left*) and a DIN rat (*right*). In both rats, background EMG (indicated by the value at 0 msec) and M response do not change with conditioning. The H-reflex of the normal rat is much smaller after down-conditioning, while that of the DIN rat is not.

($P < 0.01$ for both by Dunnett's test). Figure 2C contrasts the effects of down-conditioning in a normal and a DIN rat. Down-conditioning reduces the H-reflex in the normal rat, but not in the DIN rat.

The results summarized in Figure 2 show that DIN ablation, like CST transection and unlike LC transection, entirely prevents acquisition of a smaller H-reflex in response to the down-conditioning protocol. Finally, it should be noted that, in two rats in which DIN ablation was not effective (i.e., 3/4 of the cells remained; see Materials and Methods), down-conditioning was successful (i.e., final H-reflex sizes were 52% and 57% of control).

Figure 3 shows H-reflex size prior to and over the course of down-conditioning for the six DIN rats. There is no evidence that a rapid phase I drop in H-reflex size occurs in the first few days of down-conditioning. Thus, DIN ablation prevents both phase I and phase II decrease in the H-reflex.

Discussion

The results lead to two conclusions about the effects of ablation of the cerebellar output nuclei dentate and interpositus (DIN). The first is that DIN ablation itself has little or no long-term effect on H-reflex size. This conclusion is consistent with the results of McLeod and Van Der Meulen (1967), who studied motoneuron excitability following cerebellar ablation in cats. While cerebellar ablation reduces muscle spindle sensitivity (Van Der Meulen and Gilman 1965; Gilman and McDonald 1967), this does not directly affect the H-reflex, which is induced by nerve stimulation rather than by muscle stretch. This negative result is important, for it facilitates assessment of the effects of DIN ablation on H-reflex conditioning. The second conclusion is that DIN ablation entirely prevents down-conditioning of the H-reflex. This effect on acquisition is comparable to that of CST transection, and suggests that DIN ablation might prevent the change in CST activity that is normally induced by the down-conditioning protocol and leads to the H-reflex decrease (Chen and Wolpaw 2002).

These conclusions raise two questions. First, what is the destination of the DIN output that is essential for acquisition of down-conditioning? Does it descend to the spinal cord or does it ascend to cortex? Second, what is the role of this output in down-conditioning? Is it simply a pre-existing output essential for the normal functioning of the cortical areas that generate the descending activity that changes the H-reflex; or is it an output that reflects cerebellar plasticity that is created by the reward contingency and guides the change in the H-reflex?

The destination of the essential cerebellar output

Through the red nucleus and other structures, DIN output descends to the spinal cord and ascends to the cerebral cortex (Voogd 1995; Voogd and Glickstein 1998). Previous studies showing that transection of the entire ipsilateral lateral column (which includes the rubrospinal, vestibulospinal, and reticulospinal tracts) does not impair H-reflex conditioning suggest that the loss of conditioning after DIN ablation is not due to loss of its descending projection. The important DIN output appears to be its ascending projection. Furthermore, the fact that the CST is the only major descending pathway essential for acquisition (and maintenance) of an H-reflex decrease (summarized in Fig. 2B; Chen and Wolpaw 1997, 2002; Chen et al. 2001b) suggests that the crucial ascending influence from the DIN affects sensorimotor cortex, which is the main origin of the CST (Kuypers 1981; Kennedy 1990; Tracey 1995). It remains possible, although improbable, that this ascending cerebellar influence acts through non-CST descending axons in the ventral column of the spinal cord (Tracey 1995).

Reciprocal pathways connect specific regions of cerebellar cortex and cerebral cortex (including primary motor cortex, premotor cortex, and prefrontal cortex) (Allen and Tsukahara 1974; Sasaki et al. 1979; Bloedel and Courville 1981; Kuypers 1981; Asanuma et al. 1983; Yamamoto et al. 1983, 1992; Orioli and Strick 1989; Tracey 1995; Voogd 1995; Middleton and Strick 1997, 1998; Schmahmann and Pandyat 1997; Voogd and Glickstein 1998; Holdefer et al. 2000). These loops are now believed to serve a variety of higher functions in addition to the simpler motor coordination functions long attributed to the cerebellum (Glickstein and Yeo 1990; Raymond et al. 1996; Houk 1997; Schmahmann and Pandyat 1997; Desmond and Fiez 1998; Middleton and Strick 1998; Thach 1998; Leggio et al. 1999; Mandolesi et al. 2001). They are a likely substrate for the cerebellar role in production of the CST activity responsible for H-reflex down-conditioning.

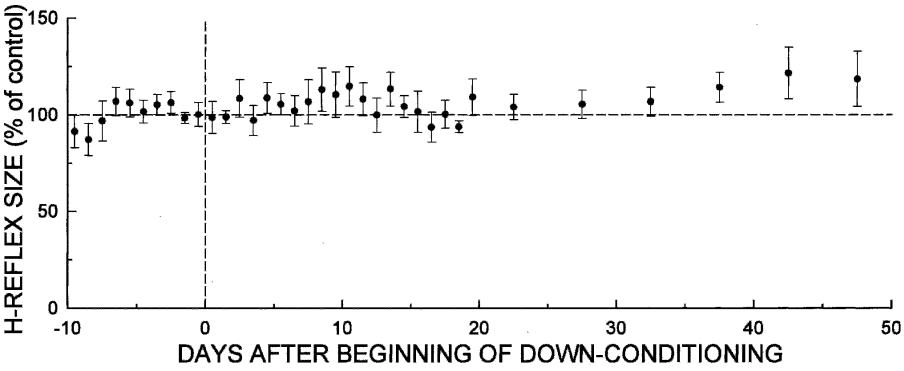

Figure 3. Average H-reflex size as percent of control H-reflex size for the six DIN rats for 10 d before and 50 d after the beginning of down-conditioning. Background EMG amplitude and M-response size were stable throughout. None of the down-conditioning values is significantly different from the pre-conditioning values ($P > 0.28$). Down-conditioning had no significant immediate or gradual effect on H-reflex size.

The contribution of the cerebellar output

In its demonstration that the cerebellum is essential for H-reflex conditioning, and thus for the responsible spinal cord plasticity, this study is consistent with evidence that cerebellar lesions can rapidly produce persistent changes in the spinal cord (DiGiorgio 1929; Chamberlain et al. 1963; Steinmetz et al. 1981). The nature of the cerebellum's role in producing H-reflex change remains to be defined. Beyond the immediate post-ablation period, DIN ablation had no apparent effect on animal well-being, gross motor behavior, or activity level. The rats continued to gain weight, walked without apparent deficit, and satisfied the background EMG requirement (see Materials and Methods) with the same daily frequency and on the same daily schedule as before ablation. The H-reflex itself showed no significant change from its pre-ablation size (Fig. 1B). Furthermore, the fact that H-reflex down-conditioning was successful in the two rats in which DIN ablation was not effective (i.e., in which 3/4 of the cells remained; see Materials and Methods) helps to rule out nonspecific effects of the ablation surgery as a cause for the failure of down-conditioning in all the rats in which DIN ablation was effective. These observations suggest that the abolition of down-conditioning by DIN ablation was not due to a nonspecific impairment of CNS function, but instead reflects a specific deficit in responding appropriately to the reward contingency.

One possibility is that cerebellar output is essential for processes in sensorimotor cortex and/or elsewhere that produce the CST activity that rapidly induces phase I change in the H-reflex and gradually induces the spinal cord plasticity that underlies phase II change. Like both simple and complex motor functions, the production of this CST activity may depend on the reciprocal pathways that connect cerebellum and motor cortex (Glickstein and Yeo 1990; Raymond et al. 1996; Houk 1997; Schmahmann and Pandyat 1997; Desmond and Fiez 1998; Middleton and Strick 1998; Thach 1998; Leggio et al. 1999; Mandolesi et al. 2001). By interrupting these pathways, DIN ablation could abolish or severely impair these processes and thus prevent H-reflex conditioning. Alternatively, cerebellar output might be needed to maintain the spinal cord's capacity to respond with appropriate plasticity to the CST activity induced by the reward contingency. While observation of locomotion and other behaviors did not reveal general motor deficits that would support these possibilities, the detection of such deficits might require highly specialized motor tests.

Another possibility is that the reward contingency induces cerebellar plasticity that changes output to sensorimotor cortex and thereby produces the CST activity that, in turn, changes the spinal cord. Studies of VOR plasticity and eyeblink conditioning suggest that conjunctions of activity in mossy and climbing fibers induce plasticity in cerebellar cortex and/or nuclei, and that the resulting changes in cerebellar output contribute to behavioral change (Kim and Thompson 1997; Pellegrini and Evinger 1997; Ito 1998, 2000; Lisberger 1998; Strata and Rossi 1998; Yeo and Hesslow 1998; Garcia et al. 1999; Mauk et al. 2000; Medina et al. 2000, 2002; Raymond and Lisberger 2000; Hansel et al. 2001; King et al. 2001; Carey and Lisberger 2002; van Alphen and De Zeeuw 2002; Blazquez et al. 2003). In these models, both mossy and climbing fiber inputs are believed to be sensory. If cerebellar plasticity plays a similar role in H-reflex conditioning, the mossy fiber input may not be sensory, since conditioning is not impaired by transecting the major ascending spinal cord pathways (Chen and Wolpaw 1997, 2002). While the effect of transecting the portion of the ventral spinocerebellar tract that ascends on the contralateral side of the spinal cord (Yamada et al. 1991; Tracey 1995) has not been studied, it appears more likely that the mossy fiber input is an efference copy of sensorimotor cortex output conveyed to the cerebellum by cortico-pontine-cerebellar connections (Allen and Tsukahara 1974; Ruigrok and Cella 1995; Brodal and Bjaalie 1997; Schmahmann and Pandyat 1997). The climbing fiber input could originate from the lower probability of reward immediately after CST activity that incorrectly influences H-reflex size. The resulting cerebellar plasticity could modify DIN output to cortex so as to decrease future production of that CST activity. The initial development of this cerebellar plasticity would account, through its effect on CST activity, for phase I change in the H-reflex (Wolpaw and O'Keefe 1984; Chen et al. 2001a). Furthermore, the persistence of this cerebellar plasticity could account for the development and maintenance of phase II H-reflex change (J.R. Wolpaw, L. Chen, and X.Y. Chen, in prep).

Conclusion

By showing that DIN output is essential for down-conditioning of the H-reflex, this study extends the role of the cerebellum in skill acquisition to include its participation in induction of plasticity in the spinal cord. The nature of the cerebellum's contribution is as yet unknown. It is possible that cerebellar output is essential for processes in sensorimotor cortex or elsewhere that produce the spinal cord plasticity associated with H-reflex conditioning. Alternatively, it is possible that the reward contingency induces cerebellar plasticity that conveys that contingency to the spinal cord via the sensorimotor cortex and CST, and thereby creates the spinal cord plasticity directly responsible for H-reflex change.

Materials and Methods

The study used 13 adult Sprague-Dawley rats (10 females weighing 216–307 g initially and three males weighing 336–429 g initially). 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 had been reviewed and approved by the Institutional Animal Care and Use Committee of the Wadsworth Center. The H-reflex conditioning protocol, described in detail elsewhere (Wolpaw and

Herchenroder 1990; Chen and Wolpaw 1995, 1997, 2002), is briefly summarized here. Other procedures are described in detail.

The H-reflex conditioning protocol

Under general anesthesia (80 mg/kg ketamine HCl and 10 mg/kg xylazine, both i.p.), each rat was implanted with chronic stimulating and recording electrodes in the right hindleg. To elicit the H-reflex, a nerve-stimulating cuff was placed on the right posterior tibial nerve just above the triceps surae branches. To record soleus EMG activity, fine-wire electrodes were 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 head. Data collection started at least 20 d after implantation. During data collection, each animal lived in a standard rat cage with a flexible cable attached to the head 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, from which they passed to an EMG amplifier and a nerve-cuff stimulation 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 h to 0600 h each day.

A computer system continuously monitored EMG from soleus muscle and controlled the nerve-cuff stimulus. If the absolute value (i.e., equivalent to the full-wave rectified value) of background (i.e., ongoing) EMG remained within a defined range (typically 1%–2% of maximum possible EMG as assessed by maximum M response) 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. This criterion ensured that background EMG at the time of H-reflex elicitation remained the same throughout data collection. Pulse amplitude was initially set just above M-response threshold and then continuously and automatically adjusted to maintain M-response size unchanged. Thus, both the background EMG (reflecting soleus motoneuron tone at the time of H-reflex elicitation) and the M response (reflecting the effective strength of the nerve cuff stimulus) remained stable throughout the entire period of data collection. Under the control mode, the computer simply measured the absolute value of soleus EMG for 50 msec following the stimulus and determined H-reflex size. Under the down-conditioning mode, it gave a food reward 200 msec after nerve stimulation if EMG amplitude in the H-reflex interval (i.e., typically 5.5–9.0 msec after stimulation) was below a criterion value (which was initially defined on the basis of the control-mode data so as to reward the smallest 20%–30% of the rat's H-reflexes, and was subsequently adjusted as needed so as to maintain this reward percentage). In the course of its normal activity, the animal usually satisfied the background EMG requirement, and thus received nerve-cuff stimulation, 2000–8500 times per day. H-reflex size 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. As noted in the Results, each rat's number of trials per day, background EMG amplitude, and M-response size remained stable throughout data collection.

DIN ablation and post-ablation animal care and well-being

Bilateral ablation of the dentate and interpositus cerebellar nuclei (DIN) (Voogd 1995; Voogd and Glickstein 1998) was performed electrolytically with a platinum/iridium electrode (monopolar, 0.125-mm diameter, 0.100 mm exposed). (Ablation was bilateral so that, if the results were negative—i.e., if conditioning occurred in the DIN rats—they would clearly rule out an essential role for the cerebellum.) The rat was anesthetized as described above and placed in a stereotaxic frame with its head secured by ear bars and a tooth holder. Two holes (1-mm diameter) were made on each side of the skull. Coordinates for electrode placements were initially derived from Paxinos and Watson (1986) and Kruger et al. (1995) and slightly modified on the basis of histological analyses of several preliminary experiments. For dentate ablation on each side, the electrode was positioned vertical to the plane defined by the interaural line and the midline with its tip on the surface of the brain 2.70 mm caudal to the interaural line and 2.72 mm lateral to the midline, and the tip was then inserted vertically 4.10 mm into the brain. For interpositus ablation, the electrode was positioned vertical to the plane defined by the interaural line and the midline with its tip on the surface of the brain 2.70 mm caudal to the interaural line and 1.72 mm lateral to the midline, and the tip was then inserted vertically 4.50 mm into the brain. Each ablation was made by passing AC current (0.2–0.3 mA, 5-Hz sine wave) for 7 min. After ablation, the electrode was removed and the hole was filled with bone wax. After both nuclei were ablated on both sides, the muscle and skin were sutured in layers.

Immediately after ablation, the rat was placed under a heat lamp and given an analgesic (Demerol, 0.2 mg, i.m.). Once awake, it received a second dose of analgesic and was returned to its cage. Those who ate poorly in the first few post-ablation days were fed manually with water-soaked rat chow and a high-calorie dietary supplement (Nutri-Cal). All rats resumed normal eating within 5 d and remained healthy and active throughout the rest of the study. Body weight, which fell 3%–18% in the first post-ablation week, recovered to its pre-ablation level in 1–6 wk. Every rat gained weight over the time of study. For all rats, weight increased from 216–429 g at the beginning of study to 285–656 g at the end. Locomotion, which was often awkward and poorly balanced immediately after DIN ablation, appeared normal within 3–4 wk or less, well before H-reflex conditioning began.

Histology

At the end of the study, each rat was given an overdose of sodium pentobarbital (i.p.) and 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 tibial nerve were examined, and the soleus muscles of both sides were removed and weighed. Soleus muscle weights (measured as percent of body weight) were symmetrical and did not differ significantly from those of normal rats. The brain was removed and the area encompassing the cerebellum was blocked and stored in 10% sucrose in 0.2 M phosphate buffer (pH 7.3). Transverse 100-μm serial sections were cut with a Vibratome and stained with 1% neutral red. In the sections encompassing the DIN, we counted, on both right and left sides, the number of DIN cells with diameters ≥10 μm. For each rat, these counts were made without knowledge of the effect of the down-conditioning protocol on the H-reflex. Four normal (i.e., unablated) rats were similarly studied. The number of DIN cells remaining on each side of each DIN-ablated rat (i.e., DIN rat) was calculated as a percent of the average number of cells on each side of the normal rats.

Normal rats averaged 2879 (±91 SD) DIN cells on each side, with no significant difference between right and left sides ($P > 0.05$ by paired t-test). In two of the 13 DIN rats, DIN ablation was not effective: In both rats total cell counts averaged 74% of normal (74% and 80% on the right, respectively, and 74% and 68% on the left, respectively). In the other 11 DIN rats, DIN ablation was highly effective: Total cell counts averaged 10% (±14 SD) of normal (range 0%–48%). The right DIN averaged 10% (±14 SD; range 0%–50%) and the left DIN averaged 10% (±16 SD; range 0%–44%). Figure 1A shows transverse sections from a normal rat and one of these 11 DIN rats. Of these 11 rats in which DIN ablation was effective, five were used in the control study of the effects of DIN ablation alone, while the other six (and the two rats in which DIN ablation turned out to have been not effective) were used in the study of the effects of DIN ablation on H-reflex conditioning.

Other than loss of the DIN nuclei, DIN rats showed only the narrow tract of the electrode through the cerebellum (except for one rat in which the damage impinged slightly on the right lateral vestibular nucleus). Thus, it is highly probable that the ef-

fects of DIN ablation were due to DIN loss rather than to collateral damage to other structures.

Data analysis

To assess short-term effects of DIN ablation on H-reflex size (i.e., Fig. 1C), a repeated-measures ANOVA was used to detect significant variation over days for the last 10 pre-ablation days and the first 20 post-ablation days. If an effect was found, Dunnett's multiple comparisons method was used to identify post-ablation days that differed significantly from the average of the last 10 pre-ablation days. To assess the long-term effects of DIN ablation, a similar procedure was used to compare the average H-reflex size for each 10-d period (starting 20 d after DIN ablation) to the average H-reflex size for the 10-d period immediately prior to ablation. A similar procedure was used to assess the short-term and long-term effects of down-conditioning on H-reflex size in DIN rats (i.e., Fig. 3).

To assess the effects of DIN ablation on acquisition of H-reflex conditioning (i.e., Fig. 2B), a paired *t*-test was used to compare the average H-reflex size for the last 10 d of down-conditioning (i.e., days 41–50 of the 50-d down-conditioning period) to the average H-reflex size for the last 10 d prior to the beginning of the down-conditioning period; and an ANOVA followed by Dunnett's test was used to compare H-reflex sizes for the last 10 d of down-conditioning (expressed as percent of the average for the last 10 d prior to down-conditioning) from DIN rats to those from normal rats and rats with CST or LC transections. The Fisher exact test was used to compare rat groups in regard to the number successful (i.e., the number of rats in which the H-reflex decreased to ≤80% of its initial value) (Wolpaw et al. 1993; Chen and Wolpaw 1995).

Acknowledgments

We thank Lu Chen, Rongliang Liu, Gerwin Schalk, and Hesham Sheikh for excellent technical assistance; and Jonathan S. Carp, Dennis J. McFarland, and Elizabeth Winter Wolpaw for valuable comments on the manuscript. This work was supported in part by grants from the National Institutes of Health (HD36020 to X.Y.C. and NS22189 to J.R.W.), the Christopher Reeve Paralysis Foundation (to X.Y.C.), and the International Spinal Research Trust (to J.R.W.).

References

Allen, G.I. and Tsukahara, N. 1974. Cerebrocerebellar communication systems. *Physiol. Rev.* **54:** 957–1006.

Asanuma, C., Thach, W.T., and Jones, E.G. 1983. Distribution of cerebellar terminations and their relation to other afferent terminations in the ventral lateral thalamic region of the monkey. *Brain Res.* **286:** 237–265.

Blazquez, P.M., Hirata, Y., Heiney, S.A., Green, A.M., and Highstein, S.M. 2003. Cerebellar signatures of vestibulo-ocular reflex motor learning. *J. Neurosci.* **23:** 9742–9751.

Bloedel, J.R. and Courville, J. 1981. Cerebellar afferent systems. In *Handbook of physiology. The nervous system. Motor control.* Sect. 1, Pt. II, Chap. 16, pp. 735–830. American Physiology Society, Bethesda, MD.

Brodal, P. and Bjaalie, J.G. 1997. Salient anatomic features of the cortico-ponto-cerebellar pathway. *Progr. Brain Res.* **114:** 227–249.

Brown, W.F. 1984. *The physiological and technical basis of electromyography*. Butterworths, Boston.

Carey, M.R. and Lisberger, S.G. 2002. Embarrassed, but not depressed: Eye opening lessons for cerebellar learning. *Neuron* **35:** 223–226.

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

———. 1995. Motoneuron properties after operantly conditioned increase in primate H-reflex. *J. Neurophysiol.* **73:** 1365–1373.

Carp, J.S., Chen, X.Y., Sheikh, H., and Wolpaw, J.R. 2001. Operant conditioning of rat H-reflexes affects motoneuron axonal conduction velocity. *Exp. Brain Res.* **136:** 269–273.

Carrier, L., Brustein, S., and Rossignol, S. 1997. Locomotion of the hindlimbs after neurectomy of ankle flexors in intact and spinal cats. *J. Neurophysiol.* **77:** 1979–1993.

Chamberlain, T., Halick, P., and Gerard, R.W. 1963. Fixation of experience in the rat spinal cord. *J. Neurophysiol.* **22:** 662–673.

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

———. 1997. Dorsal column but not lateral column transection prevents down conditioning of H-reflex in rats. *J. Neurophysiol.* **78:** 1730–1734.

———. 2002. Probable corticospinal tract control of spinal cord plasticity in rats. *J. Neurophysiol.* **87:** 645–652.

Chen, X.Y., Chen, L., and Wolpaw, J.R. 2001a. Time course of H-reflex conditioning in the rat. *Neurosci. Lett.* **302:** 85–88.

Chen, X.Y., Feng-Chen, K.C., Chen, L., Stark, D.M., and Wolpaw, J.R. 2001b. Short-term and medium-term effects of spinal cord tract transections on soleus H-reflex in freely moving rats. *J. Neurotrauma* **18:** 313–327.

Cohen, T.E., Kaplan, S.W., Kandel, E.R., and Hawkins, R.D. 1997. A simplified preparation for relating cellular events to behavior: Mechanisms contributing to habituation, dishabituation, and sensitization of the Aplysia gill-withdrawal reflex. *J. Neurosci.* **17:** 2886–2899.

Desmond, J.E. and Fiez, J.A. 1998. Neuroimaging studies of the cerebellum: Language, learning and memory. *Trends Cogn. Sci.* **2:** 355–362.

DiGiorgio, A.M. 1929. Persistenza nell'animale spinale, di asymmetrie posturali e motorie di origine cerebellare. Nota I–III. *Arch. Fisiol.* **27:** 518–580.

Evatt, M.L., Wolf, S.L., and Segal R.L. 1989. Modification of human spinal stretch reflexes: Preliminary studies. *Neurosci. Lett.* **105:** 350–355.

Feng-Chen, K.C. and Wolpaw, J.R. 1996. Operant conditioning of H-reflex changes synaptic terminals on primate motoneurons. *Proc. Natl. Acad. Sci.* **93:** 9206–9211.

Garcia, K.S., Steele, P.M., and Mauk, M.D. 1999. Cerebellar cortex lesions prevent acquisition of conditioned eyelid responses. *J. Neurosci.* **19:** 10940–10947.

Gilman, S. and McDonald, W.I. 1967. Cerebellar facilitation of muscle spindle activity. *J. Neurophysiol.* **30:** 1494–1512.

Glickstein, M. and Yeo, C. 1990. The cerebellum and motor learning. *J. Cogn. Neurosci.* **2:** 69–80.

Halter, J.A., Carp, J.S., and Wolpaw, J.R. 1995. Operantly conditioned motoneuron plasticity: Possible role of sodium channels. *J. Neurophysiol.* **74:** 867–871.

Hansel, C., Linden, D.J., and D'Angelo, E. 2001. Beyond parallel fiber LTD: The diversity of synaptic and non-synaptic plasticity in the cerebellum. *Nat. Neurosci.* **4:** 467–475.

Henneman, E. and Mendell, L.M. 1981. Functional organization of motoneuron pool and its inputs. In *Handbook of physiology. The nervous system. Motor control.* Sect. 1, Pt. I, Chap. 11, pp. 423–507. American Physiology Society, Bethesda, MD.

Holdefer, R.N., Miller, L.E., Chen, L.L., and Houk, J.C. 2000. Functional connectivity between cerebellum and primary motor cortex in the awake monkey. *J. Neurophysiol.* **84:** 585–590.

Houk, J.C. 1997. On the role of the cerebellum and basal ganglia in cognitive signal processing. *Prog. Brain Res.* **114:** 543–552.

Ito, M. 1998. Cerebellar learning in the vestibulo-ocular reflex. *Trends Cogn. Sci.* **2:** 313–321.

———. 2000. Mechanisms of motor learning in the cerebellum. *Brain Res.* **886:** 237–245.

Kennedy, P.R. 1990. Corticospinal, rubrospinal and rubro-olivary projections: A unifying hypothesis. *Trends Neurosci.* **13:** 474–479.

Kim, J. and Thompson, R.F. 1997. Cerebellar circuits and synaptic mechanisms involved in classical eyeblink conditioning. *Trends Neurosci.* **20:** 177–181.

King, D.A.T., Krupa, D.J., Foy, M.R., and Thompson, R.F. 2001. Mechanisms of neuronal conditioning. *Int. Rev. Neurobiol.* **45:** 313–337.

Kruger, L., Saporta, S., and Swanson, L.W. 1995. *Photographic atlas of the rat brain*. Cambridge University Press, Cambridge, UK.

Kuypers, H.G.J.M. 1981. Anatomy of the descending pathways. In *Handbook of physiology. The nervous system. Motor control.* Sect. 1, Pt. I, Chap. 13, pp. 345–422. American Physiology Society, Bethesda, MD.

Leggio, M.G., Neri, P., Graziano, A., Mandolesi, L., Molinari, M., and Petrosini, L. 1999. Cerebellar contribution to spatial event processing: Characterization of procedural learning. *Exp. Brain Res.* **127:** 1–11.

Lieb, J.R. and Frost, W.N. 1997. Realistic simulation of the *Aplysia* siphon-withdrawal reflex circuit: Roles of circuit elements in producing motor output. *J. Neurophysiol.* **77:** 1249–1268.

Lisberger, S.G. 1998. Physiologic basis for motor learning in the vestibulo-ocular reflex. *Otolaryng. Head Neck Surg.* **119:** 43–48.

Magladery, J.W., Porter, W.E., Park, A.M., and Teasdall, R.D. 1951. 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. Johns Hopkins Hosp.*

88: 499–519.
Mandolesi, L., Leggio, M.G., Graziano, A., Neri, P., and Petrosini, L. 2001. Cerebellar contribution to spatial event processing: Involvement in procedural and working memory components. *Eur. J. Neurosci.* **14:** 2011–2022.
Matthews, P.B.C. 1972. *Mammalian muscle receptors and their central actions*, pp. 319–409. Williams & Wilkins, Baltimore.
Mauk, M.D., Medina, J.F., Nores, W.L., and Ohyama, T. 2000. Cerebellar function: Coordination, learning or timing? *Curr. Biol.* **10:** R522–R525.
McLeod, J.G. and Van Der Meulen, J.P. 1967. Effect of cerebellar ablation on the H reflex in the cat. *Arch. Neurol.* **16:** 421–432.
Medina, J.F., Nores, W.L., Ohyama, T., and Mauk, M.D. 2000. Mechanisms of cerebellar learning suggested by eyelid conditioning. *Curr. Opin. Neurobiol.* **10:** 717–724.
Medina, J.F., Repa, J.C., Mauk, M.D., and LeDoux, J.E. 2002. Parallels between cerebellum- and amygdala-dependent conditioning. *Nat. Rev. Neurosci.* **3:** 122–131.
Middleton, F.A. and Strick, P.L. 1997. Cerebellar output channels. *Int. Rev. Neurobiol.* **41:** 61–82.
———. 1998. Cerebellar output: Motor and cognitive channels. *Trends Cogn. Sci.* **2:** 348–354.
Orioli, P.J. and Strick, P.L. 1989. Cerebellar connections with the motor cortex and the arcuate premotor area: An analysis employing retrograde transneuronal transport of WGA-HRP. *J. Comp. Neurol.* **288:** 612–626.
Paxinos, G. and Watson, C. 1986. *The rat brain in sterotaxic coordinates*, 2nd ed. Academic Press, San Diego.
Pellegrini, J.J. and Evinger, C. 1997. Role of cerebellum in adaptive modification of reflex blinks. *Learn. Mem.* **3:** 77–87.
Raymond, J.L. and Lisberger, S.G. 2000. Hypotheses about the neural trigger for plasticity in the circuit for the vestibulo-ocular reflex. *Prog. Brain Res.* **124:** 235–246.
Raymond, J.L., Lisberger, S.G., and Mauk, M.D. 1996. The cerebellum: A neuronal learning machine? *Science* **272:** 1126–1131.
Ruigrok, T.J.H. and Cella, F. 1995. Precerebellar nuclei and red nucleus. In *The rat nervous system* (ed. G. Paxinos), pp. 277–308. Academic Press, San Diego, CA.
Sasaki, K., Jinnai, K., Gemba, H., Hashimoto, S., and Mizuno, N. 1979. Projection of the cerebellar dentate nucleus onto the frontal association cortex in monkeys. *Exp. Brain Res.* **37:** 193–198.
Schmahmann, J.D. and Pandyat, D.N. 1997. The cerebrocerebellar system. *Int. Rev. Neurobiol.* **41:** 31–60.
Steinmetz, J.E., Cervenka, J., Robinson, C., Romano, A.G., and Patterson, M.M. 1981. Fixation of spinal reflexes in rats by central and peripheral sensory input. *J. Comp. Physiol. Psychol.* **95:** 548–555.
Strata, P. and Rossi, F. 1998. Plasticity of the olivocerebellar pathway. *Trends Neurosci.* **21:** 407–413.
Thach, W.T. 1998. What is the role of the cerebellum in motor learning and cognition? *Trends Cogn. Sci.* **2:** 331–337.
Thompson, R.F., Bao, S., Chen, L., Cipriano, B.D., Grethe, J.S., Kim, J.J., Thompson, J.K., Tracy, J.A., Weninger, M.S., and Krupa, D.J. 1997. Associative learning. *Int. Rev. Neurobiol.* **41:** 151–189.
Tracey, D.J. 1995. Ascending and descending pathways in the spinal cord. In *The rat nervous system* (ed. G. Paxinos), pp. 67–80. Academic Press, San Diego, CA.
van Alphen, A.M. and De Zeeuw, C.I. 2002. Cerebellar LTD facilitates but is not essential for long-term adaptation of the vestibulo-ocular reflex. *Eur. J. Neurosci.* **16:** 486–490.
Van Der Meulen, J.P. and Gilman, S. 1965. Recovery of muscle spindle activity in cats after cerebellar ablation. *J. Neurophysiol.* **28:** 943–957.
Voogd, J. 1995. Cerebellum. In *The rat nervous system* (ed. G. Paxinos), pp. 309–350. Academic Press, San Diego, CA.
Voogd, J. and Glickstein, M. 1998. The anatomy of the cerebellum. *Trends Neurosci.* **21:** 370–375.
Wang, Y., Diao, R., Schalk, G., Wolpaw, J.R., and Chen, X.Y. 2003. Effects of H-reflex down-conditioning on GABAergic terminals on rat soleus motoneurons. Abstract Viewer/Itinerary Planner, Program No. 497.8. Society for Neuroscience, Washington, DC.
Whalen, P. and Pearson, K.G. 1997. Plasticity in reflex pathways controlling stepping in the cat. *J. Neurophysiol.* **78:** 1643–1650.
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.
———. 1997. The complex structure of a simple memory. *Trends Neurosci.* **20:** 588–594.
———. 2001. Spinal cord plasticity in the acquisition of a simple motor skill. In *Spinal cord plasticity: Alterations in reflex function* (eds. M.M. Patterson and J.W. Grau), pp. 101–125. Kluwer Academic, Boston, MA.
———. 2002. Memory in neuroscience: Rhetoric versus reality. *Behav. Cogn. Neurosci. Rev.* **1:** 130–163.
Wolpaw, J.R. and Chen, X.Y. 2001. Operant conditioning of rat H-reflex: Effects on mean latency and duration. *Exp. Brain Res.* **136:** 274–279.
Wolpaw, J.R. and Herchenroder, P.A. 1990. Operant conditioning of H-reflex in freely moving monkeys. *J. Neurosci. Meth.* **31:** 145–152.
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.
Wolpaw, J.R. and O'Keefe, J.A. 1984. Adaptive plasticity in the primate spinal stretch reflex: Evidence for a two-phase process. *J. Neurosci.* **4:** 2718–2724.
Wolpaw, J.R. and Tennissen, A.M. 2001. Activity-dependent spinal cord plasticity in health and disease. *Ann. Rev. Neurosci.* **24:** 807–843.
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., Herchenroder, P.A., and Carp, J.S. 1993. Operant conditioning of the primate H-reflex: Factors affecting the magnitude of change. *Exp. Brain Res.* **97:** 31–39.
Yamada, J., Shirao, K., Kitamura, T., and Sato, H. 1991. Trajectory of spinocerebellar fibers passing through the inferior and superior cerebellar peduncles in the rat spinal cord: A study using horseradish peroxidase with pedunculotomy. *J. Comp. Neurol.* **304:** 147–160.
Yamamoto, T., Wagner, A., Hassler, R., and Sasaki, K. 1983. Studies on the cerebellocerebral and thalamocortical projections in squirrel monkeys (*Saimiri sciureus*). *Exp. Neurol.* **79:** 27–37.
Yamamoto, T., Yoshida, K., Yoshikawa, Y., Kishimoto, Y., and Oka, H. 1992. The medial dorsal nucleus is one of the thalamic relays of the cerebellocerebral response to the frontal association cortex in the monkey: Horseradish peroxidase and fluorescent dye double staining study. *Brain Res.* **579:** 315–320.
Yeo, C.H. and Hesslow, G. 1998. Cerebellum and conditioned reflexes. *Trends Cogn. Sci.* **2:** 322–330.

Received December 27, 2004; accepted in revised form April 5, 2005.