Research

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

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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 eyelid 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 Tennyson 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, 1999; Strata and Rossi 1998; Yeo and Hesslow 1999; Garcia et al. 1999; Mauk et al. 2000; Medina et al. 2001, 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 Tennyson 2001). This study is the first step toward determining whether cerebellar plasticity of the kind described in the VOR and eyelid 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; Henne 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 Tennyson 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; Woll 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-
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 eyelink 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 rats (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 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.
and phase II decrease in the H-reflex. Thus, DIN ablation prevents both phase I and II changes in the H-reflex size.

The results summarized in Figure 2 show that DIN ablation, like CST transection and unlike LC transection, 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 changes in CST activity that is normally induced by the down-conditioning protocol and leads to the H-reflex decrease (Chen and Wolpaw 2002).

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 changes 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 serves 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, 1998; Schmahmann and Pandya 1997; 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 DIN ablation might prevent the change in CST activity that is normally induced by the down-conditioning protocol.

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 transaction 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).


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 30 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 ≈50% 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.
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 1979; 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 II 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 Pandya 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 Pandya 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 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...
Hensch and Wolpaw (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 Achilles tendon. 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 randomly varying 2.3–2.7-s periods, 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 activity 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 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 rat—they would clearly rule out an essential role for the cerebellum.) The rat was anesthetized 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 dorsal 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 rats that ate poorly in the first few post-ablation days were fed manually with water-sorted rat chow. 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 conditioning was not effective: In both rats total cell counts averaged 74% of normal (range 0%–58%).

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 of the last 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- 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

References


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