Effects of Excitatory Modulation on Intrinsic Properties of Turtle Motoneurons

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Hornby, T. George, Jennifer C. McDonagh, Robert M. Reinking, and Douglas G. Stuart. Effects of excitatory modulation on intrinsic properties of turtle motoneurons. J Neurophysiol 88: 86–97, 2002; 10.1152/jn.00551.2001. The purpose of this study was to quantify the effects of excitatory modulation on the intrinsic properties of motoneurons (MNs) in slices of spinal cord taken from the adult turtle. Responses were noted following application of an excitatory modulator: serotonin (5-HT), muscarine, trans-1-amino-1,3-cyclopentane dicarboxylic acid (tACPD), or all three combined. A sample of 44 MNs was divided into 2 groups, on the basis of whether MNs did (28/44) or did not (16/44) demonstrate a nifedipine-sensitive acceleration of discharge during a 2-s, intracellularly injected stimulus pulse. Such acceleration indicates the development of a plateau potential (PP). Excitatory modulation lowered the MNs’ resting potential, increased input resistance, decreased rheobase, reduced several afterhyperpolarization values, and shifted the conventional, one-phase stimulus current–spike frequency (I-f) relation to the left. For both MN groups, the relative efficacy of excitatory modulation on both non-PP and PP MNs was generally in the following order: combined application > 5-HT ≈ muscarine > tACPD. In many instances, the effects of modulation differed significantly for non-PP versus PP MNs, the most pronounced being in their I-f relation. To describe this difference, it was necessary to measure a two-phase relation. In PP MNs, excitatory modulation considerably increased the slope of the first (initial) phase and flattened the second (later) phase of this relation. The latter result bore similarities to that obtained in a previous study, which addressed MN firing behavior during fictive locomotion of the high-decerebrate cat.

INTRODUCTION

It is well-known that various endogenous neurotransmitters, modulators, and their agonists can substantially alter the sub- and/or suprathreshold ionic conductances responsible for the repetitive discharge of motoneurons (MNs) (for review, Binder et al. 1996; Powers and Binder 2001; Russo and Hounsgaard 1999). Previous studies have suggested that the extrinsic modulation of MN conductances active below the action potential (AP) threshold may also alter the threshold for repetitive firing, thereby causing a shift in the bias of the stimulus current (I)–spike frequency (f) relation. Conversely, modulators that alter conductances activated near or above the AP threshold may change the slope (gain) of this relationship (Binder et al. 1993). In both cases, modulation of the I-f relation changes the net synaptic current necessary to produce a given muscle force.

Many MNs possess nonlinear membrane properties that are revealed only following modulation by neuroactive agents. For example, the plateau potential (PP; due to a persistent inward current), which is defined as a sustained depolarization or discharge following a brief excitatory stimulus (for historical review, see Hornby et al. 2002a) becomes manifest in MNs only following the extrinsic application of a variety of excitatory modulator agonists, and/or following the blockade of outward currents. As a result, excitatory modulation may result in the MN responding nonlinearly to intracellularly injected and/or synaptic current.

Some of the above effects have been shown previously in a variety of in vivo and in vitro preparations but without provision of quantitative measurements of repetitive firing (see the above reviews). For this reason, little is known about the robustness of these effects, and their functional significance during natural, near-natural, and fictive movements.

In this study, intracellular recording and stimulation of MNs was undertaken in slices of turtle spinal cord, using the in vitro techniques of Hounsgaard et al. (1988b). Intrinsic MN properties, with an emphasis on the I-f relation, were measured in the control versus modulated condition. The latter was shown to have a powerful effect on the I-f relation, particularly in MNs that were generating PPs. It is argued that the present results are relevant to a previous study in which repetitive MN firing was measured during fictive locomotion in the high-decerebrate cat preparation (Brownstone 1989; Brownstone et al. 1992). Preliminary accounts of some of our results have appeared previously in abstract form (Hornby et al. 1997, 1998b, 2000; Stuart et al. 1999).

This work was part of the Ph.D. dissertation research of T. G. Hornby.

METHODS

Most of the present techniques were recently described in detail by our laboratory (Hornby et al. 2002b; McDonagh et al. 1998a). All procedures were in conformity with university, state, and federal regulations for the care and use of laboratory animals.

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Dissection and slice preparation

Spinal cord slices (2 mm thick) were obtained from the adult, North American pond turtle, Pseudemys (Trachemys) scripta elegans, while deeply anesthetized with pentobarbital sodium, and perfused intracardially. The slices were maintained in oxygenated turtle physiological saline at room temperature (25–26°C) for 2–3 h prior to the recording session. Throughout recording, the test slice was at the same temperature, and perfused continuously with the same solution at 1 ml/min. At the end of each day’s recording session (5–8 h), the slices were refrigerated (4°C) overnight in a sealed container of turtle physiological saline that had been previously saturated with 98% O2–2% CO2. For recording on subsequent days (i.e., ≤48 h following surgery), the slices were warmed to room temperature.

Recording and measurement procedures

Intracellular potentials were recorded in spinal MNs at depths of ~100–300 μm from the cut surface of the slice, using sharp microelectrodes of thin-wall borosilicate glass with filament (1.5 mm OD). Electrodes were filled with 1 M KCl acetate. Successful penetration of a spinal MN was characterized by a rapid negative shift in membrane potential, and maintenance of a resting potential more negative than ~60 mV, thereby precluding excessive damage to the cell. The cell sample was restricted to those requiring an injected current >0.4 nA for the generation of APs. This precluded the testing of ventral-horn interneurons (see following text for further MN criteria). The membrane potential of each test MN was amplified, filtered (0–3 kHz), and recorded on both a tape recorder and a computer.

MEMBRANE PROPERTIES. Spinal MNs were studied for an average of 51 min (range, 19–218 min). Passive (cell-at-rest) properties measured included resting membrane potential (in mV), input resistance (in MΩ), and membrane time constant (in ms). The “steady-state” (not peak) input resistance was measured as the slope of the linear regression of a voltage response to four, 2-s constant-current pulses (2 depolarizing, 2 hyperpolarizing; each an average of 4 successive sweeps) of varying magnitude, which were applied to the cell at its resting potential. The time constant was averaged from the decay of the voltage response at the termination of four current steps used during input resistance determination.

Transitional (rest-to-threshold) properties included the rheobase current (nA) and four afterhyperpolarization (AHP) parameters that were based on the AP generated in the rheobase test. Rheobase current was measured as the smallest-amplitude, 2-s injected current pulse that elicited a single AP. The AHP measurements included the following: fast AHP amplitude, voltage difference (mV) between AP-spike onset and peak of the fast component of the AHP; slow AHP amplitude, voltage difference (mV) between spike onset and peak of the AHP’s slow component; AHP duration, time (ms) from AP-spike onset to termination of postspike hyperpolarization; and slow AHP half-decay time, time (ms) for voltage decay from peak of the AHP’s slow component to one-half of that level.

Traditional active (repetitive firing) properties included the following: minimum current necessary for repetitive firing (Imin, nA; current required to elicit >5 APs throughout a 2-s stimulus); minimum (Imin) and maximum (Imax) firing rates (Hz) of repetitive discharge (determined by the MN discharge frequency during the 2nd s of a 2-s current pulse); Imax (nA), stimulus current at Imax and, one-phase f/I slope (Hz/nA), slope of the steady-state one-phase stimulus current (I)–spike frequency (f) relation. Note that this one-phase measurement was for the primary range of firing (terminology of Kornell for anesthetized cat MNs) (Kornell 1965a–c), because turtle MNs in a SC slice do not exhibit Kornell’s secondary range: i.e., an abrupt increase in f/I slope for firing rates at higher stimulus strengths (Hornby et al. 2002a; McDonagh et al. 1998a; cf., however, Hounsgaard et al. 1988b). Note further that the profile of the steady state I-f relation across vertebrate MNs is somewhat sigmoidal, with the f/I slope becoming progressively lower at higher current intensities (e.g., in the lamprey, Buchanan 1993; turtle, Hounskaard et al. 1988b; and cat, Granit et al. 1963; Kornell 1965b). For the issues addressed since Kornell’s seminal (Kornell 1965a–c) reports on cat MNs, it has been considered sufficient to approximate the relation as a linear one (e.g., Binder et al. 1993; Kornell 1999; Powers and Binder 2001). For the present study, too, a linear approximate was appropriate. In addition, however, it was necessary to divide the f/I slope into two phases, as described below.

TWO-PHASE MEASUREMENT OF THE f/I SLOPE. Following application of excitatory modulators, many of our turtle MNs exhibited PP behavior (see following text). The result was a transition point in their I-f relation, with the pretransition phase markedly steeper (greater) than the posttransition one. This behavior necessitated a two-phase measurement of the f/I slope as shown in Fig. 1.

The two-phase I-f relationship required three sets of data points: Imin,fmin; Itrans,ftrans, and, Imax,fmax. The Imin,fmin and Imax,fmax measurements were made as in our previous reports (e.g., McDonagh et al. 1998a). In the test (modulated) condition when PP behavior was present, Itrans,ftrans measurements were made by visual inspection, because there was usually a clear-cut transition point in the I-f relation. This point was obscure for all cells in the control state, and in those cells that did not display modulator-induced PP type behavior. In such cases, Itrans was denoted as the current midway between Imin and Imax, and ftrans was denoted as the spike-frequency at that current. For the control versus test (modulated) condition, this latter Itrans value relative to the Imin value (162%) of non-PP generating cells was quite close to the corresponding value of the PP-generating cells (167%). Our latter Itrans procedure had the advantage of allowing an objective (nonarbitrary) statistical comparison between the initial, steeper f/I slope of the non-PP generating cells (both in the control and modulated state) versus those with PP behavior in which the shift of the transition point was dramatic (see following text). Note further that the term “transition point” refers only to a change in the slope of the I-f relation. It gives no direct indication of the onset and/or time course of accelerated (PP-influenced) MN discharge both within and across the various stimulus current steps that were used in this study.

![Image](https://example.com/image.png)

**FIG. 1.** Measurement of the modulator-induced, 2-phase current-frequency (I-f) relation. Shown is a schematic of the I-f relation of a generic motoneuron (MN) in a neutral bathing medium (control, ○), and following application of 1 or more excitatory modulators (test, ●) that produced plateau potential (PP) behavior in many of the cells. Criteria for selection of the data points Itrans,ftrans, Itrans,ftrans, and Imax,fmax are described in the text. In this and several subsequent figures, the measurement (and visualization) of the 1-phase I-f relation involved determining the slope of a straight line connecting Imin,fmin to Imax,fmax. [Note: for simplicity, the mean I-f relations in this and all subsequent figures are drawn with lines connecting the relevant pairs of points. Therefore as in our previous work (McDonagh et al. 1998a), the values of the diagrammed slopes of the I-f relations in the following I-f figures differ slightly from those used in the analysis].
The pretransition \( f/I \) slope was calculated as the regression line of all the data before and including the transition point. Similarly, the posttransition \( f/I \) slope was calculated as the regression line of all the data after and including the transition point. The \( f/I \) slope ratio was calculated as the latter slope divided by the former one. This parameter provided an indication of the percentage change in the gain of the \( I-f \) relation.

Identification of MNs. For largely anatomical reasons, it is not practical to identify MNs by the antidromic activation of the short, highly dispersed ventral-root filaments in a slice of turtle spinal cord (McDonagh et al. 1998a; see their Fig. 1). Further, some MNs may have their axons cut when making the test slices. All the tested cells of the present study had an input resistance value >2.6 MΩ, a rheobase current >0.4 nA M and an \( f/I \) slope <50 Hz/\( nA \). Previously, our laboratory has shown that these values clearly separate MNs from interneurons using both electrophysiological criteria (McDonagh et al. 1998a) and morphological evidence from reconstructions of stained MNs and interneurons (McDonagh et al. 1998b, 1999a). It is conceivable, but not likely, that these cells could have been segmental or ascending-tract interneurons. For example, in our previous morphological study of the soma diameter of >350 μm turtle MNs, we saw virtually no other cells of comparable diameter in the ventral horn (Callister et al. 1996; see also Ruigrok et al. 1984).

Pharmacological procedures

Excitatory modulators were applied individually and in combination at concentrations consistent with previously published results and/or at doses that (at least in our own work) produced maximal alterations in MN behavior without damage to the cell or the onset of spontaneous discharge. We used the term “excitatory” to denote the net facilitative effect (increased input resistance, decreased rheobase, increased gain of the \( I-f \) relation) of these agents on neuronal excitability. The agents were first made up as high-concentration stock solutions in standard turtle physiological saline, and later diluted to the final concentration. They included serotonin (5-HT; 100 μM) (Hounsghaard and Kiehn 1989), muscarnic (20 μM) (Svirskis and Hounsgaard 1998), and trans-1-amino-1,3-cyclopentane, dicarboxylic acid (t-ACPD; 20 μM). While both t-ACPD (Svirskis and Hounsgaard 1995) and cis-ACPD (Svirskis and Hounsgaard 1998) have been reported to modulate MN behavior, our preliminary experiments showed that application of t-ACPD was appropriate for our present purposes; i.e., it substantially modulated MN properties, including the production of PP-like behavior.

Intrinsic MN properties were first determined in control bathing medium, then remeasured following addition of a modulator, or combination of modulators, to the bathing medium. In preliminary experiments, measurement of MN properties at 6–8 versus 30 min following modulator application revealed only small differences in properties. Therefore statistical comparisons were made between the initial control MN properties versus those measured after 6–8 min of modulator application. Note further that measurements made in a subsequent control bathing medium 1–2 h following the test measurements were almost identical to the initial control ones. Some residual modulatory effects were sometimes noted, however. For this reason, the present procedure was to use separate SC slices for the measurements made on each single MN.

To investigate PP behavior further, the ionic channel blockers TTX (1 μM; a blocker of the fast Na+ channel) and the dihydropyridine, nifedipine (15 μM; a partial blocker of the L-type Ca2+ channel) (Hounsgaard and Mintz 1988) were applied for ≤30 min before the properties of MNs were reinvestigated. Although higher nifedipine concentrations (≥50 μM) have been used to block the L-type conductance in turtle spinal neurons (Russo and Hounsgaard 1994), we used lower concentrations to reduce the possibility of an N-type Ca2+ blockade, as has been observed in other vertebrate neurons (Jones and Jacobs 1990; Wilkinson and Barnes 1996).

Both blocking agents were dissolved in DMSO and diluted with physiological saline to appropriate concentrations. Preliminary experiments revealed no changes in intrinsic MN properties following application of DMSO alone. This finding was consistent with previous studies showing that DMSO alone has no significant effect on intrinsic vertebrate neuron conductances, but rather altered glutamatergic (Lu and Mattson 2001) and cholinergic (Kubota et al. 1998) transmission.

Identification of PPs

Background. Previously, Russell and Hartline (1982) have provided 12 tests, which, in various combinations, have been used to identify PPs (see also Hartline and Graubard 1992). For turtle MNs, Hounsgaard et al. (1986) utilized a subset of these tests for verification of the presence of a PP. Importantly, Hounsgaard and Kiehn (1989) have emphasized the value of a single criterion, wherein the MN exhibits an acceleration, rather than adaptation, of its discharge in response to a constant-current stimulus. This acceleration was shown to be due primarily to an increase in an L-type Ca2+ conductance sensitive to the dihydropyridines (Hounsgaard and Kiehn 1989; Svirskis and Hounsgaard 1998). The significance of this criterion is reinforced by the previous literature across vertebrates on MN spike-frequency adaptation, because it has shown consistently that in non-PP generating vertebrate MNs, firing rate progressively decreases throughout the constant-stimulus period (e.g., Kornell and Monster 1982; Powers et al. 1999).

Present approach. In 64% of the cells tested, discharge following excitatory modulation was characterized by the appearance of accelerated and maintained discharge (as discussed above) before and/or during the 2nd s of the 2-s stimulus pulses. We interpreted this change as commensurate with PP-like behavior (hereafter termed PP behavior). Similarly, we observed a marked alteration in the \( I-f \) relation from near-linearity (1-phase) to a two-phase relation with a steep initial slope followed by a shallower one at higher current intensities. Although this behavior was concomitant with PP behavior, the criterion for PP manifestation was the acceleration of frequency during a 2-s current pulse. This interpretation required some control experiments using previously established criteria (see above). For example, Fig. 2 demonstrated a modulator-induced, nifedipine-sensitive accelerated MN discharge during a 2-s, constant-current intracellular stimulus.

In Fig. 2C, note that the control response is at a relatively low spike frequency, adapting throughout the 2-s stimulus epoch progressively from an initial ~20-Hz rate to <5 Hz after 1.5 s of the 2-s stimulus pulse. This is in sharp contrast to the PP behavior brought on in the test (muscarnic-modulated) condition. In the latter case, the cell first demonstrated an initial adaptation of spike frequency (from ~60 to 35 Hz) in the first 0.5 s of stimulation. Then, throughout the next 0.5 s, firing rate accelerated back to the initial higher level. The subsequent application of nifedipine blocked the L-type Ca2+ conductance to a large, but not complete extent, as revealed by the reduction in spike frequency and the loss of the modulator-induced acceleration phase.

The Fig. 2 result was consistent with the previous results of Hounsgaard and Kiehn (1989), and it was obtained in several MNs following application of excitatory agents. We further obtained qualitatively similar results for some TTX-applied MNs, in which a gradually increasing depolarization of membrane potential was observed during a constant-current, 2-s stimulus (for details, see Hornby 2000). Based on the above findings, and consistent with the previous description of PPs in the in vitro turtle SC slice (Hounsgaard and Kiehn 1989), PP MNs were identified as those cells demonstrating accelerated firing responses during the 2-s, constant-current intracellular stimulus pulses used for determination of the \( I-f \) relation.

Membrane potential oscillations. For 3/46 MNs, oscillatory membrane behavior was observed following excitatory modulation, as has been observed previously following application of muscarine to
turtle MNs in a spinal cord slice preparation (Guerin and Hounsgaard 1999; Svirskis and Hounsgaard 1998). In two of three cells, which were excluded from this study, such activity was manifested sub-threshold (i.e., without depolarizing current injection), and stable recording was not maintained for determination of the full array of electrophysiological properties. The third cell was included because its oscillatory behavior was observed only during long-duration (30-s) intracellular stimulation. Since the protocol for determination of its oscillatory behavior was observed only during long-duration (30-s) intracellular stimulation. Since the protocol for determination of intrinsic MN properties involved the use of current stimuli ≥2 s in duration, this cell’s measured properties were not affected by the oscillatory behavior.

Statistical analysis

Due to the size-related variability of most intrinsic MN properties (Zengel et al. 1985), changes in measured parameters are expressed below as a percentage of the control values. The significance of these differences for the control versus test condition was evaluated by use of standard paired t-test. The signifi cance of differences in the relative extent of modulation by the various modulators was tested by the use of a standard unpaired t-test. In both cases, significance was noted at P < 0.05 and <0.01.

RESULTS

The database for this study consisted of 44 MNs whose properties were within the range of MNs values reported previously (Hornby et al. 2002b; McDonagh et al. 1998a). AHP measurements could not be made accurately in 2/44 MNs, a problem encountered in previous reports from this and other laboratories (e.g., Kernell 1966; McDonagh et al. 1998a). For two other MNs, PP behavior began at $I_{\text{min}}$ as has been seen previously (Bennett et al. 1998b) for PPs activated at or below the threshold for AP generation. For these two latter cells, values are reported for only the one-phase $I_f$ relation (i.e., the data point for $I_{\text{trans}}f_{\text{trans}}$ was considered to coincide with that for $I_{\text{min}}f_{\text{min}}$).

The results are reported below in the order of the effects of excitatory modulation on the following: 1) the 44-cell sample to a single modulator or combination thereof, 2) 16/44 non-PP versus 28/44 PP MNs, and 3) selected low- versus high-threshold PP MNs. The analysis featured systematic, tabular statistical data, with one table presented below and another five available on request (i.e., Tables 4.1B to 4.3A in Hornby 2000).

Effects of excitatory modulation on MNs

PASSIVE AND TRANSITIONAL PROPERTIES. Excitatory modulation reduced the resting membrane potential (range of the 4 mean modulator effects, 1.2–6.8%), increased the input resistance (17–32%), decreased rheobase current (−23 to −38%), and decreased the values for the four AHP parameters (−0.1 to −23%). The combined relative extents of change and their statistical significance were in the parameter order: rheobase current (3/4 modulator-induced changes significant at $P < 0.05$) > input resistance (2/4 significant) > slow AHP amplitude (3/4 significant) > slow AHP half-time decay (2/4 significant) > AHP duration (1/4 significant) > resting potential (2/4 significant) > membrane time constant and fast AHP amplitude (consistently slight and insignificant effects). The relative efficacy of the four modulator applications was in the order as follows: 5-HT (significant changes in 6/8 parameters) > combined 3 modulators (5/8 parameters) > muscarine (2/8) > t-ACPD (consistently modest and relatively insignificant effects).

ACTIVE PROPERTIES: ONE-PHASE $I_f$ RELATION. The effect of excitatory modulation was relatively stronger on the five parameters of the one-phase $I_f$ relation than on the cells’ passive and transitional properties. The values of $I_{\text{min}}$ and $f_{\text{max}}$ were
particularly affected, with $I_{\text{min}}$ reduced significantly for three of four modulator applications (range of the 4 mean modulator effects, −21 to −42%), and $f_{\text{max}}$ increased significantly for all four applications (23–59%). The overall effect of modulation was to shift the $I$-$f$ relation to the left and upward, with a lesser (but consistent) increase in the slope of the relation. These changes were similar for 5-HT, muscarine, and the combined modulators, with all such effects much greater than those evoked by t-ACPD.

**ACTIVE PROPERTIES: TWO-PHASE $I$-$f$ RELATION.** For the five additional parameters of the two-phase $I$-$f$ relation, Fig. 3 shows that only the posttransition $f/I$ slope was not altered significantly by the four-modulator applications (mean changes, 0.8–19%). The changes were significant for $I_{\text{trans}}$ (−14 to −31%, 3/4 applications), $f_{\text{trans}}$ (30–90%, 3/4 applications), pretransition $f/I$ slope (55–238%, 4/4 applications), and $f/I$ slope ratio (35 to −55%, 4/4 applications). The result of these changes was to not only shift the $I$-$f$ relation to the left and upward, but to also elicit a more pronounced two-phase profile of the $I$-$f$ relation. The relative extent of these changes was in the order as follows: muscarine = combined modulators > 5-HT > t-ACPD.

The impact of PP behavior on the $I$-$f$ relation shows the effects of each modulator on combined groups of non-PP and PP MNs. In such samples, the possibility existed that the excitatory modulators affected the non-PP and PP MNs differently. This was clearly the case, as shown in Fig. 4, A—D, for the effects of 5-HT and muscarine on non-PP versus PP MNs (with similar results obtained for t-ACPD and the combined 3 modulators).

There are three main features to the Fig. 4, A—D, comparison of non-PP versus PP cells. 1) The modulators had a greater effect on the $I$-$f$ relation of PP versus non-PP cells. This was attributable largely to the greater modulator-induced reduction in $I_{\text{trans}}$ values and increase in $f_{\text{trans}}$ of PP cells. The result was to increase the pretransition $f/I$ slope, flatten the posttransition $f/I$ slope, thereby reducing the $f/I$ slope ratio of the PP cells to a greater degree than that observed in non-PP cells. 2) The modulators increased the $f_{\text{max}}$ value to the same relative extent for PP versus non-PP cells, thereby indicating that the maximum discharge capability of the MNs was not dependent on the presence of a PP. 3) The relative efficacy of the modulators was generally in the order as follows: combined modulators > muscarine = 5-HT > t-ACPD.

**EFFECTS OF NIFEDIPINE ON PP MNS.** The effects of nifedipine were two-fold on PP-generating MNs. First, it consistently reduced but did not abolish the acceleration of MN discharge during the 2-s depolarizing current (Fig. 2C above). Second, nifedipine consistently modified the modulator-induced two-phase $I$-$f$ relation profile back toward but not reaching the one-phase profile (i.e., similar to the control condition). This change (Fig. 2D) involved a migration of the transition point downward (decreased $f_{\text{trans}}$) and to the right (increased $I_{\text{trans}}$), as compared with the PP-generating state. Previous studies have shown similar decreases in the acceleration of discharge following the application of nifedipine (Hounsgaard and Kiehn 1989; Hounsgaard and Mintz 1988). Such demonstrations of changes in the $I$-$f$ relations are quite rare, however (e.g., as in Hounsgaard and Mintz 1988). Our results suggest that the alteration in the $I$-$f$ relation in PP MNs is not solely dependent on manifestation of PP behavior. Rather, other conductances must also contribute, at least in part, as occurs in the non-PP cells following extrinsic modulation (see following text).

**GROUPED EFFECTS OF THE FOUR EXCITATORY MODULATORS.** The two samples of non-PP versus PP cells in Fig. 4, A—D, together with those for t-ACPD and the combined modulators, were often too small (range, 2–9 cells) for a statistical verification of trends. Previous reports have emphasized, however, that the mechanisms by which the three presently used modu-
lators induce PP behavior are relatively similar. In particular, previous results support the often-stated argument that changes in PPs are dependent, at least in part, on an alteration in outward K⁺ currents (Hounsgaard and Kiehn 1989; Svirsksis and Hounsgaard 1998). While the possibility of direct modulation of the L-type Ca²⁺ conductance by excitatory agonists is still open to debate (Russo and Hounsgaard 1999), the following analysis grouped the responses of the 16 non-PP versus the 28 PP MNs based on the likelihood of similar mechanisms being responsible for PP generation.

**Passive and transitional properties.** The extent of modulation of the resting potential (non-PP, 5.0 ± 6.5%, mean ± SD; PP, 3.6 ± 5.1%; both at $P < 0.01$) and input resistance (non-PP, 26 ± 28%, $P < 0.01$; PP, 24 ± 22%, $P < 0.05$) was quite similar for the cell groups. While rheobase current was modulated significantly in both non-PP (−22 ± 22%) and PP (−33 ± 22%) MNs ($P < 0.01$), there was a significant difference in the extent of alteration between the groups ($P < 0.05$). Strikingly, the changes induced in AHP parameters were quite different for non-PP versus PP MNs (details in Hornby et al. 2001a). For PP MNs, only the value of slow AHP amplitude was changed significantly (−11 ± 12%, $P < 0.01$). In contrast, the values for all four AHP parameters in non-PP MNs changed significantly (range, −9 to −22%; $P < 0.01$). Accordingly, differences in the extent of change in AHP properties (other than slow AHP amplitude) were significantly greater for non-PP MNs ($P < 0.05$; see also Hornby et al. 2001a).

**Active properties. Analysis of the one-phase $I-f$ relation.** Table 1 shows that for three of five parameters of the one-phase $I-f$ relation ($I_{\text{min}}, I_{\text{max}}, 1$-phase $f/I$ slope), the grouped modulator effect was significant (20–30%) but similar for both non-PP and PP cells. For the remaining two parameters, $f_{\text{min}}$ and $I_{\text{max}}$, this effect was significant for PP (60 and 10%, respectively) but not non-PP cells. As a result, the overall modulator effect on the one-phase $I-f$ relation was to shift the relationship upward and leftward. These effects occurred to a greater degree in PP versus non-PP cells. There was virtually no difference in the extent of change in the one-phase $f/I$ slope between the two cell groups, however.

**Active properties. Analysis of the two-phase $I-f$ relation.** Figure 4, E and F, and Table 1 show the powerful effects of excitatory modulation on the grouped non-PP and PP MNs. In non-PP cells, significant modulator-induced changes were observed in three of five of the additional $I-f$ parameters: i.e., $I_{\text{trans}}, f_{\text{trans}},$ and pretransition $f/I$ slope. For PP cells, significant

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**FIG. 4.** Excitatory modulation of the 2-phase $I-f$ relation of non-PP vs. PP MNs. A: 5-HT induced a clear leftward shift in the $I-f$ relation of non-PP (7/11) MNs and a trend toward a steeper pretransition $f/I$ slope, with changes in some $I-f$ parameters. B: 5-HT–induced effects were greatly augmented in PP MNs (4/11), with pronounced changes in most $I-f$ parameters. C: muscarine induced a less-pronounced leftward shift in the mean $I-f$ relation of non-PP (3/11) MNs, and no trend toward a 2-phase relation. D: muscarine–induced effects were greatly augmented in the PP (8/11) cells, with pronounced changes in nearly all $I-f$ parameters. In summary on A–D, both 5-HT and muscarine had a far greater effect on the $I-f$ relation of PP vs. non-PP MNs, albeit the effects on the latter were clear-cut. E and F: comparison of the average 2-phase $I-f$ relation of all of the non-PP (E, 16/44) vs. PP (F, 28/44) cells. Responses are again shown for control (○) vs. test (●) condition (i.e., for the latter, sets of 11 cells were tested after the application of either 5-HT, muscarine, t-ACPD, or their combination, with $x$-axes values scaled to illustrate the differential effects). Asterisks (∗∗) indicate statistical significance ($P < 0.01$) of a spike-frequency difference for a control vs. modulated condition (for further statistics, see Table 1). Note that the 2 control $I-f$ relation plots had a similar, relatively modest 2-phase profile, whereas the 2 test ones (particularly that for PP cells) had 2 clear phases.
TABLE 1. Active properties of non-PP versus PP MNs: grouped control versus modulated responses

<table>
<thead>
<tr>
<th>Active Property, Units</th>
<th>Non-PP Cells</th>
<th>PP Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{min}}$, nA</td>
<td>2.2 ± 1.3</td>
<td>2.7 ± 2.2</td>
</tr>
<tr>
<td>$\Delta%$</td>
<td>-23 ± 20*</td>
<td>-34 ± 24*</td>
</tr>
<tr>
<td>$f_{\text{min}}$, Hz</td>
<td>6.9 ± 2.4</td>
<td>6.3 ± 2.0</td>
</tr>
<tr>
<td>$\Delta%$</td>
<td>17 ± 57</td>
<td>60 ± 94*</td>
</tr>
<tr>
<td>$I_{\text{trans}}$, nA</td>
<td>3.5 ± 1.9</td>
<td>4.5 ± 2.7</td>
</tr>
<tr>
<td>$\Delta%$</td>
<td>-12 ± 9.9*</td>
<td>-35 ± 27†</td>
</tr>
<tr>
<td>$f_{\text{max}}$, Hz</td>
<td>26 ± 6.9</td>
<td>27 ± 6.7</td>
</tr>
<tr>
<td>$\Delta%$</td>
<td>38 ± 34*</td>
<td>63 ± 49*</td>
</tr>
<tr>
<td>$I_{\text{max}}$, nA</td>
<td>4.8 ± 2.5</td>
<td>6.3 ± 3.3</td>
</tr>
<tr>
<td>$\Delta%$</td>
<td>-2.4 ± 10</td>
<td>-10 ± 19†</td>
</tr>
<tr>
<td>$f_{\text{max}}$, Hz</td>
<td>37 ± 10</td>
<td>42 ± 13</td>
</tr>
<tr>
<td>$\Delta%$</td>
<td>38 ± 29*</td>
<td>39 ± 31*</td>
</tr>
<tr>
<td>One-phase $I/f$ slope,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hz/nA</td>
<td>16 ± 12</td>
<td>12 ± 6.3</td>
</tr>
<tr>
<td>$\Delta%$</td>
<td>29 ± 32†</td>
<td>28 ± 85‡</td>
</tr>
<tr>
<td>Pretransition $I/f$ slope,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hz/nA</td>
<td>18 ± 13</td>
<td>15 ± 8.7</td>
</tr>
<tr>
<td>$\Delta%$</td>
<td>43 ± 42*</td>
<td>216 ± 205†</td>
</tr>
<tr>
<td>Posttransition $I/f$ slope,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hz/nA</td>
<td>13 ± 11</td>
<td>9.5 ± 5.6</td>
</tr>
<tr>
<td>$\Delta%$</td>
<td>14 ± 36</td>
<td>-27 ± 40‡</td>
</tr>
<tr>
<td>$I/f$ slope ratio</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>$\Delta%$</td>
<td>-16 ± 29</td>
<td>-67 ± 25†</td>
</tr>
</tbody>
</table>

For each property, the means ± SD control value for non-PP vs. PP cells is provided, together with the modulator-induced mean change (Δ ± SD; in %). Number of Non-PP cells is 16 and PP cells is 28. This table groups the effects of excitatory modulation on active properties of non-plateau potential (non-PP) vs. PP motoneurons (MN) (i.e., as in Fig. 4 and F). Paired comparisons were made separately for the control vs. modulated MN properties of both the non-PP and PP groups, and unpaired comparisons were made between the groups. Significance: * $P < 0.01$; † $P < 0.05$. Non-PP vs. PP significance: ‡ $P < 0.01$.

Changes were observed in all five parameters (i.e., including the posttransition $I/f$ slope and the $I/f$ slope ratio).

Figure 4, E and F, and Table 1 demonstrate that among the full complement of 10 parameters of the 2-phase $I/f$ relation, 6 exhibited a grouped modulator effect that was nearly or greater than twice as pronounced in PP versus non-PP cells. Particularly noteworthy was the difference in the shift of the transition point leftward and upward. Specifically, there was a significant reduction in 5.5 to 3.1 nA for the $I_{\text{trans}}$ value of non-PP cells versus 4.5 to 2.9 nA for PP cells ($P < 0.01$ for greater effect on the PP cells). These changes were evident with similar relative $I_{\text{trans}}$ values as compared with $I_{\text{min}}$ for the PP cells ($I_{\text{trans}}$ in control state was 167% of $I_{\text{min}}$ vs. 162% of $I_{\text{min}}$ for the test condition) due to the decreasing $I_{\text{min}}$ values as described above. Conversely, the relative changes in $I_{\text{min}}$, as compared with $I_{\text{trans}}$ in the non-PP cells was increased for the modulated condition (180 vs. 159% in control condition) due to the decreased $I_{\text{min}}$ (from 2.2 to 1.7 nA) values with a nearly similar absolute change in the $I_{\text{trans}}$ (3.5 to 3.1 nA). Further, significant increases in $f_{\text{trans}}$ of both non-PP (26 to 36 Hz) and PP (27 to 44 Hz) cells were evident, with no significant differences between the groups, however. These combined results primarily account for the significant difference between the groups in the increased pretransition $I/f$ slope (PP, 216% vs. non-PP, 38%) and significant opposite changes in the posttransition $I/f$ slope (PP, -27% vs. non-PP, 14%). For the latter, note that the negative PP change was significant, whereas the positive non-PP change was not. Accordingly, the change in the $I/f$ slope ratio was also far more pronounced for PP versus non-PP MNs. To further emphasize the flattening of the $I/f$ slope beyond the transition point, for 7/28 of the PP cells, the posttransition $I/f$ slopes were <2.0 Hz/nA (vs. 10–15 Hz/nA in the control state). In addition, in 10/28 PP MNs, excitatory modulation induced a negative posttransition $I/f$ slope for greater than or equal to three consecutive increasing stimulus current steps, which were applied well before that producing the $I_{\text{trans}}$ value. None of the entire population of cells exhibited this behavior in the control condition.

Summary. There are five features to the above grouped non-PP versus PP MN comparisons. 1) The passive properties of non-PP versus PP cells were essentially similar, and they did not predict the differences observed in their $I/f$ relation. 2) There were significant differences in the effects of modulation on the transitional properties of non-PP versus PP MNs, with greater effects on the AHP of non-PP MNs, and the rheobase current of PP MNs. 3) Differences in rheobase were correlated with the extent of the leftward shift in the $I/f$ relation (i.e., thereby lowering the $I_{\text{min}}$ value) of the two cell groups. 4) There was a significantly greater modulator-induced alteration in the transition point of PP versus non-PP MNs. It included a greater increase in $f_{\text{trans}}$ values (albeit not significant), and a significant leftward shift (i.e., greater decrease) in $I_{\text{trans}}$ values. This difference in the relative modulation of the transition point underscored the change in the fundamental $I/f$ relation from a more-traditional linear one-phase $I/f$ relation to the newly presented two-phase one. 5) A substantial proportion (10/28) of PP MNs exhibited a negative input-output transformation of stimulus current to spike frequency during a component of the posttransition-phase of the $I/f$ relation.

Effects of excitatory modulation on low- versus high-threshold PP MNs

In recent reports on PP behavior in hindlimb MNs in the decerebrate cat, Lee and Heckman (1998a,b) reported a greater propensity and extent of PP behavior in low- versus high-threshold MNs. The present experimental protocol was not designed to address this issue. Nonetheless, one analysis demonstrated an interesting difference, which is shown in Fig. 5. It compares the effects of modulation of the $I/f$ relation of the 5/28 lowest-threshold PP MNs to that of the 5/28 highest-threshold PP MNs.

Three relevant properties had widely different control values for the low- versus high-threshold PP cells of Fig. 5. These mean values were, respectively, input resistance (MΩ), 23 versus 4.4; rheobase current (nA), 0.6 versus 5.2; and, one-phase $I/f$ slope (Hz/nA), 16 versus 4.0. Clearly, the comparison was of the near extremes of the low- versus high-threshold cells for turtle hindlimb MNs (Hornby et al. 2002b; McDonagh et al. 1998a).

Figure 5 shows that the low-threshold cells were modulated to a greater extent in their spike-frequency responses, particularly $f_{\text{trans}}$ (84% increase vs. 9% for high-threshold cells) and $I_{\text{max}}$ (27 vs. 6% increase). In contrast, the high-threshold cells exhibited relatively greater changes (by 3- to 8-fold) in their $I_{\text{min}}$, $I_{\text{trans}}$, and $I_{\text{max}}$ (values in Fig. 5 legend) and in the steepening of their pretransition slope (409% increase vs. 206% for low-threshold cells), flattening of their posttransition $I/f$ slope (29 vs. 9% reduction), and lowering of the slope ratio.

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Effects of excitatory modulation on non-PP versus PP MNs

The present work supported previous findings demonstrating a modulator-induced reduction in resting potential, increase in input resistance, reduction in rheobase current, and reduction in various AHP parameters (for review: Binder et al. 1996; Powers and Binder 2001; Russo and Hounsgaard 1999). These changes could result primarily from alterations in two key potassium conductances. First, and most consistently across studies and vertebrate species, is a reduction of the resting $K^+$ conductance, noted primarily by a decrease in resting potential and increase in input resistance. Application of 5-HT has generated such effects in rat spinal (Elliott and Wallis 1992; Wang and Dun 1990), phrenic (Lindsay and Feldman 1993), and facial (Larkman and Kelly 1992) MNs, and in cat (White and Fung 1989) MNs. Similar results have also been noted in mouse spinal neurons (Nowak and MacDonald 1983), and turtle (Svirskis and Hounsgaard 1998) and cat (Ziegglansberger and Reiter 1974) MNs following modulation by muscarine. Second, and perhaps less consistently, is a reduction in the $K_{Ca}$ current, which is primarily responsible for the slow component of the AHP, and which may also be active in a MN at rest. Modulation of AHP amplitude and duration following application of 5-HT has been observed in spinal MNs of the lamprey (Wallén et al. 1989), turtle (Hounsgaard and Kiehn 1989), and cat (White and Fung 1989), and in rat hypoglossal MNs (Berger et al. 1992). Depression of the AHP following modulation by muscarine has been observed in hippocampal (Fraser and MacVicar 1996; Madison et al. 1987) and neocortical (Schwindt et al. 1988) cells and has been thought to indicate a reduction in $K^+$ conductances, as well.

Effects of excitatory modulation on the $I-f$ relation

The quantitative differences between the modulator-induced changes in the $I-f$ responses of non-PP versus PP MNs have not been reported previously. The major differences were due largely to the greater effect of excitatory modulation on the transition values ($I_{trans}/f_{trans}$) associated with PP behavior. The changes in these parameters were much more substantial in PP versus non-PP MNs, and they were reflected in the greater modulator-induced changes in the pre- and posttransition $I/f$ slope values between cell types. Note also that the effect of excitatory modulation on non-PP cells was to increase their posttransition $I/f$ slope, whereas the corresponding effect on PP cells was a significant reduction in this slope. Indeed, for 7/28 of the PP cells, the modulated posttransition $I/f$ slope value was $<2.0$ Hz/nA, and 10/28 PP MNs demonstrated a negative
posttransition slope value for greater than or equal to three consecutive steps of increasing stimulus strength. This latter finding is particularly relevant to the previous high-decerebrate cat results of Brownstone et al. (1992) (see following text).

Mechanisms underlying generation of the two-phase I-f relation in PP MNs

In the present study of the I-f relation, we used a current-step paradigm like the classical one of Granit et al. (1966) and Kernell (1965a–c, 1966, 1979, 1999); i.e., step-wise increasing, 2-s current pulses at 0.1 Hz. Our procedure may have brought on the “warm-up” phenomenon (Russo and Hounsgaard 1994), which can include a decreasing threshold for PP activation near the AP threshold (Bennett et al. 1998b). The resultant I-f relation should then be characterized by a pronounced increase in the f/I slope and a shift of the transition point to a lower level of stimulus intensity. These changes were both demonstrated in the present work. Two recent studies on decerebrate cat MNs provided additional, compelling evidence for pronounced modulator-induced alterations in the I-f relation following PP activation (Bennett et al. 1998a; Lee and Heckman 1998a). Their results were qualitatively similar to the present ones, but a further comparison must await a study that evaluates the contribution of the stimulation paradigm to the I-f relation of PP MNs; e.g., by comparing the effects of current-step versus triangular-wave paradigms in the same test cells.

The pronounced modulator-induced flattening of the f/I slope following the transition point was a key feature of our present results. While all our present PP MNs demonstrated profound reductions in the f/I slope after the transition point, negative f/I slopes were observed for only a few data points, and posttransition f/I slopes were always >0 Hz/nA.

Three mechanisms may contribute to the flattening of the f/I slope observed following generation of PP behavior. 1) The mechanism underlying PPs is predominantly a persistent inward current resulting in a sustained depolarization ~20–30 mV less negative than the resting membrane potential (Hounsgaard and Mintz 1988). In dorsal horn cells of the turtle spinal cord slice, Russo and Hounsgaard (1994) have also demonstrated a two- to fourfold reduction in input resistance concurrent with PP activation. Although not tested directly in our present sample, such an alteration in input resistance would render additional synaptic or intracellular current impressed on the MN less effective in altering discharge frequency (Lee and Heckman 1998a; cf., however, Bennett et al. 1998a,b). 2) Regulation of intracellular Ca^{2+} levels may control PP activation and alter MN discharge rate during intracellular stimulation. Previous studies have demonstrated reductions in Ca^{2+} currents via elevation of intracellular Ca^{2+} levels (Tillotson 1979), or second messengers generated by a ryanodine receptor (Chavis et al. 1996; Nakai et al. 1996). The regulation of intracellular Ca^{2+} may suppress PP activity following its generation, thereby decreasing discharge frequency and flattening of the f/I slope. Such behavior could also account for the modulator-induced negative f/I slope that was observed in 10/28 of our PP MNs. 3) Saturation of MN discharge capability has been observed previously in cat MNs during strong intracellular current injection (Kernell 1965c), sensory afferent stimulation (Burke 1968; Cordo and Rymer 1982), and electrical stimulation of brain stem centers (Tansey and Botterman 1996; Zajac and Young 1980). Such “rate-limiting” behavior of MNs has also been observed during voluntary activation in both cats (Hoffer et al. 1987) and humans (De Luca et al. 1982; Monster and Chan 1977). Saturation of MN discharge capability is a less likely possibility for the present results, however, because f_max was always greater than f_trance and the overall f/I slope never reached 0 Hz/nA. The mechanisms for the observed rate-limiting in other studies may be linked to PP generation, however (for review, see Hornby et al. 2002a).

In summary, at least three mechanisms, and likely further as-yet-undetermined ones, can account for the pronounced rate-limiting behavior observed in the PP MNs of the present study. These same mechanisms may also explain the Brownstone et al. (1992) results, as discussed in the following text.

Ubiquity of PP behavior

It is not clear whether all vertebrate MNs can generate PPs. Early in the cellular/systems-level PP literature, it was argued that PPs should assist MNs involved in postural tasks because their proportion was found to be greater in MNs innervating extensor musculature (Hounsgaard et al. 1988a). Similarly, this value was emphasized in the prediction that they should be more prevalent in proximal versus distal limb muscles, and utilized more in tasks requiring static versus dynamic force production (Heckman and Lee 1999b). Alternatively, PPs have been implicated in segmental interneuronal and MN behavior during rhythmically changing pattern-generating activity (Hartline et al. 1988; Kiehn 1991; Selverston 1999). To this point, no quantitative evidence is available on the relative occurrence of PPs in MNs supplying different muscles, and when MNs are involved in different tasks (for further review, see Hornby et al. 2001b).

For the adult cat, early research on a persistent inward current evident in anesthetized preparations indicated that such behavior might be present in ~50% of MNs studied (Schwindt and Crill 1980a–c). More recently, there is evidence that PPs are more fully developed and longer lasting, and hence more efficacious, in spinal, low-threshold MNs (Lee and Heckman 1998a,b). Other evidence has shown, however, that high-threshold cat MNs can also generate substantial PPs (Bennett et al. 1998a). The present work shows that very-high threshold MNs are just as prone to exhibit PPs, as are low-threshold ones (Fig. 5). Note further that Lee and Heckman (2000) have recently demonstrated that most MNs studied under modulatory influences did not generate self-sustained PPs, although such input allowed strong amplification of excitatory (Ia) synaptic input in all the tested cells.

The purpose of the various studies cited above was not to directly address the generality of PPs among selected MN populations, and whether PP-generation is task specific. Similarly, there is no evidence at the cellular-molecular level as to why the MNs of a given motor nucleus may or may not generate PPs. Therefore these important functional issues remain open.

Relation between current and previous results on MN behavior in reduced preparations

In a study on the paralyzed high-decerebrate cat (Brownstone 1989; Brownstone et al. 1992), depolarizing current was
intracellularly injected into spinal extensor MNs during the active phase of their fictive locomotor cycle (i.e., as brought on by repetitive brain stem stimulation). In five of six MNs there was no further increase in spike frequency with increasing stimulus strength; i.e., the f/I slope became zero. This controversial finding, and the surprisingly limited directly relevant subsequent studies (i.e., see ad seriatim: Bennett et al. 1998a,b; Brownstone et al. 1994; Edwards et al. 1997; Fedirchuk et al. 1998; Heckman and Lee 1999a; Lee and Heckman 1998a,b; Schmidt 1994) have been reviewed elsewhere (Hornby 2000). It is sufficient here to point out that the rate-limiting behavior observed by Brownstone et al. (1992) has been confirmed recently by Lee and Heckman (1998a), as described above. Notably, such rate-limiting behavior is not readily observed in anesthetized, spinalized cat preparations, probably because general anesthetics reduce PPs (Guertin and Hounsgaard 1999). The present Figs. 4 and 5 add further evidence in support of the original Brownstone et al. (1992) observations on this rate-limiting phenomenon, particularly if their results are explained by their argument that their MNs exhibited PPs during controlled fictive locomotion (see also Brownstone et al. 1994). It seems likely that the several mechanisms proposed above for the flattening of the posttransition f/I slope could all come into play during elaboration of fictive locomotion in the high-decerebrate cat.

In conclusion, the present study showed that modulation of turtle MNs dramatically altered their fundamental input-output relation, particularly during PP generation. Still open are the issues of the MN type that exhibited rate-limiting behavior in the present study and the previous one of Brownstone et al. (1992). Was it the low-threshold group, as favored by Heckman and Lee (1999b) and suggested indirectly by the human motor unit results of Monster and Chan (1977)? Conversely, was it the high-threshold group, as suggested by our Fig. 5 results (see also Bennett et al. 1998a,b; Hultborn 1999)? For technical reasons (McDonagh et al. 1999b), the latter were the most likely ones studied by Brownstone et al. (1992).

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