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Motoneuron Properties After Operantly Conditioned Increase in Primate H-Reflex

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SUMMARY AND CONCLUSIONS

1. Monkeys can increase (HRup conditioning mode) or decrease (HRdown conditioning mode) the triceps surae (TS) H-reflex in response to an operant conditioning task. This conditioning modifies the spinal cord. To define this spinal cord plasticity and its role in the behavioral change (H-reflex increase or decrease), we have recorded intracellularly from TS motoneurons in conditioned animals. The present report describes data from HRup animals and compares them with data from previously studied naive (NV; i.e., unconditioned) animals.

2. Thirteen monkeys (*Macaca nemestrina*, male, 3.8–7.1 kg) were exposed to the HRup conditioning mode, in which reward occurred when H-reflex size in one leg (i.e., the trained leg) was above a criterion value. Conditioning was successful (i.e., increase of ≥20%) in 12 of the 13 animals. At the end of conditioning, H-reflex size in the trained leg averaged 188% of its initial value, whereas size in the control leg averaged 134% of its initial value.

3. Intracellular recordings were obtained from 136 TS motoneurons on trained (UT+ motoneurons) and control (UC+ motoneurons) sides of the successful animals. Measurements included axonal conduction velocity, input resistance, time constant, electrotonic length, rheobase, firing threshold to current injection, afterhyperpolarization duration and amplitude, and composite homonymous and heteronymous excitatory postsynaptic potential (EPSP) size and shape. Results were compared with intracellular data from NV animals.

4. Although HRup motoneurons displayed several significant differences from NV motoneurons, analysis of these and other intracellular properties alone or in combination did not explain the behavioral change evident in the awake behaving animal (i.e., a much larger H-reflex in the trained leg). Firing threshold to current injection was more positive (i.e., depolarized; $P < 0.01$) in UT+ motoneurons than in NV motoneurons and tended to be more negative ($P < 0.05$) in UC+ motoneurons than in NV motoneurons. Non-TS heteronymous EPSPs were much smaller in both UT+ and UC+ motoneurons than in NV motoneurons.

5. These results from HRup animals combined with earlier data from HRdown animals indicate that H-reflex increase under the HRup mode and H-reflex decrease under the HRdown mode are not mirror images of each other, but rather depend on different mechanisms. Although conditioned H-reflex decrease appears ascribable to changes in motoneuron properties and EPSPs, conditioned H-reflex increase is due either to motoneuron or EPSP changes that went undetected by the present intracellular methods or to changes elsewhere in the spinal cord. One possibility is that H-reflex increase was caused by a negative shift in threshold to synaptic input that was not accompanied by a corresponding shift in threshold to current injection. A second possibility is that HRup increase was caused by a change in the interneurons that mediate group I disynaptic input from TS muscles to TS motoneurons. Such disynaptic input could reach the motoneuron quickly enough to affect the H-reflex.

6. The intracellular data from HRup animals add to the evidence indicating that both HRup and HRdown conditioning involve changes at multiple spinal and supraspinal sites. These changes probably comprise primary changes that are responsible for the mode-appropriate H-reflex change and secondary changes that are consequences of the primary changes.

INTRODUCTION

The H-reflex, the electrical analogue of the spinal stretch reflex (SSR), is produced primarily by the two-neuron, monosynaptic pathway consisting of the Ia afferent neuron, the α-motoneuron, and the synapse between them (Brown 1984; Matthews 1972). Primates can gradually increase or decrease triceps surae (TS) H-reflex size in one leg in response to an operant conditioning task (Wolpaw 1987; Wolpaw et al. 1993). Conditioned reflex asymmetry persists even when all supraspinal influence is removed (Wolpaw and Lee 1989; Wolpaw et al. 1989). Thus this learning modifies the spinal cord. Although the available data suggest that changes occur at several spinal cord sites, the observation that conditioned reflex asymmetry is still evident under deep pentobarbital anesthesia, which suppresses all or most spontaneous activity, has focused initial attention on the reflex pathway itself, i.e., the Ia synapse on the α-motoneuron and the α-motoneuron.

A previous report (Carp and Wolpaw 1994) described TS motoneuron properties and excitatory postsynaptic potentials (EPSPs) of animals that had been exposed to the HRdown conditioning mode, in which reward occurs when TS H-reflex size in one leg (the "trained leg") is below a criterion value, and compared them with those of naive (NV; i.e., unconditioned) animals. Operantly conditioned H-reflex decrease was found to be associated with more positive (i.e., depolarized) motoneuron firing threshold and reduced motoneuron axonal conduction velocity. The threshold change increased the depolarization needed to fire the motoneuron, and this increase, combined with a slight reduction in EPSP amplitude, appeared sufficient to account for the change in behavior, i.e., a smaller H-reflex. Theoretical analysis (Halter et al. 1995) suggested that both the threshold change and the change in axonal conduction velocity could have resulted from alteration in the voltage dependence of motoneuron sodium channels.

In the present study, TS motoneuron properties were measured in animals that had been exposed to the HRup conditioning mode, in which reward occurred when TS H-reflex size in the trained leg was above a criterion value. The goal

was to determine whether conditioned H-reflex increase, like conditioned decrease, was associated with changes in motoneuron properties or Ia EPSPs and, if so, whether these changes could account for the H-reflex increase. In accord with this goal, this report compares motoneurons from the trained and control sides of HRup animals with previously studied motoneurons from NV animals (Carp 1992, 1993). The results indicate that HRup conditioning is not a mirror image of HRdown conditioning. Portions of this work have appeared in abstract form (Carp et al. 1994).

METHODS

Subjects were 13 monkeys (*Macaca nemestrina,* male; weight, 5.7 ± 0.9 kg, mean ± SD; range, 3.8–7.1 kg). 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 DHEW Publication No. (National Institutes of Health) 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 TS H-reflex

Each animal was implanted under general anesthesia with chronic fine-wire electromyographic (EMG) electrodes in the TS muscles, i.e., lateral and medial gastrocnemii (LG and MG) and soleus (SOL), and chronic nerve cuff stimulating electrodes on the posterior tibial nerve. Both trained and control legs were implanted and studied in 10 animals. In the other three, data were obtained only from the trained leg. Bilaterally and unilaterally studied animals show comparable H-reflex changes in the trained leg (Wolpaw et al. 1993). The wires from all electrodes traveled subcutaneously to an exit in the midback and passed from there to a connector plug in a custom-made jacket. A very flexible stainless steel tether cable conveyed the connections to an electronic swivel mounted in the top of a standard primate cage, from which they traveled to EMG amplifiers and nerve cuff stimulators. A computer system digitized and rectified EMG and also controlled nerve cuff stimulation.

Garbed in the jacket with tether attached, the animal moved freely around its cage. It was given standard monkey chow and fresh fruit several times per day and obtained water mainly by performing a simple computer-controlled task. It stood, or clung to the cage, so that its mouth was at the water port, and generated a specified level of TS background EMG activity in both legs for a randomly varying 1.2- to 1.8-s period. Then, a 0.1-ms square-wave voltage pulse, kept by computer just above M response (direct muscle response) threshold, was delivered to each leg's posterior tibial nerve to elicit the H-reflex. The computer digitized EMG activity from each of the six TS muscles (i.e., LG, MG, and SOL in each leg) for 100 ms after the stimulus. Two hundred milliseconds after the stimulus, a solenoid-powered syringe sent a reward squirt directly into the animal's mouth. This task was available 24 h/day. It was the animal's standard method for drinking and was completely integrated into life in the cage. An animal usually performed 2,000–6,000 trials/day and thereby obtained sufficient water, so that supplements were only occasionally required.

Each animal performed the task for 3–5 mo. Background EMG and M response size remained stable throughout. To determine initial H-reflex size defined as average EMG in the H-reflex interval (typically 12–20 ms after stimulus onset) minus average background EMG, the animal worked under the control mode for the 1st 10–20 days. Then it worked for at least 45 days under the HRup mode, in which reward occurred only if the absolute value of EMG during the H-reflex interval in each of the three muscles of one leg (the trained leg) was greater than a criterion value. The criterion values were chosen on the basis of the control mode data so that reward occurred in 50–70% of the trials. As H-reflex size rose over days and weeks, the criterion values were repeatedly increased to keep reward probability around 60%. As indicated above, both trained and control legs received comparable stimulation. The difference between them was that, under the HRup mode, the trained leg's H-reflexes determined whether reward occurred, whereas the control leg's H-reflexes were only measured. Also, as noted above, background EMG and M response size were kept stable over the months of task performance. For each of the six TS muscles, daily H-reflex sizes under the HRup mode were expressed as percent of the muscle's initial H-reflex size. The values for the three muscles of each leg were averaged to obtain the leg's daily H-reflex size.

TABLE 1. *Final H-reflex size*

	Final H-Reflex Size	
	Trained leg	Control leg
Successful	386	
	234	136
	223	45
	216	94
	200	
	198	100
	161	342
	136	149
	131	68
	126	178
	124	100
	121	
Unsuccessful	81	59
All animals	180 ± 78	127 ± 86
Successful only	188 ± 75	134 ± 88

Values for All animals (13) and for Successful only animals (12) are means ± SD. For each animal, final H-reflex is expressed in percent of its average preconditioning value.

All animals remained healthy and active and gained weight from electrode implantation to terminal intracellular study. At autopsy, the mean TS muscle weight in the trained leg was 101 ± 7% (mean ± SD) of that in the control leg. This finding was consistent with that in NV animals, in which the mean interleg weight difference was only 6 ± 7% of each animal's mean TS muscle weight, as well as with that in HRdown animals (Carp and Wolpaw 1994). 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.

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 percent 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 in trained and control legs at the time of chronic electrode implantation. Animals are listed according to the magnitude of H-reflex increase in the trained leg. By our standard criterion, based on observation of the range of spontaneous variation in H-reflex size, successful conditioning was defined as a change of ≥20% in the appropriate direction. According to this criterion, HRup conditioning was successful in 12 animals and unsuccessful in the one in which H-reflex size remained within 20% of its initial value. The average final values (±SD) for the successful animals and for all animals together are shown.

As Table 1 also shows, H-reflex size in the control leg tended

to increase with HRup exposure. On the average it increased about one-third as much as that in the trained leg. At the same time, final size in the control leg varied widely across animals and was not related to final size in the trained leg ($r^2 = 0.06$, $P > 0.5$ for linear regression of control leg H-reflex on trained leg H-reflex). In the 10 animals in which control leg data were collected, H-reflex size decreased in 3 animals, did not change in 3 animals, and increased in 4 animals (in 3 of these it increased more than in the trained leg). These findings were similar to those from a large population of HRup animals (Wolpaw et al. 1993). On average, operantly conditioned increase, although focused on the trained leg, tended to occur to a lesser extent in the control leg as well. In this respect, HRup conditioning contrasted with HRdown conditioning, in which operantly conditioned decrease was usually confined to the trained leg (Wolpaw et al. 1993). As indicated above, 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.

Intracellular study

At the completion of HRup-mode exposure, the animal was deeply anesthetized and surgically prepared for terminal intracellular studies of TS motoneuron properties and Ia EPSPs as described in detail elsewhere (Carp 1992, 1993; Carp and Wolpaw 1994). Anesthesia was induced with intramuscular ketamine (7 mg/kg) accompanied by atropine (0.03 mg/kg) and then deepened and maintained with intravenous pentobarbital (15 mg/kg initially and then 4–6 mg/kg/30 min). Throughout the experiment, heart rate, expired CO_2 and urine volume, specific gravity, and pH were monitored. Deep surgical anesthesia verified continually by lack of response to vigorous palpebral or tracheal stimulation and by absence of respiratory or cardiac response to surgery (Green 1979; Steffey 1983) was maintained. At the end of study, the anesthetized animal was killed with an overdose of intravenous pentobarbital.

The MG, LG, and SOL nerves, and the tibial nerve distal to the TS nerves (dTIB[1]) on both sides were cut and placed on bipolar stimulating electrodes. An electrode for recording the cord dorsum potential to stimulation was placed at the dorsal root entry zone. A bilateral pneumothorax was induced, and the animal was placed on a positive-pressure respirator that used room air. Neuromuscular blockade was achieved with intravenous gallamine triethiodide (3 mg/kg initially, and then 1.5 mg/kg/30 min). Continued deep surgical anesthesia was ensured by maintaining the previous pentobarbital dosage schedule and by monitoring heart rate and pupillary light reflex (Committee on Pain and Distress in Laboratory Animals 1992). On the rare occasions when heart rate increased or a pupillary light reflex was noted, pentobarbital dosage was increased. Intravenous administration of dexamethasone (0.25 $mg \cdot kg^{-1} \cdot h^{-1}$) shown previously (Carp 1992) to have no detectable effect on motoneuron properties was begun at the time of pneumothorax to reduce spinal cord edema over the period of intracellular study.

Intracellular recordings from motoneurons on both sides of the spinal cord were conducted with glass micropipettes containing 3 M K acetate and 0.01 M KCl and having DC resistances of 2–8 MΩ. Motoneurons were accepted for study only if they had an antidromically or current-evoked (0.5-ms pulse duration) action potential of at least 60 mV and a stable resting membrane potential. Recording normally began on the right side (10 out of 13 animals) and switched sides once (11 animals) or twice (2 animals).

For each motoneuron we set out to determine axonal conduction velocity, current and voltage thresholds for action-potential initiation, input resistance, time constant, electrotonic length, afterhyperpolarization (AHP) characteristics, and maximum composite EPSPs to stimulation of homonymous and heteronymous nerves both individually and together. Full details of the methods used to obtain these measures are provided in previous reports (Carp 1992, 1993). Deviation from the acceptance criteria (e.g., loss of resting membrane potential or decrease in action-potential amplitude) sometimes prevented obtaining a complete set of measurements from a motoneuron.

Data analysis

The goal of this study was to determine whether operantly conditioned H-reflex increase 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 analysis compared motoneurons from the trained sides of successful HRup animals (UT+ motoneurons) and the control sides of successful HRup animals (UC+ motoneurons) with motoneurons from NV motoneurons. The data from the one unsuccessful animal (Table 1) were not included in this evaluation.

UT+ and UC+ 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 (Carp and Wolpaw 1994). Comparisons for which P was <0.01 were considered to indicate significant differences, and those for which P was <0.05 but >0.01 were noted. Note that for firing threshold and resting potential (both of which are negative), depolarization relative to NV values is indicated by normalized values <1, and hyperpolarization relative to NV values is indicated by normalized values >1.

RESULTS

Intracellular data base

In the 13 conditioned animals, data were gathered from 149 TS motoneurons that had action potentials ≥60 mV and stable resting potentials.[2] These comprised 83 motoneurons on the trained sides (UT, i.e., up-trained) of all 13 animals, and 66 motoneurons on the control sides (UC, i.e., up-control) of 11 of the 13. As shown in Table 1, 12 of the 13 animals met the criterion for successful H-reflex conditioning (i.e., ≥20% increase in H-reflex on the trained side). From these successful animals, 136 motoneurons were recorded: 74 on the trained side (i.e., UT+ motoneurons) and 62 on the control side (i.e., UC+ motoneurons). These data were compared with previously reported data from 109 TS motoneurons of NV animals (Carp 1992, 1993). The limited data from the one unsuccessful HRup animal (9 motoneurons from the trained side, 4 from the control side) were not included in these analyses.

Motoneuron properties and EPSPs

Table 2 shows the average values and standard deviations of the intrinsic properties and EPSPs of NV, UT+, and

[1] Distal tibial afferents were abbreviated as DT in an earlier paper on EPSP properties in NV animals (Carp 1993). We have changed this abbreviation to dTIB here to avoid confusion with the abbreviations used in Carp and Wolpaw (1994) to denote motoneurons from the trained sides of HRdown conditioned animals.

[2] This motoneuron population presumably consisted largely, if not exclusively, of α-motoneurons, because EPSPs of monosynaptic latency were elicited in all motoneurons in which they were sought (including 1 with a conduction velocity of 45 m/s).

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

Property	Gastrocnemius Motoneurons			Soleus Motoneurons		
	NV	UT+	UC+	NV	UT+	UC+
Conduction velocity, m/s	73 ± 7 (70)	73 ± 5 (55)	70 ± 8 (39)	68 ± 6 (39)	71 ± 7 (19)	63 ± 6 (23)
Input resistance, MΩ	0.9 ± 0.4 (59)	0.9 ± 0.3 (52)	1.0 ± 0.5 (34)	1.2 ± 0.5 (35)	1.3 ± 0.7 (18)	1.4 ± 0.5 (21)
Time constant, ms	4.3 ± 1.4 (51)	4.3 ± 1.4 (50)	4.2 ± 1.4 (34)	5.1 ± 1.8 (33)	4.8 ± 1.9 (18)	5.0 ± 1.2 (19)
Rheobase, nA	13 ± 7 (52)	14 ± 7 (45)	12 ± 7 (32)	10 ± 6 (32)	9 ± 8 (17)	8 ± 4 (20)
Electrotonic length, λ	1.3 ± 0.2 (51)	1.3 ± 0.2 (49)	1.4 ± 0.1 (34)	1.4 ± 0.1 (31)	1.3 ± 0.1 (17)	1.4 ± 0.2 (19)
Resting potential, mV	−66 ± 5 (70)	−66 ± 7 (53)	−68 ± 6 (38)	−66 ± 7 (39)	−66 ± 6 (19)	−66 ± 6 (22)
Firing threshold, mV	−55 ± 5 (39)	−54 ± 7 (37)	−58 ± 6 (25)	−56 ± 6 (21)	−53 ± 8 (14)	−58 ± 6 (18)
Action-potential amplitude, mV	75 ± 6 (70)	75 ± 7 (55)	75 ± 7 (39)	75 ± 7 (39)	76 ± 8 (19)	79 ± 7 (23)
AHP duration, ms	75 ± 23 (44)	72 ± 23 (39)	70 ± 19 (30)	86 ± 34 (22)	113 ± 48 (17)	107 ± 37 (19)
AHP amplitude, mV	−3.4 ± 1.3 (44)	−3.5 ± 1.1 (39)	−3.3 ± 1.0 (30)	−3.5 ± 1.5 (22)	−4.0 ± 1.3 (17)	−3.9 ± 1.9 (19)
Homonymous EPSP amplitude, mV	2.1 ± 1.3 (40)	1.8 ± 1.4 (32)	2.1 ± 1.6 (23)	4.4 ± 1.9 (18)	4.9 ± 2.5 (14)	3.8 ± 1.6 (15)
EPSP rise time, ms	0.7 ± 0.2 (40)	0.7 ± 0.1 (32)	0.7 ± 0.2 (23)	0.8 ± 0.1 (17)	0.7 ± 0.2 (14)	0.9 ± 0.2 (14)
EPSP rise time/time constant	0.18 ± 0.07 (38)	0.16 ± 0.06 (30)	0.19 ± 0.06 (22)	0.18 ± 0.08 (16)	0.18 ± 0.12 (14)	0.18 ± 0.08 (12)
EPSP half width, ms	3.3 ± 0.9 (40)	3.0 ± 1.2 (32)	3.0 ± 0.8 (23)	3.9 ± 1.2 (17)	3.6 ± 1.0 (14)	4.0 ± 1.2 (14)
EPSP half width/time constant	0.83 ± 0.24 (38)	0.67 ± 0.28 (30)	0.77 ± 0.27 (22)	0.84 ± 0.36 (16)	0.87 ± 0.42 (14)	0.85 ± 0.43 (12)
Maximum afferent conduction velocity, m/s	91 ± 5 (37)	94 ± 4 (29)	94 ± 4 (21)	90 ± 5 (12)	96 ± 6 (11)	88 ± 7 (11)
Intraspinal latency, ms	0.9 ± 0.2 (37)	0.9 ± 0.1 (29)	0.9 ± 0.2 (21)	0.9 ± 0.1 (12)	0.8 ± 0.1 (11)	1.0 ± 0.1 (11)
Aggregate EPSP amplitude, mv	3.5 ± 2.0 (41)	3.7 ± 2.3 (32)	3.7 ± 2.7 (23)	5.2 ± 2.0 (18)	6.0 ± 2.8 (14)	4.5 ± 1.5 (15)
Heteronymous dTIB EPSP, mV	0.8 ± 0.7 (26)	0.4 ± 0.3 (25)	0.2 ± 0.2 (18)	1.4 ± 1.7 (12)	0.9 ± 0.9 (12)	0.8 ± 0.8 (9)

Values are means ± SD, with the number of motoneurons measured in parentheses. NV, naive animals; UT+, trained side of successful conditioned animals; UC+, control side of successful conditioned animals; AHP, afterhyperpolarization; EPSP, excitatory postsynaptic potential; dTIB, tibial nerve distal to triceps surae nerves. NV data are from Carp (1992, 1993).

UC+ motoneurons innervating gastrocnemius (LG/MG) and SOL muscles, and the numbers of neurons in which each parameter was measured. For the parameters in this table, LG and MG motoneurons are presented together because earlier analysis of NV motoneurons (Carp 1992, 1993) did not reveal any significant differences between them.

As Table 2 shows, the different motoneuron groups had different proportions of LG/MG and SOL motoneurons. Thus, before statistical analysis, each motoneuron's data were normalized to the sample mean obtained for all NV motoneurons innervating the same muscle group. For example, an LG motoneuron with a conduction velocity of 70 m/s 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), whereas a SOL motoneuron with the same conduction velocity would have a normalized conduction velocity of 1.03 (i.e., 3% faster than the average NV SOL conduction velocity of 68 m/s). Table 3 shows the means and standard errors of the normalized data for NV, UT+, and UC+ motoneurons and indicates for which measurements UT+ or UC+ motoneurons are statistically different from NV motoneurons.

UT+ motoneurons versus NV motoneurons

Table 3 indicates that UT+ motoneurons differed from NV motoneurons ($P < 0.01$) in firing threshold and heteronymous dTIB EPSP amplitude and suggests ($P < 0.05$) a small difference in homonymous EPSP rise time. As Table 2 shows, the more positive firing threshold was more evident in SOL motoneurons than in LG/MG motoneurons. Firing threshold was measured as the difference in amplitude between action potentials elicited by short and long current pulses (Carp 1992). The voltage level at which action potentials are initiated by long current pulses is time dependent, in that longer latencies to action-potential onset are associated with more positive thresholds (Burke and Nelson 1971; Gustafsson and Pinter 1984). In the present data the action-potential latency during long current pulses was slightly longer in UT+ motoneurons (17 ± 13 ms) than in NV motoneurons (14 ± 12 ms). Nevertheless, when this difference was taken into account, threshold was still significantly more positive in UT+ motoneurons than in NV motoneurons ($P < 0.01$ by analysis of covariance, with the use of action-potential latency as the covariate). As a result of the difference in firing threshold and the minimal difference in resting potential, the depolarization required to reach threshold was 13% higher in UT+ motoneurons than in NV motoneurons. An analysis of covariance that took into account the dependence of this depolarization on resting potential (Carp 1992; Gustafsson and Pinter 1984) found the increase to be of borderline statistical significance ($P < 0.02$).

The substantial reduction in heteronymous dTIB EPSPs in UT+ motoneurons contrasts with the apparent lack of change in their homonymous EPSPs or in their heteronymous TS EPSPs (as reflected in aggregate TS EPSP amplitude).

UC+ motoneurons versus NV motoneurons

Table 3 suggests ($P < 0.05$) that conduction velocity was decreased and firing threshold was increased (i.e., more

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

Property	NV	UT+	UC+
Conduction velocity	1.00 ± 0.01	1.01 ± 0.01	0.95 ± 0.01*
Input resistance	1.00 ± 0.05	0.99 ± 0.05	1.08 ± 0.07
Time constant	1.00 ± 0.04	0.99 ± 0.04	0.98 ± 0.04
Rheobase	1.00 ± 0.06	1.05 ± 0.07	0.86 ± 0.06
Electrotonic length	1.00 ± 0.01	0.98 ± 0.01	1.01 ± 0.01
Resting potential	1.00 ± 0.01	1.00 ± 0.01	1.02 ± 0.01
Firing threshold	1.00 ± 0.01	0.96 ± 0.02†	1.05 ± 0.02*
Action-potential amplitude	1.00 ± 0.01	1.01 ± 0.01	1.01 ± 0.01
AHP duration	1.00 ± 0.04	1.07 ± 0.06	1.06 ± 0.05
AHP amplitude	1.00 ± 0.05	1.06 ± 0.04	1.02 ± 0.06
Homonymous EPSP amplitude	1.00 ± 0.07	0.92 ± 0.10	0.96 ± 0.10
EPSP rise time	1.00 ± 0.04	0.95 ± 0.03*	1.05 ± 0.05
EPSP rise time/time constant	1.00 ± 0.06	0.93 ± 0.07	1.04 ± 0.06
EPSP half width	1.00 ± 0.04	0.91 ± 0.05	0.94 ± 0.04
EPSP half width/time constant	1.00 ± 0.05	0.88 ± 0.06	0.95 ± 0.07
Maximum afferent conduction velocity	1.00 ± 0.01	1.04 ± 0.01	1.01 ± 0.01
Intraspinal latency	1.00 ± 0.02	0.97 ± 0.02	1.05 ± 0.04
Aggregate EPSP amplitude	1.00 ± 0.07	1.09 ± 0.09	0.97 ± 0.10
Heteronymous dTIB EPSP amplitude	1.00 ± 0.16	0.68 ± 0.09†	0.33 ± 0.07†

Values are means ± SE. For abbreviations, see Table 2.

* $P < 0.05$; statistical significance of differences between NV mean values and UT+ and UC+ mean values (assessed by nested analysis of variance).

† $P < 0.01$.

negative) in UC+ motoneurons (i.e., those innervating the TS muscles on the control side of successful animals) compared with NV motoneurons. The action-potential latency to long current pulses for UC+ motoneurons (15 ± 13 ms) was nearly identical to that found in NV motoneurons and thus cannot account for the difference in firing threshold (see above). At the same time, because resting potential was also slightly more negative, the depolarization required to reach threshold was only 10% less in UC+ motoneurons than in NV motoneurons ($P > 0.05$).

The most striking and significant feature of the UC+ data was the marked decrease in heteronymous dTIB EPSPs. Heteronymous dTIB EPSPs in UC+ motoneurons were less than one-half the amplitude of those in NV motoneurons. Like the decrease seen in UT+ motoneurons, this decrease contrasts sharply with the lack of change in homonymous and heteronymous TS EPSPs.

Additional analyses

As the preceding subsections indicate, the intracellular data did not reveal significant differences among NV, UT, and UC motoneurons that could readily account for the larger H-reflexes seen on the trained side in awake behaving animals after HRup conditioning. In a further effort to detect relationships between motoneuron properties and behavior (i.e., final H-reflex size), we performed three additional analyses.

First, we analyzed the data from those motoneurons that had action potentials ≥75 mV (39 UT+, 34 UC+, and 54 NV motoneurons). Because they were probably less damaged by the intracellular penetration, their data were presumed to be more reliable (Carp 1992). The findings from this restricted set of motoneurons were comparable with those for the entire population presented in Table 3 and above.

Second, we analyzed the data from the most successful HRup animals. Recent studies of the range and course of H-reflex change in trained and control legs of many HRup and HRdown animals (Wolpaw et al. 1993, 1994) suggested that exposure to either mode may cause, in addition to the large mode-appropriate change in the trained leg, a small nonspecific increase of 10–15% in both legs. Thus the standard ≥20% criterion may be insufficiently rigorous to detect successful HRup conditioning. On the basis of this consideration, the present intracellular data were also evaluated by using the more stringent criterion of a ≥50% increase in the trained leg to define a subgroup of seven very successful animals. In these animals, average final H-reflex sizes were 231 ± 72% of initial value in the trained legs and 143 ± 116% in the control legs. Motoneuron measurements from the trained and control sides of these very successful animals (i.e., UT++ and UC++ motoneurons) were compared with those from NV motoneurons with the use of the nested analysis of variance described above. The 32 UT++ and the 33 UC++ motoneurons displayed differences from NV motoneurons comparable with those already described for UT+ and UC+ motoneurons. The only additional finding was that UT++ motoneurons had significantly ($P < 0.01$) shorter time constants (average normalized value of 0.85) than NV motoneurons.

Third, for all HRup animals, linear relationships between the by-animal average values of each measurement and the final H-reflex size were evaluated by regression analysis. This analysis did not reveal any significant relationships between intracellular measures and final H-reflex size on the trained or on the control side ($P \geq 0.1$ for all regressions). Inclusion of the data from the one unsuccessful animal did not substantially alter any of these relationships.

DISCUSSION

Operant conditioning can increase or decrease the TS H-reflex in the trained leg of the awake behaving animal. HRup conditioning can nearly double it, whereas HRdown conditioning can halve it (Wolpaw et al. 1993). Study of nerve volley responses in anesthetized animals after spinal cord transection (Wolpaw and Lee 1989; Wolpaw et al. 1989) showed that the reflex asymmetry created by exposure to the HRup or HRdown mode survives the removal of supraspinal influence. Thus part of the CNS plasticity responsible for the learned change in behavior, i.e., a larger or smaller H-reflex, is in the spinal cord. At the same time, differences between the H-reflexes of the awake behaving animal and the nerve volley data from the anesthetized transected animal indicated that operant conditioning produces both spinal plasticity and supraspinal plasticity, and that the altered H-reflex seen in the awake behaving animal is the product of their interaction. By focusing on the α-motoneuron and its Ia synaptic connection, the present intracellular study and its companion (Carp and Wolpaw 1994) attempted to clarify the spinal cord plasticity.

The present results combined with the previous intracellular study of HRdown animals (Carp and Wolpaw 1994)

indicate that HRup and HRdown conditioning do not result from the same mechanism operating in opposite directions. The more positive firing threshold and the modest decrease in EPSP amplitude that appear to account for H-reflex decrease under the HRdown mode are not mirrored in HRup animals by a more negative (i.e., hyperpolarized) threshold and an EPSP increase. Indeed, UT+ motoneurons showed a positive threshold shift. Furthermore, motoneurons from the trained side of HRdown animals showed a decreased axonal conduction velocity that theoretical analysis indicated was related to their positive shift in firing threshold (Halter et al. 1995). In contrast, UT+ motoneurons did not show a change in conduction velocity. This intracellular evidence of different mechanisms for HRup and HRdown conditioning complements behavioral data, indicating that reflex changes under the up and down modes differ in time course (Wolpaw and O'Keefe 1984; Wolpaw et al. 1994), in persistence (Wolpaw et al. 1986), and in dependence on animal age (Wolpaw et al. 1993).

Most important in the present context, the intracellular data from anesthetized HRup animals provide no explanation for the trained-leg H-reflex increase seen in awake behaving HRup animals, nor for the asymmetry evident in the nerve volley data from anesthetized transected animals. This lack of correlation of the intracellular data with the behavioral and nerve volley data has two possible explanations. First, this intracellular study may simply have failed to detect important changes in motoneuron properties or EPSPs. Alternatively or in addition, plasticity elsewhere in the spinal cord may have been responsible for the nerve volley results and, in combination with supraspinal plasticity, for the H-reflex changes.

Limitations of the intracellular analysis

The simplest possibility is that the samples of TS motoneurons studied (i.e., UT+, UC+, and NV) were simply too small to allow detection of significant differences in the properties measured in the face of their high inherent variability. However, even if the issue of statistical significance is set aside, few prominent differences are evident among the motoneuron groups, and none of them can account for the H-reflex changes seen in the awake behaving animal or for the nerve volley results.

Despite the absence of prominent and/or explanatory differences in individual properties, a particular combination of relatively modest changes in several properties might still account for the increased motoneuron recruitment into the H-reflex on the trained side in the awake behaving animal. In an effort to assess this possibility, we calculated an index of the likelihood of motoneuron recruitment (V_{REC}), defined as the aggregate EPSP amplitude (i.e., the effect of the combined stimulation of all 3 TS nerves) plus the resting potential minus the firing threshold (Carp and Wolpaw 1994). If the values of these measurements obtained in the anesthetized animal were identical to those in the awake behaving animal, a motoneuron in which V_{REC} is greater than zero would be expected to be recruited into the H-reflex. However, motoneuron properties are almost certainly not the same in these two situations. The EPSP in the awake behaving animal is probably smaller because it is shunted by the greater membrane conductance accompanying tonic motoneuron activity and because stimulus intensity is lower (i.e., near M response threshold). In addition, resting potential is presumably more positive in the awake behaving animal because tonic motoneuron activity is present. Thus V_{REC} provides a relative rather than an absolute measure of likelihood of recruitment (Carp and Wolpaw 1994 for discussion). Motoneurons for which V_{REC} is more positive are probably more likely to participate in the H-reflex.

Previous measurements of H-reflexes and maximum M responses and analysis of available data on the relationship between EMG amplitude and motoneuron recruitment suggested that under the control mode (i.e., before conditioning) ~25% of the motoneuron population participate in the H-reflex (Carp and Wolpaw 1994). Twenty-five percent of NV motoneurons had values of V_{REC} greater than −4.0 mV. If we assume that the same V_{REC} requirement applies to motoneurons of HRup animals, the distribution of V_{REC} values of UT+ and UC+ motoneurons predict that 21 and 30%, respectively, should have participated in the H-reflex in the awake behaving animal. Although the prediction for UC+ motoneurons is consistent with the modest control-leg increase shown in Table 1, the prediction for UT+ suggests that the trained-leg reflex should have been slightly decreased, not greatly increased as it actually was. Calculations of V_{REC} that took into account differences in proportions of LG/MG and SOL motoneurons and/or included the dTIB EPSPs in the calculation provided similar results. Although the numbers of motoneurons in which V_{REC} could be calculated were limited (i.e., 38 UT+, 33 UC+, and 41 NV), this analysis indicates that the measurements of those intracellular properties that reflect the probability of recruitment of UT+ motoneurons do not account for the large trained-leg H-reflex found in the awake behaving animal.

The changes noted in the nerve volley responses and the behavioral data might have resulted from alterations within the TS motoneuron population that did not alter the mean UT+ values shown in Table 3, but did alter the order of recruitment. For example, HRup conditioning could have altered the distribution of synaptic inputs and/or threshold properties so that a higher proportion of large motoneurons (with large axons and large motor units that contribute more to the nerve volley and the EMG) was recruited by Ia afferent input. However, properties that tend to vary with motor-unit type in cats and presumably in primates (Carp 1992), including AHP time course, input resistance, time constant, and rheobase (Zengel et al. 1985), did not differ among the entire NV, UT+, and UC+ motoneuron populations nor among the subsets that would be predicted by their V_{REC} values to have contributed to the H-reflex.

Finally, it is possible that motoneuron or EPSP modifications did occur that were responsible for the volley results and the behavioral change, but that the intracellular measurements used here were simply not able to detect them. One possibility is that the firing threshold measurements, which were obtained with the use of current injection, did not accurately reflect the thresholds for synaptic activation. For some cat motoneuron populations, the depolarization required to reach firing threshold appears to be lower for synaptic activation (Pinter et al. 1983) than for intracellularly injected current (Gustafsson and Pinter 1984). Therefore it is conceiv-

able that HRup conditioning altered the voltage dependence of transmitter-gated conductances so that firing threshold was more negative for synaptic input, but not for injected current. Thus a difference between synaptic and current firing thresholds could theoretically account for the lack of correlation between the HRup intracellular data and the corresponding behavioral and nerve volley data.

Changes at other sites in the spinal cord

If HRup conditioning does not arise from some as yet undetected change in TS motoneurons or their primary afferent EPSPs, the nerve volley asymmetry must arise, and the behavioral asymmetry may arise, from change at one or more other sites in the spinal cord. We have previously divided such change into two categories (Carp and Wolpaw 1991). The first is steady-state change in interneuronal activity impinging on the TS motoneuron or its Ia synapse (e.g., tonic presynaptic inhibition). However, the nerve volley asymmetry was evident under deep pentobarbital anesthesia, which largely suppresses spontaneous interneuronal activity and therefore should have prevented change in such activity from affecting the volleys. Furthermore, even if interneuronal activity persisted or had effects that lasted through the several days of recording, it should presumably have affected the intracellular measurements of the present study.

The second category of change elsewhere in the spinal cord is change in oligosynaptic pathways that are activated by the stimuli responsible for the H-reflex. The possibility that the primate H-reflex is not purely monosynaptic has been raised in several careful studies over the past decade (Burke et al. 1984; Fournier et al. 1986). Disynaptic pathways beginning with group Ib and/or Ia afferents from the TS muscles (Baldissera et al. 1981; Jankowska 1992) might have conveyed stimulus-evoked excitation or inhibition to the TS motoneurons or to their primary afferent contacts quickly enough to affect the response to the monosynaptic excitation produced by the same stimulus. This interaction was possible because the long peripheral afferent pathway (average length, 340 mm) and the wide range in group I fiber conduction velocities caused considerable temporal dispersion of the monosynaptic and disynaptic input reaching the motoneuron. Calculations based on earlier control data (Carp 1992, 1993) indicate that the monosynaptic EPSPs peaked in 4.7–6.0 ms, whereas disynaptic excitation or inhibition from group I fibers, delayed ~1.0 ms by the additional synaptic connection (Eccles et al. 1956, 1957), would be expected to have reached the motoneuron in 5.2–6.1 ms and thus should have largely overlapped the monosynaptic excitation. An interneuronal change that increased disynaptic excitation or decreased disynaptic inhibition could have enhanced the likelihood of motoneuron discharge and thereby increased H-reflex size. Furthermore, this possibility is compatible with the observation that H-reflex conditioning does not noticeably increase reflex duration (Wolpaw 1987). Change in disynaptic input would be expected to affect larger, less readily recruited motoneurons more than smaller, more readily recruited motoneurons. The higher conduction velocities of these larger motoneurons could prevent an increase in their recruitment from broadening the H-reflex.

This hypothesis can account for the fact that the present intracellular data provide no explanation for the larger H-reflex seen in the trained leg of the awake behaving HRup animal nor for the nerve volley asymmetry found in the anesthetized transected HRup animal. The absence of change in intracellular response to TS nerve stimulation could reflect pentobarbital depression of spinal interneurons. In these circumstances, even combined stimulation of the TS nerves could have failed to activate the interneurons that convey disynaptic input to the TS motoneurons in the behaving animal. On the other hand, the intense dorsal root stimulus used in the nerve volley study presumably excited all group I non-TS afferents in addition to all group I TS afferents. Furthermore, the short conduction path provided a more synchronized input to the spinal interneurons. In cats the interneurons in question receive group I inputs from numerous muscle groups and are readily driven to discharge by spatial and temporal summation of that input (Jankowska 1992). Thus the magnitude and synchrony of the input provided by the dorsal root stimulus of the nerve volley study may have overcome the depressant effect of the pentobarbital anesthesia on the interneurons of the disynaptic pathway, and thereby permitted expression of conditioning-induced changes in them.[3]

HRup and HRdown conditioning both involve changes at multiple sites

That both HRup and HRdown conditioning produce changes at multiple spinal and supraspinal sites was first indicated by the contrasts between the H-reflexes of the awake behaving animal and the nerve volleys obtained from the anesthetized transected animal (e.g., the very large volley response on the control side of HRdown animals) (Wolpaw and Lee 1989). The intracellular data from both HRdown and HRup animals provide additional direct and indirect evidence for changes at multiple sites. Although the threshold and EPSP changes seen in HRdown animals appear sufficient to explain the H-reflex decrease, they cannot account for other aspects of task performance nor for the very large nerve volley responses seen on the control side (see Carp and Wolpaw 1994 for full discussion).

In the present study the striking decrease in dTIB heteronymous EPSPs seen in both UT+ and UC+ motoneurons does not have any obvious connection to the behavioral changes caused by HRup conditioning, especially because similar decreases were present in motoneurons of HRdown animals (unpublished data). Thus this effect may be a result of some feature that both HRup and HRdown tasks have in common (e.g., requirement for maintenance of background EMG). In addition, motoneurons from both trained and control sides of HRdown animals tended to have lower AHP amplitudes (Carp and Wolpaw 1994), whereas (as Table 3

[3] A second effect of the proximal site of stimulation in the nerve volley study would be reduced overlap between the arrivals of the mono- and disynaptic inputs. This would diminish the ability of disynaptic inhibition to modify monosynaptic recruitment of motoneurons, because inhibition must arrive before the time the motoneuron would have fired (i.e., before the peak of the monosynaptic EPSP). However, a mechanism involving disynaptic excitation might not be as greatly affected by a decrease in overlap between the mono- and disynaptic inputs, because arrival of disynaptic excitation beyond the peak of the monosynaptic EPSP could still bring the motoneuron to threshold.

indicates) AHP amplitude was normal in HRup motoneurons. This apparent difference, which also bears no obvious relation to the behavioral change, appears to be a bilateral effect of exposure to the HRdown mode. Finally, the positive threshold shift detected in UT+ motoneurons and the negative shift that may be present in UC+ motoneurons do not have obvious behavioral correlates or rationales (unlike the larger positive shift evident on the trained side of successful HRdown animals).

These results comprise part of the growing evidence that even the simplest behavioral changes involve plasticity at multiple sites within the CNS. Recent studies indicate that other apparently simple behavioral changes, including vestibuloocular reflex plasticity, nictitating membrane conditioning, and sensitization in *Aplysia* (Broussard et al. 1992; Frost et al. 1988; Perrett et al. 1993), are also accompanied by changes at multiple locations. The occurrence of complex plasticity in association with seemingly simple behavioral changes was not generally anticipated when these experimental models were first developed. However, as discussed in detail elsewhere (Wolpaw and Carp 1993), two considerations indicate that such complexity is certainly understandable and probably inevitable. First, a conditioning protocol does not merely require the CNS to modify a specific behavior, such as the TS H-reflex, but rather to modify that behavior without seriously disturbing performance of other previously learned behaviors. Because most CNS elements, especially the motoneuron and structures closely related to it, participate in a wide variety of behaviors, any change is likely to affect more than one behavior and thereby trigger compensatory changes, which may bear little apparent relation to the behavioral change that increases reward probability (e.g., a larger or smaller H-reflex on the trained side). Second, a change in any CNS element is likely to affect the pattern or intensity of activity impinging on other elements and thereby produce additional changes, which may also bear little apparent relation to the intended behavioral change, and might even appear maladaptive. At present, it seems likely that the threshold change noted in UT+ motoneurons, the decreased AHP amplitude of HRdown motoneurons, the decreased dTIB EPSPs of both HRdown and HRup motoneurons, and whatever change is responsible for the very large nerve volley responses found on the control side of HRdown animals fall into these categories.

Conclusions

The intracellular data from HRup animals reveal some changes in motoneuron properties and EPSPs. However, none of these changes can readily explain the marked increase in H-reflex size produced by HRup conditioning. Thus they contrast with intracellular data from HRdown animals, which do provide a reasonable explanation for the H-reflex decrease produced by HRdown conditioning. The intracellular measurements of the present study may have failed to detect a crucial motoneuron change, such as a negative shift in firing threshold to synaptic input. Alternatively, H-reflex increase may be due to change in the interneurons conveying disynaptic input to the TS motoneurons.

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REFERENCES

BALDISSERA, F., HULTBORN, H., AND ILLERT, M. Integration in spinal neuronal systems. In: *Handbook of Physiology. The Nervous System. Motor Control.* Bethesda, MD: Am. Physiol. Soc., 1981, sect. 1, vol. II, part 1, p. 509–595.

BROUSSARD, D. M., BRONTE-STEWART, H. M., AND LISBERGER, S. G. Expression of motor learning in the response of the primate vestibuloocular reflex pathway to electrical stimulation. *J. Neurophysiol.* 67: 1493–1508, 1992.

BROWN, W. F. *The Physiological and Technical Basis of Electromyography.* Boston, MA: Butterworths, 1984.

BURKE, D., GANDEVIA, S. C., AND MCKEON, B. Monosynaptic and oligosynaptic contributions to human ankle jerk and H-reflex. *J. Neurophysiol.* 52: 435–448, 1984.

BURKE, R. E. AND NELSON, P. G. Accommodation to current ramps in motoneurons on fast and slow twitch motor units. *Int. J. Neurosci.* 1: 347–256, 1971.

CARP, J. S. Physiological properties of primate lumbar motoneurons. *J. Neurophysiol.* 68: 1121–1131, 1992.

CARP, J. S. Monosynaptic EPSPs in primate lumbar motoneurons. *J. Neurophysiol.* 70: 1585–1592, 1993.

CARP, J. S., CHEN, X. Y., AND WOLPAW, J. R. Motoneuron properties after operantly conditioned increase in primate H-reflex. *Soc. Neurosci. Abstr.* 20: 788, 1994.

CARP, J. S. AND WOLPAW, J. R. Possible mechanisms of operantly conditioned plasticity in motoneuron response to Ia afferent input. In: *Plasticity of Motoneuronal Connections. Restorative Neurology,* edited by A. Wernig. Amsterdam: Elsevier, 1991, vol. 5, p. 435–442.

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

Committee on Pain and Distress in Laboratory Animals. *Recognition and Alleviation of Pain and Distress in Laboratory Animals*. Washington, DC: Commission on Life Sciences, National Research Council, National Academy Press, 1992, p. 79–82.

ECCLES, J. C., ECCLES, R. M., AND LUNDBERG, A. Synaptic actions on motoneurones caused by impulses in Golgi tendon organ afferents. *J. Physiol. Lond.* 138: 227–252, 1957.

ECCLES, J. C., FATT, P., AND LANDGREN, S. Central pathway for direct inhibitory action of impulses in largest afferent nerve fibres to muscle. *J. Neurophysiol.* 19: 75–98, 1956.

FOURNIER, E., MEUNIER, S., PIERROT-DESEILLIGNY, E., AND SHINDO, M. Evidence for interneuronally mediated Ia excitatory effects to human quadriceps motoneurons. *J. Physiol. Lond.* 377: 143–169, 1986.

FROST, W. N., CLARK, G. A., AND KANDEL, E. R. Parallel processing of short-term memory for sensitization in *Aplysia. J. Neurobiol.* 19: 297–334, 1988.

GREEN, C. J. *Animal Anesthesia*. London: Lab. Anim., 1979, p. 217–226.

GUSTAFSSON, B. AND PINTER, M. J. An investigation of threshold properties among cat spinal alpha-motoneurones. *J. Physiol. Lond.* 357: 453–483, 1984.

HALTER, J. A., CARP, J. S., AND WOLPAW, J. R. Operantly conditioned motoneuron plasticity: possible role of sodium channels. *J. Neurophysiol.* 73: 867–871, 1995.

JANKOWSKA, E. Interneuronal relay in spinal pathways from proprioceptors. *Prog. Neurobiol.* 38: 335–378, 1992.

MATTHEWS, P. B. C. *Mammalian Muscle Receptors and Their Central Actions.* Baltimore, MD: Williams & Wilkins, 1972, p. 319–409.

PERRETT, S. P., RUIZ, B. P., AND MAUK, M. D. Cerebellar cortex lesions disrupt learning-dependent timing of conditioned eyelid responses. *J. Neurosci.* 13: 1708–1718, 1993.

PINTER, M. J., CURTIS, R. L., AND HOSKO, M. J. Voltage threshold and excitability among variously sized cat hindlimb motoneurons. *J. Neurophysiol.* 50: 644–657, 1983.

STEFFEY, E. P. Concepts of general anesthesia and assessment of adequacy of anesthesia for animal surgery. In: *Animal Pain: Perception and Alleviation,* edited by R. L. Kitchell, H. H. Erickson, E. Carstens, and L. E. Davis. Bethesda, MD: Am. Physiol. Soc., 1983, p. 133–150.

WOLPAW, J. R. Operant conditioning of primate spinal reflexes: the H-reflex. *J. Neurophysiol.* 57: 443–458, 1987.

WOLPAW, J. R. AND CARP, J. S. Adaptive plasticity in spinal cord. In: *Advances in Neurology,* edited by F. J. Seil. New York: Raven, 1993, p. 163–174.

WOLPAW, J. R. AND HERCHENRODER, P. A. Operant conditioning of H-reflex in freely moving monkeys. *J. Neurosci. Methods* 31: 145–152, 1990.

WOLPAW, J. R., HERCHENRODER, P. A., AND CARP, J. S. Operant conditioning of the primate H-reflex: factors affecting the magnitude of change. *Exp. Brain Res.* 97: 31–39, 1993.

WOLPAW, J. R. AND LEE, C. L. Motoneuron response to dorsal root stimulation in anesthetized monkeys after spinal cord transection. *Exp. Brain Res.* 68: 428–433, 1987.

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

WOLPAW, J. R., LEE, C. L., AND CALAITGES, J. G. Operant conditioning of primate triceps surae H-reflex produces reflex asymmetry. *Exp. Brain Res.* 75: 35–39, 1989.

WOLPAW, J. R., MANICCIA, D. M., AND ELIA, T. Operant conditioning of the primate H-reflex: course of development. *Neurosci. Lett.* 170: 203–207, 1994.

WOLPAW, J. R. AND O'KEEFE, J. A. Adaptive plasticity in the primate spinal stretch reflex: evidence for a two-phase process. *J. Neurosci.* 4: 2718–2724, 1984.

WOLPAW, J. R., O'KEEFE, J. A., NOONAN, P. A., AND SANDERS, M. G. Adaptive plasticity in the primate spinal stretch reflex: persistence. *J. Neurophysiol.* 55: 272–279, 1986.

ZAR, J. H. *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice-Hall, 1984, p. 194–195.

ZENGEL, J. E., REID, S. A., SYPERT, G. W., AND MUNSON, J. B. Membrane electrical properties and prediction of motor-unit type of medial gastrocnemius motoneurons in the cat. *J. Neurophysiol.* 53: 1323–1344, 1985.