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RESEARCH NOTE

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Operant conditioning of rat H-reflex affects motoneuron axonal conduction velocity

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Abstract This study assessed the effects of operant conditioning of the H-reflex on motoneuron axonal conduction velocity in the rat. After measurement of the control H-reflex size, rats were either exposed for at least 40 days to the HRup or HRdown conditioning mode, in which reward occurred only if the soleus H-reflex was greater than (HRup mode) or less than (HRdown mode) a criterion or continued under the control condition (HRcon mode) in which the H-reflex was simply measured. We then measured axonal conduction velocity of triceps surae motor units of HRup, HRdown, and HRcon rats by stimulating the axon in the ventral root and recording from the tibial nerve. Conduction velocity was 8% less in successful HRdown rats than in HRcon rats (P=0.02). Conduction velocity in HRup rats and unsuccessful HRdown rats was not significantly different from that in HRcon rats. Since recording bypassed the intraspinal portion of the motoneuron, the change was clearly in the axon. This decrease was similar to the 6% decrease previously found in successful HRdown monkeys. Unsuccessful HRdown rats and monkeys did not show this decrease. This result suggests that the mechanism of HRdown conditioning is similar in rats and monkeys and provides further support for the hypothesis that HRdown conditioning decreases motoneuron excitability by producing a positive shift in firing threshold. While traditional theories of learning emphasize synaptic plasticity, neuronal plasticity may also contribute to operantly conditioned behavioral changes.

Keywords Plasticity · H-reflex · Conduction velocity · Motoneuron · Spinal cord

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Introduction

Theories of the mechanisms underlying learning traditionally focus on synaptic plasticity (Kandel et al. 2000). However, recent data in a simple model of vertebrate learning suggest that plasticity in neuronal properties can play an important role. The spinal reflex pathway mediating the first response to limb displacement (i.e., the spinal stretch reflex) or to electrical stimulation of large diameter sensory afferents (i.e., the H-reflex) can be operantly conditioned (Wolpaw 1997). Rats, monkeys, and humans can gradually increase or decrease the size of the spinal stretch reflex or the H-reflex in order to increase the probability of reward (Wolpaw et al. 1983; Wolpaw 1987; Chen and Wolpaw 1995; Wolf and Segal 1996). H-reflex change, once established, survives removal of supraspinal influence, indicating that it involves plasticity in the spinal cord (Wolpaw and Lee 1989). Monkeys that have decreased the H-reflex have altered motoneuron properties, including a more depolarized threshold for action potential initiation, which is able to account for much of the change in H-reflex size (Carp and Wolpaw 1994). The data are consistent with an operantly conditioned change in sodium-channel kinetics in the motoneuron (Halter et al. 1995). In addition, H-reflex conditioning induces changes in synaptic contacts on motoneurons (Feng-Chen and Wolpaw 1996).

In addition to its demonstrated effects within the spinal cord, H-reflex operant conditioning also appears to affect peripheral components of the reflex pathway. Motoneurons of monkeys that have decreased the H-reflex have lower conduction velocities (Carp and Wolpaw 1994). This decrease is consistent with a change in the firing threshold of the axon comparable to the change found in the firing threshold of the motoneuron cell body (Carp and Wolpaw 1994; Halter et al. 1995). However, because conduction velocity measurement in the monkey study was based on the antidromic latency of the motoneuron intracellular response to peripheral nerve stimulation, it was unclear whether the conduction velocity decrease was due

to change throughout the axon or to change at or near the cell body (i.e., initial segment or axon hillock).

The goals of the present study were: first, to determine whether a change in the conduction velocity is a consistent feature of H-reflex operant conditioning; and second, to determine whether conditioning affects conduction velocity in the motoneuron axon beyond the spinal cord. To achieve these goals, we studied the conduction velocity of motoneuron axons innervating the triceps surae (TS) muscles [i.e., medial gastrocnemius (MG), lateral gastrocnemius (LG) and soleus (SOL)] in rats that had been rewarded for increasing (HRup rats) or decreasing (HRdown rats) the size of the SOL H-reflex and in control rats in which the SOL H-reflex had been measured without imposition of any reward contingency (HRcon rats). Portions of the data from HRup and HRdown rats have been reported in abstract form (Carp et al. 1999a).

Materials and methods

Experiments were performed in 26 male Sprague-Dawley rats. All procedures adhered to the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Academy Press, Washington DC 1996) and had been reviewed and approved by the Institutional Animal Care and Use Committee of the Wadsworth Center. Animal preparation, H-reflex conditioning, and motor unit data collection methods have been described elsewhere (Chen and Wolpaw 1995; Carp et al. 1999b) and are summarized here, except where methodological differences require a more detailed description.

Rats weighing 312–475 g were anesthetized (ketamine/xylazine i.p.) and implanted in the right leg with a nerve cuff on the posterior tibial nerve at the knee and with fine-wire electromyographic (EMG) recording electrodes in the TS muscles (one pair in SOL and usually a second pair with one wire in LG and another in MG). All wires were routed subcutaneously to a skull-mounted plug for connection to EMG amplifiers and a nerve-cuff stimulation unit. Water and standard rat chow were available ad libitum, except during H-reflex conditioning, when rats obtained food primarily by performing the task described below.

SOL EMG was monitored continuously by computer, which recorded the EMG during the H-reflex and controlled the nerve-cuff stimulus. After each stimulus, the computer determined if M-response size was larger or smaller than a just-above threshold target value and then decremented or incremented, respectively, the digital-to-analog converter controlling the stimulus intensity by a single step (Wolpaw and Herchenroder 1990). This procedure maintained a stable M-response throughout the experiment. Data were collected for 10–28 days prior to H-reflex conditioning to determine each rat's initial H-reflex size. Nineteen rats were then exposed for at least 40 days to the HRup mode (HRup rats, n=8) or the HRdown mode (HRdown rats, n=11), in which the computer triggered delivery of a reward (a 20-mg food pellet) when average H-reflex size was above (HRup) or below (HRdown) a criterion value. The remaining rats continued under the initial condition in which the H-reflex was simply measured without any reward contingency (HRcon rats, n=7) for 71–225 days (except for one rat in which data collection stopped after 24 days due to failure of the skull-mounted connector plug). To determine final the H-reflex size for HRup, HRdown, and HRcon rats, the average H-reflex size for the final 10 days of data collection was calculated as the percent of the initial H-reflex size. HRcon, HRdown, and HRup rats remained healthy and active throughout the several months of data collection and displayed similar weight gains (i.e., mean gains ±SEM were 213±14, 211±10, and 213±19 g, respectively). Weight ranges at the end of data collection were 507–700, 529–745, and 540–667 g, respectively.

At the end of H-reflex data collection, rats were anesthetized with pentobarbital and secured in the prone position in a rigid frame with ear and jaw bars, hip clamps, and pins through the tibia and malleolus. The TS muscles of the right hindlimb were dissected from surrounding tissue (but not from each other). The chronic EMG electrode wires were cut where they entered the muscles, and a pair of fine wires was inserted into each of the TS muscles (i.e., MG, LG, and SOL) for recording EMGs. The tibial nerve was dissected from surrounding tissue and a sub-epineurial electrode was applied just proximal to the chronic nerve cuff to record electroneurographic activity (ENG). A bath filled with 36–37°C mineral oil surrounded the entire rear portion of the rat up to its abdomen (which was secured to a U-shaped opening in the bath with cyanoacrylate glue).

After dorsal laminectomy of the L1-L5 vertebrae and longitudinal incision of the dura, L5 and/or L6 ventral root axons (or their myelin sheaths) were impaled with glass microelectrodes (3 M potassium acetate, 12–25 MΩ). ENG was bandpass filtered (0.003–10 kHz), and the axonal voltage recording was low-pass filtered (10 kHz). All signals were amplified to maximize resolution during analog-to-digital conversion by computer (sampling rate ≥20 kHz for ENG and axon voltage). TS motor units were identified by the presence of reproducible all-or-none EMG and twitch responses to single stimuli over at least a 4-fold range of current intensities. (Contractile properties of these motor units were also recorded, and will be reported elsewhere.) Axonal conduction time was assessed by eliciting individual action potentials by injection of a 2- to 3-ms depolarizing current pulses at 8–16 Hz and recording axon voltage and tibial nerve ENG. Usually, 400–800 responses were averaged, but as many as 4000 were averaged when the ENG had a low signal-to-noise ratio and recording stability permitted. Orthodromically conducted action potentials evoked by intra-axonal current injection were usually detected as triphasic ENG waveforms. Axonal conduction time was calculated as the latency from the onset of the current-evoked action potential to the onset of the large negative component (usually the second peak) of the ENG waveform. Axonal conduction velocity was calculated as the ratio of the conduction distance (measured postmortem with the rat still in the apparatus) to the conduction time.

Differences among H-reflex conditioning groups were assessed by nested analysis of variance, with conditioning group as the main factor, animals nested within conditioning groups, and individual conduction velocity measurements nested within animals. This hierarchical design permits assessing the effects of the main factor (i.e., conditioning group) with respect to between-subject differences independently of effects due to within-subject differences (i.e., those due to differences in conduction velocity measurements among rats) (Sokal and Rohlf 1981). Because the between-subject effects reflect the average values of each rat, this analysis is independent of differences in the numbers of conduction velocity measurements performed in individual rats. Differences among the conditioning groups made by inter-group contrasts for which P was less than 0.05 were considered to be statistically significant.

Results

Table 1 shows, for each group, the average value for the H-reflex size at the end of conditioning in percent of their initial value. Successful H-reflex conditioning [i.e., a change in the correct direction of at least 20% (Wolpaw et al. 1993)] occurred in six of the eight HRup rats (final values of 133–221%) and in eight of the 11 HRdown rats (final values of 18–78%). In the remaining two HRup rats and three HRdown rats, final H-reflex

Table 1 Mean values ±SEM of final H-reflex size and of average motor unit axonal conduction velocity from each rat in control (*HRcon*), successfully conditioned (*HRup+* and *HRdown+*), and unsuccessfully conditioned (*HRup–* and *HRdown–*) rats. Conduction velocity data are adjusted to reflect the results of the nested analysis of variance by removing the effect of rats within conditioning groups (i.e., by subtracting from each conduction velocity measurement the difference between each rat's mean value and the conditioning group mean). This adjustment allows evaluation of differences among conditioning groups with respect to the variation among rats, making it independent of the number of motor units recorded within rats. The *asterisk* (*) indicates a significant difference (P=0.02) from the HRcon group by inter-group contrasts among the five groups

Rat group	Final H-reflex % of control	Conduction velocity	
		m/s	% of HRcon
HRcon	101±4	72.4±1.4	100.0±1.9
HRdown+	47±8	66.6±1.9*	92.0±2.6
HRdown-	95±7	75.2±4.4	103.9±6.1
HRup+	174±14	69.4±1.2	95.9±1.7
HRup-	115±3	71.0±2.8	98.1±3.9

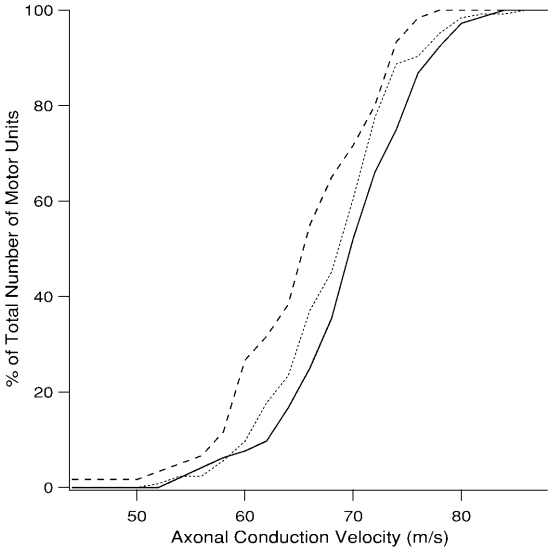

Fig. 1 Cumulative distributions of conduction velocity in motor units of control rats (HRcon, *solid line*), rats with a decreased SOL H-reflex size (HRdown+, *dashed line*), and those with an increased SOL H-reflex size (HRup+, *dotted line*). There was a shift to the left in conduction velocity in HRdown+ rats with respect to HRcon rats, but not in HRup+ rats (see text for statistical analysis). The approximately parallel shift in conduction velocity indicates a generalized effect across axons of different sizes that occurs only in rats that produce a substantial decrease in H-reflex size

sizes were within 20% of their initial values. Successful HRup and HRdown rats are subsequently referred to as HRup+ and HRdown+ rats, respectively, while unsuccessful HRup and HRdown rats are referred to as HRup- and HRdown- rats, respectively. In HRcon rats, H-reflex sizes remained within 20% of their initial values (final values of 91–114%). No significant differences were observed in background EMG, M-response size, number of trials performed per day, or stimulus intensity between the initial and final recording periods for any of the five groups of rats.

We studied 389 single motor units from the 26 rats. While in most rats only the SOL muscle was monitored during H-reflex conditioning, we studied motor units from all three TS muscles (i.e., SOL, MG, or LG) because previous data from monkeys have indicated that conditioning of SOL, MG, or LG has comparable effects on all three (Carp and Wolpaw 1994, 1995) and because EMG electrodes in SOL reflect activity in MG and LG as well. The median number of TS motor units per rat was 16 (range of 1–29). Analysis focused on the 144 motor units from the seven HRcon rats, the 124 from the six HRup+ rats, and the 60 from the eight HRdown+ rats. The small populations from the two HRup- and > the three HRdown- rats (23 and 38 motor units, respectively) provided some insight into the specificity of the association between the behavioral change (i.e., change in H-reflex size) seen in HRdown+ rats and decreased axonal conduction velocity.

The results indicate that, in rats as in monkeys, successful HRdown conditioning affects axonal conduction velocity. Table 1 shows that axonal conduction velocity was significantly lower in HRdown+ rats than in HRcon rats (P=0.02). The 8.0% decrease in HRdown+ rats was similar to the 6% decrease observed in HRdown+ monkeys (Carp and Wolpaw 1994). In contrast, no significant change was found in HRdown– rats or HRdown- monkeys. Furthermore, no significant change was found in HRup+ or HRup- rats, or in HRup+ monkeys [only one HRup- monkey was studied (Carp and Wolpaw 1995)]. The fact that a conduction velocity decrease of similar magnitude occurs in rats and monkeys with, and only with, successful HRdown conditioning suggests that it is a characteristic feature of HRdown+ conditioning.

Figure 1 shows the effects of conditioning mode on the cumulative distributions of motor unit axonal conduction velocities. This distribution is shifted markedly to the left in HRdown+ rats with respect to HRcon rats (dashed and solid lines, respectively, in Fig. 1; χ^2=15.8, P=0.0004 by a two-sample Kolmogorov-Smirnov test). The similarity of the shapes of the cumulative probability distributions in HRcon and HRdown+ rats indicates that the change in conduction velocity is broadly distributed across the data sample and, thus, is similar in motor axons of different diameters (although slowly conducting axons may have been slightly more affected than rapidly conducting axons). On the other hand, no significant shift in conduction velocity distribution occurs in HRup+ rats with respect to HRcon rats (dotted and solid lines, respectively, in Fig. 1).

While the conditioning groups were similar in initial and final weights (as noted above), intergroup differences in age or in implant duration might conceivably affect their relative conduction velocities. Conduction velocity varies directly with body weight (and presumably age) in unimplanted rats (Chen et al. 1992). The time from implantation surgery until motor unit study (i.e.,

implant duration) was longer in HRcon rats (mean ±SEM =178±35 days) than in either HRdown or HRup rats (122±12 or 108±12 days, respectively). (Three of the HRcon rats were part of a study evaluating the control H-reflex over a prolonged period.) To evaluate the possible confounding effects of these factors, we performed a multiple regression of each rat's average conduction velocity upon H-reflex conditioning group (i.e., HRcon, HRdown+, HRdown-, HRup+, and HRup-), body weight, and implant duration. After removal of the contributions of body weight and implant duration, axonal conduction velocity was still 7.2% slower in HRdown+ rats than in HRcon rats (P=0.02 by contrast of means after multiple regression; average conduction velocity ±SEM =67.0±1.4 and 72.2±1.5 m/s for HRdown+ and HRcon rats, respectively). No other group differed significantly from the HRcon group. Thus, differences among rats in weight and/or implant duration did not account for the effects of successful HRdown conditioning on motor unit axonal conduction velocity.

Discussion

The effects of successful HRdown conditioning on conduction velocity in the rat and monkey elucidate as well as confirm each other. In the monkey study, conduction velocity was calculated from the antidromic latency from peripheral nerve stimulation to the onset of the action potential recorded intracellularly from the motoneuron. Thus, the relative contributions of H-reflex conditioning effects on the somatic and axonal components of the motoneuron could not be distinguished. The present rat study bypassed the motoneuron cell body, axon hillock, and initial segment and, thus, demonstrates that HRdown conditioning affects the axon in the ventral root and/or the peripheral nerve. In addition, the rat methodology eliminated two potential sources of variability present in the monkey study. In the monkey, the beginning of conduction time was the time of delivery of the stimulus to the peripheral nerve, and the point on the axon where this stimulus elicited the antidromic action potential could have been slightly proximal to the stimulating electrode, thereby reducing the conduction distance. In addition, systematic variation in passive or active subthreshold properties could have affected estimation of the time of action potential initiation in the monkey motoneuron and, thus, affected the end of the conduction time. In the rat, the onset of the action potential in the ventral root defined the beginning of the conduction time and the arrival of the action potential at the nerve cuff defined its end, and the distance between these two sites was clear.

Because it indicates that the conduction velocity change in rats is due to a change in the axon itself, the present study implies that the conduction velocity change found in monkeys also reflects axonal change. Computer simulation based on an increased firing threshold (and other data) in HRdown+ monkeys indicated that a shift in sodium-channel activation voltage provided a substantially better explanation for conditioning-induced changes in axonal conduction velocity than did changes in other properties (e.g., fiber diameter, myelin thickness, potassium- or other sodium-channel properties) (Halter et al. 1995). This data led to the hypothesis that a conditioning-induced change in sodium-channel activation voltage was responsible for the threshold change in the motoneuron cell body and throughout the axon, the latter accounting for the change in conduction velocity. In turn, the similar conduction velocity change in rats suggests that a similar shift in firing threshold also occurred in the cell bodies of rat motoneurons. Because it decreases the probability that group-I input produced by the nerve-cuff stimulation will excite the motoneuron, a shift in firing threshold might, in both rats and monkeys, account for the decrease in H-reflex size that occurs with HRdown+ conditioning.

A change in axonal excitability could indirectly affect H-reflex size through a change in the stimulus eliciting the group-I input. An increase in firing threshold would be expected to decrease the size of the M-response, and the ensuing computer-controlled increase in stimulus intensity designed to maintain M-response size (see Materials and methods) would also increase the group-I volley. However, stimulus intensity was unaffected by any of the conditioning modes, indicating that any contribution of this hypothetical mechanism was minimal.

The link between conditioning-induced changes in somatic firing threshold and axonal conduction velocity may not be present in all circumstances. Conduction velocity was unaffected in HRup+ monkeys, but the same motoneurons did show a modest positive shift in firing threshold (Carp and Wolpaw 1995). The apparent dissociation in HRup monkeys between effects on conduction velocity and firing threshold suggests that, in this setting, different mechanisms contribute to these axonal and somatic effects. If H-reflex conditioning were associated with changes in the amount or pattern of motor unit activity, conduction velocity could conceivably change due to retrograde influences from the muscle (Lewis et al. 1977; Foehring et al. 1987; Foehring and Munson 1990). Such an effect could be mediated by muscle-derived trophic factors, which can have differential effects on axonal and somatic properties (Gonzalez and Collins 1997; Mendell et al. 1999).

In conclusion, the decrease in axonal conduction velocity found in HRdown+ rats supports the hypothesis, originally based on primate data, that changes in the excitability of the motoneuron cell body is an essential feature of successful HRdown conditioning. To the extent that it reflects changes in the cell body, this change in the peripheral axon could provide convenient experimental access to a mechanism of activity-dependent CNS plasticity. In a broader context, the present data are further evidence that simple learning does not completely depend on synaptic plasticity (Halter et al. 1995; Spitzer 1999). Plasticity in neuronal properties such as firing threshold may also play an important role in operantly conditioned behavioral change.

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