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Motoneuron Plasticity Underlying Operantly Conditioned Decrease in Primate H-Reflex

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SUMMARY AND CONCLUSIONS

1. Monkeys can gradually increase or decrease the size of the triceps surae H-reflex in response to an operant conditioning task. This conditioning modifies the spinal cord. To determine the location and nature of the spinal cord plasticity and define its role in the behavioral change (i.e., H-reflex increase or decrease) we have recorded intracellularly from triceps surae motoneurons in conditioned animals and compared the results with data from naive (i.e., unconditioned) animals.

2. Eleven monkeys (*Macaca nemestrina*, male) were exposed to the HRdown conditioning mode, in which reward occurred when H-reflex size in one leg (i.e., the trained leg) was below a criterion value. In six animals (5.1–8.2 kg) H-reflex size in the trained leg fell to 24–58% of its initial value, whereas in the other five animals (4.0–5.5 kg) it remained at 92–114% of its initial value. This outcome, which was in accord with recent data indicating that success in HRdown conditioning is age dependent, allowed comparison of intracellular data from successful HRdown animals with data from unsuccessful animals as well as with data from naive (i.e., unconditioned) animals.

3. Intracellular recordings were obtained from 221 triceps surae motoneurons on trained and control sides of successful and unsuccessful HRdown animals. Measurements included axonal conduction velocity, input resistance, time constant, electrotonic length, rheobase, firing threshold, afterhyperpolarization duration and amplitude, and composite homonymous and heteronymous excitatory postsynaptic potentials to peripheral nerve stimulation. Results were compared with data from 109 triceps surae motoneurons in naive animals.

4. Motoneurons from the trained side of successful HRdown animals had a significantly more positive average firing threshold (−52 vs. −55 mV) and a significantly lower average conduction velocity (67 vs. 71 m/s) than those from naive animals. In contrast, motoneurons from the trained side of unsuccessful HRdown animals were not significantly different from naive motoneurons.

5. These data are consistent with the hypothesis that operantly conditioned decrease in H-reflex size is due to a positive shift in motoneuron firing threshold and a consequent increase in the depolarization needed to reach that threshold.

6. The more positive firing threshold, if present in the axon as well as in the soma, could also account for the decreased conduction velocity observed in motoneurons from the trained side of successful animals.

INTRODUCTION

CNS mechanisms responsible for the acquisition and maintenance of adaptive behavioral changes are under investigation in both vertebrates and invertebrates (Dudai 1989; Martinez and Kesner 1991; Wolpaw et al. 1991). The central problem is to link specific behavioral changes to specific neuronal and/or synaptic modifications and to define the processes that create these modifications. Up to the present, invertebrate studies have been more successful in locating the modified neurons and synapses and in describing the modifications and the processes that produce them. This difference is normally ascribed to the simplicity and accessibility of the pathways mediating specific invertebrate behaviors. Vertebrate systems, however, also possess adaptive behaviors subserved by simple and accessible CNS pathways. In addition, their behaviors are more directly relevant to the adaptive behaviors of highest interest, those occurring in humans.

The initial response to sudden muscle stretch, known as the spinal stretch reflex (SSR) or tendon jerk, is mediated primarily by a two-neuron, monosynaptic pathway—the Ia afferent fiber from the muscle spindle, the α-motoneuron, and the synapse between them (Henneman and Mendell 1981; Lee and Tatton 1975; Magladery et al. 1951; Matthews 1972). Over the past decade studies in several laboratories have shown that this behavior possesses the capacity for adaptive change or learning (Evatt et al. 1989; Meyer-Lohmann et al. 1986; Wolf and Segal 1990; Wolpaw 1987; Wolpaw et al. 1983). Monkeys and humans can gradually increase or decrease the SSR or its electrical analogue, the H-reflex, when reward depends on reflex size. Reflex size in the trained muscle increases (up mode conditioning) or decreases (down mode conditioning) steadily over weeks. This change is relatively specific to the trained muscle and occurs without change in background activity of the trained muscle, its synergists, or its antagonists.

Most importantly in the present context, evidence of H-reflex conditioning persists in the spinal cord of a conditioned animal after removal of all supraspinal influence (Wolpaw and Lee 1989; Wolpaw et al. 1989). Thus part of the memory trace—the CNS plasticity that develops during conditioning and underlies adaptive increase or decrease of the reflex—is in the spinal cord. The simplicity of the reflex pathway and the accessibility of the spinal cord provide the opportunity to locate and define this plasticity.

The observation that effects of H-reflex conditioning are still present in the transected spinal cord under deep pentobarbital anesthesia (Wolpaw and Lee 1989; Wolpaw et al. 1989), which severely depresses spontaneous interneuronal activity, suggests that the sites of spinal cord plasticity include the components of the reflex arc—the Ia synapse on the motoneuron, the immediate postsynaptic region, and/or the entire motoneuron. To evaluate these possibilities

we have measured motoneuron properties and primary afferent (mainly group Ia) excitatory postsynaptic potentials (EPSPs) in animals after conditioning of the triceps surae (TS) H-reflex.

In the present study we report results from animals that were exposed to the HRdown conditioning mode, in which reward occurred when TS H-reflex size in one leg (the "trained leg") was below criterion. The H-reflex in the other leg (the "control leg") was also elicited and measured, but its size did not affect reward probability. The original intent was to compare motoneurons from the trained side and from the control side of HRdown animals to motoneurons from naive (NV) (i.e., unconditioned) animals studied in earlier work (Carp 1992, 1993). Chance, however, furnished a third group of animals that provided a valuable additional form of control data. Earlier studies of H-reflex conditioning used mainly young adult animals weighing $\geq$6 kg and observed successful conditioning (i.e., change in required direction $\geq$20% of initial value) in 87% of them (summarized in Wolpaw et al. 1993). More recent studies, including the present one, have used a number of lighter [probably late adolescent (Sirianni and Swindler 1985)] animals weighing <6 kg. Such animals do as well as heavier animals at HRup conditioning but are much less likely to be successful at HRdown conditioning. In ~50% of lighter animals (<6 kg) exposed to the HRdown mode, H-reflex size remains within 20% of initial size (Wolpaw et al. 1993). The distribution of H-reflex sizes in these unsuccessful animals is clearly separate from that in successful animals. Owing to this previously unknown effect of animal age on HRdown conditioning, in the present study we are able to compare five motoneuron populations from three groups of animals. We compare motoneurons from the trained side of successful HRdown animals, the control side of successful HRdown animals, the trained side of unsuccessful HRdown animals, the control side of unsuccessful HRdown animals, and the previously studied (Carp 1992, 1993) NV animals. The additional control group, unsuccessful HRdown animals, provides an opportunity to determine whether any changes detected in intracellular measurements are simply nonspecific effects of exposure to the HRdown mode or are specific to H-reflex decrease.

The results provide a plausible, although surprising, explanation for the smaller TS H-reflex found in the trained leg of successful HRdown animals. Portions of this work have appeared in abstract form (Carp et al. 1993).

METHODS

Subjects were 11 monkeys (*Macaca nemestrina*, male, 4.0–8.2 kg). They came from the same source as and were comparable in weight with the animals from which NV motoneuron data were obtained (Carp 1992, 1993). Both the chronic conditioning protocol and the acute intracellular procedures have been described completely elsewhere (Carp 1992, 1993; Wolpaw and Herchenroder 1990). They are summarized here. All chronic and acute animal procedures were in accord with Department of Health, Education, and Welfare Publication (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 Committee of the Wadsworth Center.

Operant conditioning of the H-reflex

To prepare each animal for conditioning, chronic stimulating and recording electrodes were implanted under general anesthesia (intravenous pentobarbital). To elicit the TS H-reflex, a silicon rubber cuff containing stainless steel fine-wire electrodes was placed on the tibial nerve just proximal to the knee (Stein et al. 1977, 1980). Fine-wire EMG electrodes were inserted in the TS muscles [i.e, medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SOL)]. In 9 of the 11 animals, right and left legs were similarly implanted, and six electromyographic (EMG) channels were normally monitored simultaneously (i.e., MG, LG, and SOL muscles of each leg). In the remaining two animals data were obtained from only one leg. The wires from all stimulating and recording electrodes passed subcutaneously to an exit in the midback and into a pocket in a custom-made monkey jacket. There they connected to wires that went through a very flexible stainless steel tether cable to an electronic swivel and thence to EMG amplifiers and nerve cuff stimulators.

The animal wore the jacket and attached tether continuously and lived in a standard primate cage. It could move freely about the cage and was provided with standard monkey chow three times per day, fresh fruit twice per day, and environmental enrichment. It could see and interact with monkeys in adjacent cages via mirrors. Water was provided primarily by the task described below. On the rare days when task performance did not provide sufficient water, supplements were given. Laboratory lighting was reduced between 2100 and 0600 h. Animal wellbeing and jacket integrity were monitored by close visual inspection several times per day. Weight, skin condition, and jacket condition were assessed under light ketamine anesthesia after the first 2 wk and subsequently at longer intervals or whenever indicated.

A minicomputer system interfaced with up to four monkeys simultaneously and continuously. The computer recorded the absolute value of each muscle's digitized EMG (equivalent to full-wave rectification). It controlled three outputs: a large light in the animal's cage that illuminated when one channel of TS background EMG was in a specified range (see below), bilateral tibial nerve stimuli at M (direct muscle) response threshold and simultaneous flash of a small round light in the water port of the cage, and a reward squirt of water from the syringe-solenoid system mounted in the water port.

The animal drank by performing a simple task. It stood or clung to the cage so that its mouth was in front of the water port and provided a specified level of TS background EMG activity in both legs for a randomly varying 1.2- to 1.8-s period. The background level required (see below) was sufficient to give substantial H-reflexes. (Unlike humans, monkeys usually have minimal or absent TS H-reflexes in the absence of background tone.) The large light helped the animal to maintain correct background EMG, particularly early in conditioning. It was controlled by a 250-ms running average of the absolute value of EMG from one pair of TS electrodes (usually the LG pair of the right leg). If correct EMG was present in the muscles of both legs at the end of the 1.2- to 1.8-s period, an 0.1-ms square-wave voltage pulse at an amplitude kept by computer just above M response threshold was delivered to each leg's tibial nerve through the nerve cuff and elicited the H-reflex. Because these stimuli were barely perceptible to the animal, the small round light in the water port flashed at the same time. This visual signal, much too late to affect the bilateral H-reflexes, signaled the animal to open its mouth for the reward squirt. The computer digitized each muscle's EMG for 100 ms after the stimulus. Two hundred milliseconds after the stimulus, the solenoid-powered syringe delivered the reward squirt directly into the animal's mouth. After each trial the computer determined the average absolute value of the EMG for the M-response interval (usually 3–9 ms after stimulus onset). It compared this value for

one muscle in each leg (usually LG) with a target M response value and appropriately adjusted each leg's stimulus amplitude for the next trial. By this means stimulus strength was kept just above M response threshold and nerve excitation by the stimulus was stable over time. In summary, the animal held correct TS background EMG in both legs for 1.2–1.8 s, received bilateral nerve cuff stimuli, opened its mouth, and received water. This task was the animal's standard method for drinking and was fully integrated into its normal life in the cage.

The animal mastered the task in standard operant fashion. Initially the TS background EMG criteria were set very low and applied to only one leg, so that the animal often satisfied them and caused a reward squirt as it moved about the cage. At this stage no nerve cuff stimuli were given. Only the flash of the small light next to the squirter indicated that reward was imminent. As the monkey learned to orient to the squirter, so that the reward actually reached its mouth and reward frequency increased, the TS background EMG criteria were gradually raised and applied to both legs until substantial background EMG levels were achieved bilaterally. At this point the bilateral nerve cuff stimuli were added and the M response targets were set so as to give small (i.e., threshold) M responses and substantial H-reflexes in both legs.

As indicated above, the task was available 24 h/day and became part of the animal's normal routine. Animals normally completed 2,000–6,000 trials per day and usually obtained all required fluid in this fashion, so that supplements in the form of wet monkey chow were needed only rarely.

The computer provided a daily summary, including average background EMG amplitude and average poststimulus course of EMG amplitude. (As noted above, all EMG measurements were absolute values.) Daily H-reflex size was defined as average EMG amplitude in the H-reflex interval minus average background EMG amplitude, and calculated in units of background EMG level. The background EMG criteria and the computer control of stimulus strength ensured that background EMG levels and M response amplitude remained stable over the months of data collection. In those cases when change in EMG electrode function for a given channel (i.e., LG, MG, or SOL) made it difficult for the animal to maintain the required level of background EMG on that channel, the background EMG criteria for that channel were altered so that the animal could continue to work; however, determination of a leg's H-reflex size was based only on those channels in which background EMG level did not change.

Once an animal learned the task, data were collected over 3–6 mo. For the first 10–20 days the animal continued to perform the task under the control mode, in which reward followed every H-reflex elicitation. These data established control H-reflex sizes for each muscle in each leg. Then the animal worked for ≥50 days under the HRdown mode, in which reward occurred only if the absolute value of EMG during the H-reflex interval (typically 12–20 ms after stimulus onset) in each of the three muscles of one leg (the trained leg) was less than a criterion value. The three criterion values were selected on the basis of the control mode data so that reward occurred on 50–70% of the trials. As H-reflex size fell over days and weeks and reward probability rose, the criterion values were repeatedly reduced to keep reward probability at ~60%. It is important to note that both trained and control legs received comparable stimulation. The difference between the two legs was that under the HRdown mode the trained leg's H-reflexes determined whether reward occurred, whereas the control leg's H-reflexes were simply measured. Also, as noted above, background EMG and M response amplitude were kept stable over the months of data collection.

All animals remained healthy and active from electrode implantation to terminal intracellular study. Over this period, body weight and calf and thigh circumferences remained stable or increased. Leg circumferences remained symmetrical. At autopsy, the mean TS muscle weight in the trained leg was 102 ± 11% (mean ± SD) and 101 ± 4% (mean ± SD) that in the control leg for successful and unsuccessful animals, respectively. These results were consistent with findings in five NV animals, in which the mean interleg weight difference was only 6 ± 7% of each animal's mean TS muscle weight. Postmortem examination of the nerve cuffs revealed the expected connective tissue investment of the fine-wire electrodes and good preservation of the nerve within this connective tissue sheath.

TABLE 1. *Final H-reflex sizes*

	Final H-Reflex Size		
	Trained Leg	Control Leg	Weight, kg
Successful animals	24	77	6.4
	38	159	8.2
	43	71	5.9
	45	77	6.1
	47	76	6.7
	58	285	5.1
Unsuccessful animals	92	—	5.5
	101	141	4.0
	103	128	5.1
	104	—	4.0
	114	129	4.9
All successful animals	43 ± 11	124 ± 86	6.4 ± 1.1
All unsuccessful animals	103 ± 8	133 ± 7	4.7 ± 0.7

Values for all successful animals and all unsuccessful animals are means ± SD. Final H-reflex is expressed as a percent of the average control period value for individual animals. Body weight is that recorded on the day of chronic electrode implantation.

Final H-reflex size for each leg was determined by averaging each muscle's daily H-reflex size for the final 20 days, expressing the result as a percentage of the muscle's average H-reflex size for the control mode period and then averaging the results for the three muscles. Thus a value of 100% indicated no change in the leg's H-reflex.

Table 1 shows for each animal final H-reflex sizes (i.e., average values for the last 20 days of HRdown conditioning as percent of control mode values) in trained and control legs and body weight at the time of chronic electrode implantation. Animals are listed according to the magnitude of H-reflex decrease in the trained leg. They fall into two distinct groups. The first six animals were successful; final H-reflex size in the trained leg was 24–58% of its control mode size. The remaining five animals were unsuccessful; final H-reflex size in the trained leg was 92–114% of its control mode size. This division corresponded well with our standard criterion (based on observation of the range of spontaneous variation in H-reflex size), according to which successful conditioning is defined as a change of ≥20% in the appropriate direction. It also matched the division evident in a much larger population of HRdown animals (Wolpaw et al. 1993).[1] The average final values

[1] Comparisons of the distribution of the final H-reflex sizes in 26 HRdown animals with those predicted by least-square fits to bimodal or unimodal normal distributions indicated that the distribution was drawn from two different populations ($\chi^2 = 4.4$, df = 11, $P > 0.95$ for observed vs. predicted bimodal) rather than from a single population ($\chi^2 = 22.2$, df = 11, $P < 0.03$ for observed vs. predicted unimodal). The predicted means and standard deviations for the bimodal fit were 51 ± 18% and 111 ± 5% and thus corresponded well to the mean values for successful and unsuccessful animals of the present smaller population (i.e., Table 1). On the basis of the bimodal fit, the probability that an animal classified as successful in Table 1 was actually a member of the unsuccessful group is <0.0001, whereas the probability that an animal classified as unsuccessful in Table 1 was actually a member of the successful group is 0.01.

(±SD) for each group are shown at the *bottom*. The accompanying average weights display the strong positive relationship between probability of success in HRdown conditioning and body weight that was also present in a larger population (Wolpaw et al. 1993). This relationship could not be ascribed to other factors (such as differences in background EMG or initial H-reflex size).

As Table 1 also shows, final H-reflex sizes in the control legs varied widely and did not correlate with trained leg behavior. These findings of great variation in final control leg H-reflex size and lack of correlation with the final trained leg H-reflex were also noted in a much larger population (Wolpaw et al. 1993). The only difference was that the average final H-reflex size in the control leg was higher in the present population (i.e., 127%) than in the larger population (i.e., 108%), owing primarily to the single very high value. As previously indicated, H-reflexes in trained and control legs were elicited and measured simultaneously throughout conditioning, but reward depended only on H-reflex size in the trained leg.

Animal preparation for intracellular study

At the end of HRdown mode exposure the animal was deeply anesthetized for terminal intracellular studies of TS motoneuron properties and Ia EPSPs. Anesthesia was induced with ketamine (7 mg/kg im) accompanied by atropine (0.03 mg/kg) and then deepened and maintained with pentobarbital (15 mg/kg iv initially and then 4–6 mg/kg iv every 30 min). An endotracheal tube was inserted to maintain airway patency and the brachial vein was cannulated for fluid replacement and drug administration. Animals were supported in a rigid frame by stereotaxic headholder, hip pins, and clamps on the T_{10}–T_{11} spinous processes and both knees. A urinary catheter was inserted. Throughout the experiment heart rate, expired CO_2, and urine volume, specific gravity, and pH were monitored and deep surgical anesthesia [verified repeatedly by lack of response to vigorous palpebral or tracheal stimulation and by lack of respiratory or cardiac response to surgery (Green 1979; Steffey 1983)] was maintained. At the end of study the anesthetized animal was killed by an overdose of intravenous pentobarbital.

An L_1–L_6 dorsal laminectomy and dural incision gave access to the spinal cord. The lumbosacral dentate ligaments were cut and the L_5–L_7 spinal cord segments were lifted ~4 mm above the vertebral column on a plastic platform to reduce respiratory and cardiac movement artifacts. The nerves innervating the MG, LG, and SOL muscles were dissected from surrounding tissue in both hindlimbs, cut, and placed on bipolar stimulating electrodes. The cord dorsum potential evoked by peripheral nerve stimulation was recorded with a monopolar electrode at the dorsal root entry zone. Mineral oil pools made by lifting and retracting the cut skin edges of the incisions in the back and legs were kept at 37–39°C by heat lamps.

After this preparation and just before the beginning of intracellular study, a bilateral pneumothorax was created and the animal was placed on a positive-pressure respirator that used room air. Respiratory rate and volume were set to keep expired CO_2 at or slightly below prepneumothorax levels (typically 29–33 mmHg). Neuromuscular blockade was established with intravenous gallamine triethiodide (3 mg/kg initially and then 1.5 mg/kg every 30 min). Because neuromuscular blockade prevented assessment of motor response to painful stimulation, continuation of deep surgical anesthesia was ensured by continuing the established pentobarbital dosage schedule and by monitoring heart rate and pupillary light reflex (Committee on Pain and Distress in Laboratory Animals 1992). On the few occasions when heart rate increased or a pupillary light reflex was observed, pentobarbital dosage was increased. At the same time as creation of the pneumothorax, hourly intravenous administration of dexamethasone (0.25 mg/kg) was begun to reduce spinal cord edema over the 18- to 24-h period of intracellular study. In previous studies of NV animals (Carp 1992) this use of dexamethasone had no detectable effects on motoneuron physiological properties.

Physiological measurements

Intracellular recordings from motoneurons on both sides of the spinal cord were performed with glass micropipettes filled with 3 M potassium acetate and 0.01 M KCl and having DC resistances of 2–8 MΩ. Motoneurons were included in this study only if they had an antidromically or current-evoked (0.5-ms pulse duration) action potential of ≥60 mV and a stable resting membrane potential. Motoneuron recording normally began on the right side and switched sides once (5 animals) or twice (6 animals) over the 18–24 h of study. Previous studies (Carp 1992) indicated that motoneuron properties are not dependent on the time of recording.

For each motoneuron we set out to measure axonal conduction velocity, current and voltage thresholds, input resistance, time constant, electrotonic length, afterhyperpolarization (AHP) characteristics, and composite homonymous and heteronymous EPSPs to peripheral nerve stimulation. Deviation from the acceptance criteria (e.g., loss of resting membrane potential or decrease in action potential amplitude) sometimes precluded obtaining a full set of measurements from a motoneuron. Thus the RESULTS section indicates for each motoneuron population and for each measurement how many neurons were actually measured.

Conduction velocity was calculated from the conduction path distance (measured along the nerve postmortem) and the antidromic action potential latency.

The AHP after the action potential was defined by the average of 50 action potentials elicited by short (0.5 ms) current pulses. From this average two measurements were obtained: maximum amplitude, defined as the difference between the resting potential and the most negative membrane potential during the AHP; and duration, defined as the time between the repolarization of the action potential to the resting potential and the recovery from the AHP to within 2 SD of the resting potential.

Input resistance and membrane time constant were calculated from averages of 75–125 voltage responses to two to six different small-amplitude (0.5–3.0 nA) 100-ms hyperpolarizing current pulses. Input resistance was defined as the ratio of the maximal membrane potential deflection during the current pulse to the amplitude of the current pulse. Membrane time constant was determined from the membrane potential trajectory after the current pulse by a curve-peeling method (Carp 1992) based on that of Zengel et al. (1985). The slowest voltage decay component (excluding the membrane potential "sag" back toward its resting level) was assumed to reflect the motoneuron membrane time constant. The membrane time constant and the next faster decay constant determined by the curve-peeling method were used to estimate electrotonic length, using a method based on Rall's equivalent cylinder model of neuronal electrotonic behavior for sealed-end boundary conditions (Rall 1969). Voltage response averages were obtained as soon as the membrane potential and action potential amplitude stabilized after cell penetration and when possible were obtained again at the end of the cell's recording session. In cases in which a second set of averages was obtained, its values for input resistance and time constant were averaged with those of the first set to provide final values.

To determine the absolute voltage threshold for action potential initiation, the depolarization from resting potential required for action potential initiation was calculated as the difference between the action potential amplitude elicited by a short (0.5 ms) current pulse and that elicited by a long (100 ms) current pulse (Gustafsson and Pinter 1984). The absolute voltage threshold was then

calculated as the sum of this depolarization and the resting membrane potential. Because of the dependence of action potential amplitude on resting membrane potential (Carp 1992), threshold was calculated only for cells in which the resting membrane potentials for long and short current pulse trials differed by ≤1 mV. Rheobase was defined as the amplitude of a long (100 ms) current pulse that elicited an action potential in 50% of the trials.

Homonymous and heteronymous EPSPs were evoked by stimulation of individual TS muscle nerves at 0.5 Hz with 0.05-ms square pulses. To ensure that the maximum EPSPs were recorded, averages of 15–25 responses were recorded at several stimulus intensities usually ranging from 1.5 to 3.0 times that producing a just-threshold response at the cord dorsum electrode. The following measurements were obtained from the maximum EPSPs: amplitude, risetime (i.e., the time required to rise from 10 to 90% of the peak amplitude), half-width (i.e., EPSP width at half of the peak amplitude), intraspinal latency (i.e., the time from cord dorsum potential onset until EPSP onset), and maximum afferent conduction velocity (i.e, the ratio of the conduction distance to the onset latency of the cord dorsum potential). In addition, the aggregate EPSP amplitude was determined either by measurement of the response to simultaneous stimulation of all three TS nerves (71 motoneurons) or by prediction from the individual EPSPs (125 motoneurons). Evaluation of the data from 69 motoneurons in which EPSPs were elicited by both individual and simultaneous stimulation showed that the aggregate EPSP could be predicted accurately for the 79 motoneurons for which the homonymous EPSP and both heteronymous EPSPs were available ($r^2 = 0.98$), for the 15 motoneurons for which the homonymous EPSP and one heteronymous EPSP were available ($r^2 = 0.93$), and for the 31 motoneurons for which only the homonymous EPSP was available ($r^2 = 0.88$).

Data analysis

The goal of this study was to determine whether operantly conditioned H-reflex decrease in the trained leg was associated with one or more alterations in motoneuron properties or primary afferent EPSPs on the trained side and/or on the other (i.e., control) side. Thus the motoneurons studied fell into five groups according to whether the animal had been conditioned and, if it was a conditioned animal, the side from which the motoneuron came (i.e., trained or control) and the animal's behavioral performance (i.e., successful or unsuccessful, Table 1). These five motoneuron groups were: NV, trained side of successful HRdown animals (DT+), control side of successful HRdown animals (DC+), trained side of unsuccessful HRdown animals (DT−), and control side of unsuccessful HRdown animals (DC−).

DT+, DC+, DT−, and DC− motoneurons were compared with NV motoneurons by a nested analysis of variance with group as the main factor, animals nested within groups, and motoneurons nested within animals (Zar 1984). This analysis allowed us to compare motoneuron groups despite the fact that a different population of animals contributed to each group and interanimal variation was present for some properties.[2] Comparisons for which $P < 0.01$ were considered to indicate significant differences, and those for which $P < 0.05$ but >0.01 were noted.

RESULTS

Description of intracellular database

In the 11 HRdown animals, data were gathered from 221 TS motoneurons that had action potentials ≥60 mV and stable resting potentials. Of the 117 motoneurons from successful HRdown animals, 69 were from the trained side (DT+) and 48 were from the control side (DC+). Of the 104 motoneurons from unsuccessful HRdown animals, 66 were from the trained side (DT−) and 38 were from the control side (DC−). The data from these four groups (i.e., DT+, DC+, DT−, and DC−) were compared with previously reported data from a fifth group of 109 NV motoneurons from unconditioned animals (Carp 1992, 1993).

Evaluation of the NV motoneuron data ruled out several factors that could conceivably have complicated comparison of these five groups of motoneurons. First, motoneurons studied early in the 18- to 24-h recording session were no different from motoneurons studied later (when the spinal cord could have been less healthy because of prolonged deep anesthesia and multiple electrode penetrations). Second, properties of motoneurons from right and left sides were not significantly different. The lack of right-left differences in motoneuron properties (Carp 1992) indicates that our consistent use of the right leg as the trained leg in conditioned animals was not a confounding factor for data analysis. Third, motoneuron properties in NV animals were not significantly related to body weight [$P > 0.05$ for linear regressions of each motoneuron property (average of all motoneurons in each animal) on body weight]. The NV animal weight range (4.5–9.3 kg) nearly encompassed the range of the present HRdown animals (4.0–8.2 kg). Thus, although Table 1 shows that successful and unsuccessful HRdown animals differed in average body weight, this weight difference should not have interfered with the comparison of motoneurons from the two groups of animals.

Although no physiological criteria have been established for distinguishing between α- and γ-motoneurons in primates, γ-motoneurons in cats do not receive monosynaptic inputs (Baldissera et al. 1981). Because EPSPs of monosynaptic latency were elicited in all motoneurons in which they were sought, including a motoneuron with a conduction velocity of 39 m/s, it is probable that the motoneuron population described here consisted largely, if not exclusively, of α-motoneurons.

Physiological properties of DT+, DC+, DT−, DC−, and NV motoneurons

The purpose of this study was to determine whether HRdown conditioning affected TS motoneuron properties and, if effects were found, to determine whether any of them were associated with the conditioning-induced change in behavior (i.e., with H-reflex decrease in the trained leg). Thus the analysis compares the data from DT+, DT−, DC+, and DC− motoneurons with data from NV motoneurons.

Table 2 shows for gastrocnemius (LG/MG) motoneurons and SOL motoneurons in each of the five groups (i.e., DT+, DT−, DC+, DC−, and NV) the average value and standard deviation of each parameter and the number of cells in which that parameter was measured. LG and MG motoneurons are presented together because earlier analysis of NV motoneurons (Carp 1992, 1993) did not reveal any significant differences between them.

Comparisons of DT+, DT−, DC+, and DC− motoneu-

[2] A number of properties, i.e., conduction velocity, input resistance, length constant, and EPSP halfwidth/time constant, varied significantly across NV animals (analysis of variance, $P < 0.05$).

TABLE 2. *Physiological properties of gastrocnemius and soleus motoneurons from conditioned and naive animals*

	Gastrocnemius Motoneurons					Soleus Motoneurons				
Property	NV	DT+	DC+	DT−	DC−	NV	DT+	DC+	DT−	DC−
Conduction velocity, m/s	73 ± 7 (70)	69 ± 5 (42)	72 ± 4 (35)	71 ± 5 (46)	71 ± 6 (31)	68 ± 6 (39)	63 ± 5 (27)	62 ± 9 (13)	68 ± 7 (20)	65 ± 8 (7)
Input resistance, MΩ	0.9 ± 0.4 (59)	0.9 ± 0.3 (37)	0.8 ± 0.3 (30)	1.0 ± 0.3 (40)	0.9 ± 0.4 (28)	1.2 ± 0.5 (35)	1.5 ± 0.4 (22)	1.4 ± 0.4 (12)	1.4 ± 0.6 (19)	1.6 ± 0.4 (6)
Time constant, ms	4.3 ± 1.4 (51)	4.1 ± 0.9 (37)	4.5 ± 1.3 (29)	4.6 ± 1.7 (36)	3.7 ± 1.1 (26)	5.1 ± 1.8 (33)	5.6 ± 1.3 (22)	5.8 ± 1.0 (10)	4.6 ± 1.6 (19)	5.4 ± 0.5 (5)
Rheobase, nA	13 ± 7 (52)	15 ± 7 (33)	14 ± 7 (27)	13 ± 7 (38)	15 ± 6 (28)	10 ± 6 (32)	8 ± 5 (19)	7 ± 3 (10)	11 ± 10 (18)	6 ± 2 (5)
Electrotonic length, λ	1.3 ± 0.2 (51)	1.4 ± 0.1 (36)	1.4 ± 0.1 (29)	1.4 ± 0.2 (36)	1.4 ± 0.1 (24)	1.4 ± 0.1 (31)	1.4 ± 0.1 (22)	1.3 ± 0.1 (10)	1.4 ± 0.1 (18)	1.3 ± 0.1 (5)
Resting potential, mV	−66 ± 5 (70)	−65 ± 6 (42)	−66 ± 7 (35)	−65 ± 6 (45)	−66 ± 4 (30)	−66 ± 7 (39)	−66 ± 5 (26)	−68 ± 4 (13)	−68 ± 5 (20)	−65 ± 5 (7)
Firing threshold, mV	−55 ± 5 (39)	−52 ± 6 (29)	−55 ± 7 (23)	−55 ± 4 (33)	−53 ± 4 (23)	−56 ± 6 (21)	−54 ± 4 (16)	−58 ± 4 (9)	−57 ± 5 (17)	−53 ± 3 (5)
Action potential amplitude, mV	75 ± 6 (70)	74 ± 7 (42)	74 ± 7 (35)	73 ± 6 (46)	74 ± 6 (31)	75 ± 7 (39)	75 ± 8 (27)	77 ± 6 (13)	76 ± 6 (20)	73 ± 7 (7)
AHP duration, ms	75 ± 23 (44)	69 ± 17 (31)	75 ± 25 (25)	79 ± 25 (35)	77 ± 23 (25)	86 ± 34 (22)	110 ± 39 (17)	102 ± 20 (10)	106 ± 34 (18)	106 ± 39 (5)
AHP amplitude, mV	−3.4 ± 1.3 (44)	−3.1 ± 0.9 (31)	−2.7 ± 0.9 (25)	−3.0 ± 1.0 (35)	−2.8 ± 0.9 (25)	−3.5 ± 1.5 (22)	−3.1 ± 0.9 (17)	−3.0 ± 1.0 (10)	−3.5 ± 1.4 (18)	−2.5 ± 1.2 (5)
Homonymous EPSP amplitude, mV	2.1 ± 1.3 (40)	1.4 ± 0.9 (22)	1.9 ± 1.1 (19)	2.4 ± 2.0 (28)	1.0 ± 0.8 (22)	4.4 ± 1.9 (18)	4.6 ± 2.5 (15)	4.7 ± 2.2 (11)	7.1 ± 2.9 (15)	2.4 ± 1.5 (4)
EPSP risetime / Time constant	0.18 ± 0.07 (38)	0.20 ± 0.06 (19)	0.16 ± 0.06 (19)	0.18 ± 0.05 (26)	0.19 ± 0.10 (20)	0.18 ± 0.08 (16)	0.15 ± 0.04 (15)	0.14 ± 0.04 (10)	0.19 ± 0.09 (16)	0.13 ± 0.05 (4)
EPSP half-width / Time constant	0.83 ± 0.24 (38)	0.69 ± 0.24 (19)	0.71 ± 0.18 (19)	0.77 ± 0.20 (26)	0.86 ± 0.38 (20)	0.84 ± 0.36 (16)	0.71 ± 0.22 (15)	0.73 ± 0.16 (10)	0.88 ± 0.31 (16)	0.69 ± 0.23 (4)
Maximum afferent conduction velocity, m/s	91 ± 5 (37)	89 ± 4 (13)	90 ± 4 (18)	92 ± 3 (26)	92 ± 5 (15)	90 ± 5 (12)	88 ± 2 (10)	89 ± 5 (9)	96 ± 4 (13)	94 ± 3 (3)
Intraspinal latency, ms	0.9 ± 0.2 (37)	0.8 ± 0.3 (12)	0.8 ± 0.2 (18)	0.8 ± 0.2 (26)	0.8 ± 0.2 (14)	0.9 ± 0.1 (12)	0.9 ± 0.1 (10)	0.8 ± 0.2 (9)	0.8 ± 0.1 (12)	1.1 ± 0.1 (3)
Aggregate EPSP amplitude, ms	3.5 ± 2.0 (41)	2.9 ± 1.7 (22)	3.4 ± 1.6 (19)	4.3 ± 2.4 (28)	1.9 ± 1.1 (23)	5.2 ± 2.0 (18)	5.1 ± 2.3 (15)	5.4 ± 2.2 (11)	8.3 ± 2.9 (15)	4.0 ± 2.5 (4)

Values are means ± SD, with number of motoneurons measured in parentheses. NV, naive animals; DT+, trained side of successful conditioned animals; DC+, control side of successful conditioned animals; DT, trained side of unsuccessful conditioned animals; DC−, control side of unsuccessful conditioned animals; AHP, afterhyperpolarization; EPSP, excitatory postsynaptic potential.

rons with NV motoneurons on the basis of the data as presented in Table 2 would have been complicated by differences between LG/MG and SOL motoneurons and the different proportions of the two types in each group. To avoid this complication we expressed each motoneuron's data in units of the average results obtained for all NV motoneurons of the same type. For example, a SOL motoneuron with a conduction velocity of 70 m/s would have a normalized conduction velocity of 1.03 (i.e., 3% faster than the average NV SOL conduction velocity of 68 m/s), whereas an LG motoneuron with the same conduction velocity would have a normalized conduction velocity of 0.96 (i.e., 4% slower than the average NV LG/MG conduction velocity of 73 m/s). This normalization allowed the data from all motoneurons in a group to be combined. Table 3 presents the means ± SE of these normalized data for each of the five groups. By definition, the mean values for NV motoneurons are all unity. For DT+, DC+, DT−, and DC− motoneuron groups, differences from the NV group are indicated by asterisks (2 for $P < 0.01$, 1 for $P < 0.05$).

DT+ and DT− motoneurons

Table 3 shows that DT+ motoneurons differed from NV motoneurons in firing threshold and conduction velocity ($P < 0.01$ for both). These differences were evident in both LG/MG and SOL motoneurons (i.e., Table 2). Threshold was 6% (i.e., 3 mV) more positive in DT+ motoneurons than in NV motoneurons. In contrast, resting membrane potential was comparable in DT+ and NV motoneurons. As a result of the difference in firing threshold and the lack of difference in resting potential, the depolarization required to reach threshold was 18% higher in DT+ than in NV motoneurons. An analysis of covariance that took into

TABLE 3. *Normalized physiological properties of motoneurons from conditioned and NV animals*

Property	NV	DT+	DC+	DT−	DC−
Conduction velocity	1.00 ± 0.01	0.94 ± 0.01†	0.97 ± 0.01	0.98 ± 0.01	0.97 ± 0.01
Input resistance	1.00 ± 0.05	1.08 ± 0.04	0.98 ± 0.05	1.08 ± 0.05	1.01 ± 0.07
Time constant	1.00 ± 0.04	1.01 ± 0.03	1.06 ± 0.04	1.01 ± 0.05	0.89 ± 0.04
Rheobase	1.00 ± 0.06	1.00 ± 0.07	0.95 ± 0.08	1.00 ± 0.10	1.07 ± 0.08
Electronic length	1.00 ± 0.01	1.02 ± 0.01	1.01 ± 0.01	1.01 ± 0.02	1.05 ± 0.02
Resting potential	1.00 ± 0.01	0.99 ± 0.01	1.01 ± 0.01	1.00 ± 0.01	0.99 ± 0.01
Firing threshold	1.00 ± 0.01	0.94 ± 0.01†	1.00 ± 0.02	1.01 ± 0.01	0.96 ± 0.01*
Action potential amplitude	1.00 ± 0.01	0.99 ± 0.01	1.00 ± 0.01	0.99 ± 0.01	0.99 ± 0.01
AHP duration	1.00 ± 0.04	1.05 ± 0.05	1.06 ± 0.05	1.12 ± 0.05	1.07 ± 0.06
AHP amplitude	1.00 ± 0.05	0.89 ± 0.04	0.79 ± 0.04*	0.92 ± 0.05	0.80 ± 0.05*
Homonymous EPSP amplitude	1.00 ± 0.07	0.83 ± 0.08	0.97 ± 0.09	1.32 ± 0.14	0.51 ± 0.07*
$\frac{\text{EPSP risetime}}{\text{Time constant}}$	1.00 ± 0.06	0.98 ± 0.06	0.86 ± 0.05	1.00 ± 0.04	1.02 ± 0.11
$\frac{\text{EPSP half-width}}{\text{Time constant}}$	1.00 ± 0.05	0.84 ± 0.05	0.86 ± 0.04	0.97 ± 0.05	1.00 ± 0.09
Maximum afferent conduction velocity	1.00 ± 0.01	0.97 ± 0.01	0.99 ± 0.01	1.03 ± 0.01	1.02 ± 0.01
Intraspinal latency	1.00 ± 0.02	0.97 ± 0.05	0.93 ± 0.05	0.91 ± 0.03	0.98 ± 0.07
Aggregate EPSP amplitude	1.00 ± 0.07	0.88 ± 0.08	0.99 ± 0.08	1.34 ± 0.10	0.57 ± 0.07*

Values are means ± SE. For each property, the statistical significance of differences between NV mean values and DT+, DC+, DT−, or DC− mean values is indicated by * ($P < 0.05$) or † ($P < 0.01$). For abbreviations, see Table 2.

account the known dependence of this depolarization on resting potential (Carp 1992; Gustafsson and Pinter 1984) found the increase to be significant ($P < 0.01$).

Firing threshold was determined from the difference in amplitude between action potentials elicited by short and long current pulses (see METHODS). The voltage level at which action potentials are initiated during long current pulses is time dependent in that longer latencies to spike onset are associated with more depolarized thresholds (Burke and Nelson 1971; Gustafsson and Pinter 1984). In the present data, the average latency to spike discharge during long current pulses was slightly longer in DT+ motoneurons than in NV motoneurons (17 ± 14 ms, mean ± SD, vs. 14 ± 12 ms, mean ± SD, respectively). However, when this difference in spike latency was taken into account there was still a significant difference in threshold between NV and DT+ motoneurons ($P < 0.01$ by analysis of covariance, using action potential latency as the covariate). Thus this small difference in spike latency did not account for the observed difference in firing threshold between NV and DT+ motoneurons.

DT+ motoneurons also differed from NV motoneurons in axonal conduction velocity. Conduction velocity was 6% slower in DT+ than in NV motoneurons. This corresponded with an average decrease of ~4 m/s.

For both firing threshold and conduction velocity of DT+ motoneurons, the by-animal average values [0.94 ± 0.05 (SD) for firing threshold and 0.94 ± 0.06 (SD) for conduction velocity] were identical to the by-motoneuron average values shown in Table 3.

In contrast with DT+ motoneurons, DT− motoneurons were not significantly different from NV motoneurons in firing threshold, conduction velocity, or any other motoneuron property. In the group data of Table 3, mean normalized DT− firing threshold is 1.01 and DT− conduction velocity is 0.98. The by-animal average values were comparable [i.e., 1.00 ± 0.02 (SD) and 0.98 ± 0.04 (SD), respectively]. Thus the changes noted in DT+ motoneurons were not nonspecific effects of the HRdown conditioning task. They were clearly associated with successful HRdown conditioning. These results suggest that final H-reflex size in the trained leg of successful HRdown animals was smaller because these motoneurons were farther from firing threshold when the Ia afferent volley elicited by the nerve cuff stimulus arrived.

The values in Table 3 for homonymous and aggregate EPSPs of DT− motoneurons are quite high. However, these values were largely due to one animal, and statistical analysis did not reveal any difference from NV values ($P > 0.6$ for both).

DC+ and DC− motoneurons

In contrast with DT+ motoneurons, DC+ motoneurons (i.e., those innervating the TS muscles on the control side in successful animals) were not significantly different from NV motoneurons. Average firing threshold was identical with that of NV motoneurons. Average conduction velocity differed in the same direction as did that of DT+ motoneurons, but the difference was smaller and did not attain statistical significance.

Table 3 suggests ($P < 0.05$) that DC− motoneurons had homonymous and aggregate EPSPs that were smaller than those of NV animals. Additional analysis indicated that this effect could not be attributed to differences in resting potential or input resistance between NV and DC− motoneurons ($P < 0.001$ by analysis of covariance of EPSP amplitude, using input resistance and resting potential as covariates). The by-animal mean values [0.55 ± 0.28 (SD) for homonymous EPSPs and 0.59 ± 0.28 (SD) for aggregate EPSPs] corresponded closely with the group means in Table 3. Table 3 also suggests that firing threshold was more positive in DC− motoneurons. The by-animal mean value [0.95 ± 0.02 (SD)] was comparable with the group mean.

AHP amplitude tended to be smaller in both DC+ and DC− motoneurons than in NV motoneurons ($P < 0.05$). The fact that AHP amplitude was also somewhat low in DT+ and DT− motoneurons (i.e., Table 3) suggests that

this reduction reflected a nonspecific effect of the HRdown operant conditioning task.

DISCUSSION

Our central goal is to define the neuronal and/or synaptic changes responsible for operantly conditioned change in H-reflex size. In accord with this purpose, the aim of this study was to determine whether operantly conditioned decrease in TS H-reflex size was associated with any changes in the intrinsic properties of TS motoneurons or in their primary afferent EPSPs. The results indicate that H-reflex decrease is accompanied by more positive firing threshold and lower axonal conduction velocity.

These changes are specific to motoneurons on the trained side of successful HRdown animals. They are not found in motoneurons from the trained side of unsuccessful HRdown animals. Thus they cannot be attributed to nonspecific effects of the chronic conditioning protocol or the acute surgical preparation and intracellular recording procedures.

The occurrence of these changes and their association with successful HRdown conditioning raises three major issues. First, are these changes detected in the anesthetized animal responsible for the behavioral change (i.e., a markedly smaller H-reflex) seen in the awake behaving animal? Second, how might they be produced? Third, how do they relate to the other spinal and supraspinal changes detected by earlier studies?

Firing threshold and H-reflex size

A change in firing threshold to a more positive level, in the absence of corresponding change in resting potential, increases the voltage change needed to reach that threshold and thereby increases the size of the smallest EPSP able to trigger an action potential. EPSPs that were just large enough to reach threshold before HRdown conditioning are not large enough after conditioning. As a result fewer motoneurons fire and the H-reflex is smaller. Thus the change in threshold found in DT+ motoneurons in the anesthetized animal is qualitatively consistent with the H-reflex decrease seen in the awake behaving animal. The effect on H-reflex size is presumably enhanced by the slight decrease in DT+ aggregate EPSP amplitude (Table 3).[3]

At the same time, the degree to which findings in the anesthetized animal translate to the awake behaving animal is not clear. In the awake behaving animal TS background EMG is present, so that a certain number of motoneurons are firing steadily and the average resting potential is doubtless more positive than the average resting potential in the anesthetized animal. On the other hand, in the awake animal the EPSP is almost certainly smaller than the maximum EPSP measured in the anesthetized animal because stimulus intensity is lower (i.e., near M response threshold)

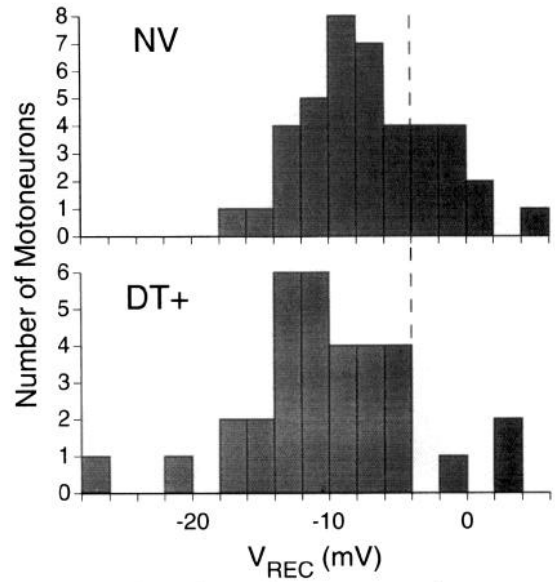

FIG. 1. Distributions of V_{REC} [i.e., aggregate excitatory postsynaptic potential (EPSP) plus resting potential minus firing threshold] for naive (NV) motoneurons and trained side of successful HRdown animals (DT+) motoneurons. Values are given for all NV and DT+ motoneurons in which resting potential, firing threshold, and aggregate EPSPs were available. The mean values were -7.2 ± 4.8 (SD) mV for NV motoneurons and -10.3 ± 6.2 (SD) mV for DT+ motoneurons. The distributions are significantly different by t test ($P < 0.02$). As explained in the text, motoneurons to the *right* of the dashed line at -4.0 mV would be expected to contribute to the H-reflex in the awake, behaving animal.

and because the EPSP is shunted by the greater membrane conductance accompanying tonic motoneuron activity. Nevertheless, by combining the present intracellular data with the EMG data from the behaving animal, we can attempt to estimate the probable effect of the change in firing threshold observed in the anesthetized animal on the H-reflex seen in the awake behaving animal.

As indicated above, a cell will fire if the EPSP plus the resting potential minus the firing threshold is >0. This quantity reflects the likelihood of recruitment and is here abbreviated V_{REC}. Figure 1 shows the distributions of V_{REC} for NV and DT+ motoneurons. (The EPSP amplitude used to calculate V_{REC} for each motoneuron was the aggregate EPSP amplitude.) Our previous measurements of H-reflexes and maximum M responses indicate that, in the control mode, H-reflex size averages 6% of maximum possible size (i.e., size if every motoneuron were recruited by the nerve cuff stimulus). According to available data on the relationship between EMG amplitude and motoneuron recruitment (Stein and Yang 1990), 6% of maximum EMG amplitude indicates 25% motoneuron recruitment (i.e., because smaller motoneurons are recruited first). As the data in Fig. 1 indicate, 25% of NV motoneurons have values of V_{REC} that are greater than -4.0 mV. This result suggests that an NV motoneuron will participate in the H-reflex of the awake behaving animal if its value of V_{REC} *under anesthesia* is greater than -4.0 mV. That the necessary value of V_{REC} is <0 is presumably due to awake/anesthetized differences in membrane potential and other factors as noted above. If we assume that this same V_{REC} requirement applies to motoneurons of HRdown animals, the V_{REC} values of DT+ motoneurons in Fig. 1 indicate that only 9% will

[3] Although this 12% decrease was not statistically significant, the ability to detect a difference was limited by the intrinsic variation in EPSP size with input resistance (Carp 1993). Analysis of covariance with input resistance as the covariate indicated that EPSP amplitudes in DT+ motoneurons tended to be lower than those in NV motoneurons ($P < 0.02$ and $P < 0.03$ for homonymous and aggregate EPSPs, respectively).

participate in the H-reflex in the awake behaving animal (i.e., for only 9% is V_{REC} greater than −4.0 mV). Because resting potential of DT+ motoneurons is comparable with that of NV motoneurons and EPSP size is only slightly less (i.e., Table 3), this difference in predicted recruitment is largely attributable to the more positive firing threshold of DT+ motoneurons. Converted into EMG amplitude (Stein and Yang 1990), the drop from 25 to 9% recruitment predicts that the H-reflex on the trained side of successful HRdown animals should be 32% of its control mode value.[4] This prediction is consistent with the observed value of 43% (i.e., Table 1).

Given the uncertain relationships between anesthetized and awake situations and the necessary use of EMG/recruitment data from non-TS muscles, this analysis must be considered very preliminary and tentative. Certainly a comprehensive effort to model the behavior of TS motoneurons after conditioning should be undertaken. Nevertheless, this initial effort supports the hypothesis that increase in motoneuron firing threshold is responsible for operantly conditioned decrease in H-reflex size.

The increase in firing threshold and consequent increase in the depolarization needed to reach that threshold were not accompanied by a corresponding increase in rheobase. The absence of a change in rheobase may be due to the fact that average input resistance was higher in DT+ motoneurons (i.e., Table 3), although this increase did not reach statistical significance. In addition, rheobase varied greatly across motoneurons (i.e., Tables 2 and 3), so that a small difference among motoneuron groups may have escaped detection by the present data set.

Conduction velocity

The most surprising result of this study was the decrease of 6% in antidromic conduction velocity that was noted only in DT+ motoneurons.[5] Because the motoneuron axon is half of the reflex pathway and some time is required for intraspinal conduction and synaptic activation, this decrease should have increased H-reflex latency by only ~0.5 ms. Although EMG was normally sampled at 2,000 Hz in the behaving animal, the amplitude measurements were subsequently averaged over 2-ms intervals. Thus our ability to detect such a small change was limited. Nevertheless, average results for trained leg H-reflexes of the six successful HRdown animals indicated that the time to the maximum value of the H-reflex increased from 15.1 ms before HRdown exposure to 15.8 ms at the end of exposure, a change consistent with the 6% decrease in motoneuron conduction velocity. In contrast, the time to maximum H-reflex value in the trained legs of unsuccessful animals was 14.3 ms before exposure and 14.0 ms at the end of exposure. The slightly shorter initial latency in unsuccessful animals is consistent with their smaller size (i.e., Table 1.)

Conduction velocity is a complex product of anatomic and physiological characteristics of the axon and its myelin sheath (Jack et al. 1983; Waxman 1980). Thus a large number of factors could account for the decrease observed in DT+ motoneurons. However, the concurrent increase in firing threshold suggests one particular explanation. If threshold is increased throughout the motoneuron, in the axon as well as in the soma, the depolarization produced at each node of Ranvier by the action potential at the adjacent node will have to proceed slightly further to reach threshold and generate its own action potential. The result should be that conduction time is prolonged. Thus the increase in firing threshold provides a qualitative explanation for the decrease in conduction velocity. Whether this explanation is also quantitatively plausible remains uncertain. Calculations based on a recent model of conduction velocity in myelinated mammalian axon (Halter and Clark 1991; Halter et al. 1993) suggest that the observed increase in firing threshold could account for the observed decrease in conduction velocity.

Although a uniform change in conduction velocity throughout the motoneuron axon is perhaps the simplest explanation for the present data, other possibilities deserve consideration. The decreased conduction velocity measured in DT+ motoneurons could also be produced by a change confined to certain parts of the axon (e.g., more proximal portions) or even to the initial segment alone. Measurements of conduction velocity over separate portions of the motor axon before, during, and after HRdown conditioning are needed to evaluate such possibilities.

Possible mechanisms of changes in motoneuron properties

Although the change in firing threshold provides a potential explanation for the change in conduction velocity, the origin of the change in firing threshold itself remains to be defined. Firing threshold is largely determined by the properties of sodium channels (Barrett and Crill 1980; Jack et al. 1983; Matzner and Devor 1992). Modeling studies suggest that a positive shift in sodium channel voltage dependence might underlie the change in firing threshold (Halter et al. 1993). Sodium channel function can be altered by protein kinases A and C (Dascal and Lotan 1991; Gershon et al. 1992; Numann et al. 1991; Schreibmayer et al. 1991; Smith and Goldin 1992). Furthermore, in vertebrate neurons, protein kinase C alters properties dependent on sodium channel function, including firing threshold and axonal conduction velocity (Meiri and Gross 1989; Szente et al. 1990; Zhang and Krnjevic 1987). Thus it is possible that the observed changes in threshold and conduction velocity are due to a protein kinase C-mediated alteration in sodium channel function. Although protein kinase C has been local-

[4] Comparable results were obtained using other data concerning the relationship between EMG amplitude and motoneuron recruitment (i.e., Brown et al. 1988; McComas et al. 1971; Sica et al. 1974). The results given here are derived from an average of the two plots in the *top graph* of Fig. 6 of Stein and Yang (1990). It should be noted that the present calculations of V_{REC} do not take into account the different proportions of LG/MG and SOL motoneurons in NV and DT+ motoneuron groups. Because the SOL proportion was somewhat larger for DT+ motoneurons and SOL motoneurons tended to have larger values of V_{REC}, the data presented in Fig. 1 slightly underestimate the predicted difference in recruitment between NV and DT+ motoneurons.

[5] Previous evaluation (Carp 1992) detected no significant right-left differences in conduction velocity (or any other motoneuron property). Nevertheless the surprising nature of the apparent effect of HRdown conditioning on conduction velocity and the fact that all DT+ motoneurons were from the right side prompted us to compare their conduction velocities with those of right-sided NV motoneurons. A comparable decrease (i.e., 5.5%) was still evident.

ized to spinal motoneurons (Hietanen et al. 1990), pathways and/or neuroactive substances that can affect protein kinase C in these neurons have yet to be defined. At the same time, it should be noted that some aspects of long-term potentiation, a commonly used model for associative plasticity, are thought to be mediated by protein kinase C (Ben-Ari et al. 1992; Colley and Routtenberg 1993).

Firing threshold might also be modified by changes in other voltage-dependent channels that affect membrane potential trajectory and thereby alter sodium channel activation indirectly. For example, spinal motoneurons possess a slowly activating inward current at ≥10 mV above resting potential (Schwindt and Crill 1980). However, further analysis of the present data showed that the extent to which firing threshold was elevated in DT+ over NV motoneurons did not differ as a function of the latency of action potentials generated by rheobase-level injected currents. The lack of a greater firing threshold difference between NV and DT+ motoneurons at longer latencies suggests that the change in firing threshold is not due to modification of this slowly activating inward current.

Evidence for additional changes associated with H-reflex conditioning

The observed change in firing threshold provides a plausible explanation for operantly conditioned H-reflex decrease. At the same time it raises other issues concerning the conditioning task specifically and the animal's behavior generally. The conditioning task requires the animal to maintain a certain level of TS background EMG activity. In the control mode, before imposition of the HRdown mode, the required level is presumably produced by TS motoneurons firing tonically in response to a particular balance of excitatory and inhibitory synaptic inputs. If HRdown conditioning increases the firing thresholds of these motoneurons without changing their resting potentials, the required level of background EMG will no longer be maintained unless the balance of excitatory and inhibitory inputs also changes. For example, excitatory synaptic input from particular supraspinal pathways might increase. Thus, because the change in threshold motivated by the HRdown reward contingency disturbs another part of task performance, other aspects of the CNS activity supporting that performance can be expected to change.[6]

Furthermore, the effects of altered TS motoneuron threshold are likely to extend beyond the conditioning task. TS motoneurons participate in many other behaviors, and modification of firing threshold is likely to alter the patterns of CNS activity underlying many of these behaviors as well. For example, because the two TS motoneuron populations are no longer functionally symmetrical (i.e., motoneurons on the trained side have acquired higher firing thresholds), their reciprocal participation in behaviors such as walking or climbing is likely to be disturbed unless compensatory changes occur in supraspinal control and/or in spinal interneuronal pathways.

The significantly smaller EPSPs and the possible change in firing threshold found in DC− motoneurons, i.e., those on the control side of unsuccessful animals, suggest that exposure to the HRdown task can produce changes not directly related to increasing reward probability. These changes might have resulted from the adoption by these unsuccessful animals of an improper, i.e., nonadaptive, response to the HRdown contingency.

Earlier studies provided evidence for plasticity at multiple sites. Monosynaptic TS nerve volley responses to supramaximal dorsal root stimulation were measured in NV and conditioned animals after anesthesia and thoracic cord transection removed all supraspinal control (Wolpaw and Lee 1989; Wolpaw et al. 1989). In HRdown animals, responses on the control side were larger than those on the HRdown side and in addition were larger than those in NV animals. Conversely, in HRup animals, responses on the control side were smaller than those on the HRup side and similar to or smaller than those in NV animals. Thus HRdown conditioning has effects on both sides of the spinal cord. The present study, which assessed the TS motoneuron and its primary afferent inputs from TS muscles, provides no explanation for the very large volley responses found on the control side of HRdown animals in the earlier study. Explanation is likely to lie elsewhere in the spinal cord.[7] One possibility is that H-reflex conditioning also affects non-TS monosynaptic excitatory pathways (or even disynaptic inhibitory pathways) to TS motoneurons. Such pathways were presumably excited by the supramaximal dorsal root stimulation used in the volley study, but not by the more specific stimulation of the present study. In addition, supramaximal dorsal root stimulation may have produced EPSPs large enough to reduce or eliminate the effect of the change in firing threshold.

The present data also provide no explanation for the very wide variation in final control leg H-reflex sizes evident in Table 1. Although the data are limited, V_{REC} calculation for DC+ and DC− motoneurons suggests that their recruitment into the H-reflex should be similar (i.e., 8 and 11%) to the 9% predicted for DT+ motoneurons. At the same time, V_{REC} predicts that recruitment of DT− motoneurons should be 45%, considerably higher than the 25% calculated for NV motoneurons, and inconsistent with the absence of behavioral change on the trained side of unsuccessful animals (i.e., Table 1). These differences between intracellular data obtained in the anesthetized animal and behavioral data obtained from the awake behaving animal probably result in large part from the fact that supraspinal influences

[6] An increase in tonic excitatory input that maintains background EMG despite the positive shift in firing threshold would presumably do so at least in part by increasing the slope of motoneuron depolarization. Thus it would not eliminate the effect of the altered threshold on H-reflex size, because at any random point in time the motoneuron would still be farther from firing threshold than it was before the threshold became more positive, and the aggregate Ia EPSP would therefore still be less likely to fire the motoneuron.

[7] Although deep pentobarbital anesthesia was present in both the earlier volley studies and the present intracellular study, transection of the thoracic spinal cord was performed only in the former. However, this difference in methodology is an unlikely explanation for the difference in results, because spinal transection in the anesthetized animal had no significant effect on the monosynaptic nerve volleys (Wolpaw and Lee 1987, 1989). This suggests that the deep pentobarbital anesthesia present in both studies was functionally equivalent to spinal cord transection.

present in the awake behaving animal were eliminated in the anesthetized animal.

Whether the changes evident on the control side do in fact reflect compensatory plasticity that reduces undesirable effects of the altered firing threshold on the trained side remains to be determined. Nevertheless, this contralateral plasticity, together with the plasticity likely to result from the obvious need to adjust task performance to continue to maintain correct background EMG, indicates that HRdown conditioning involves changes at more than one site in the CNS.

Conclusions

The results support the hypothesis that HRdown conditioning decreases the TS H-reflex by increasing the motoneuron firing threshold. This hypothesis is strengthened by the absence of altered threshold in animals in which conditioning failed to decrease the H-reflex. The decrease in axonal conduction velocity associated with successful HRdown conditioning may also result from the change in threshold. At the same time, H-reflex conditioning appears to be associated with plasticity at other sites as well. This additional plasticity may prevent the altered threshold from adversely affecting other behaviors.

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