State-dependent hyperpolarization of voltage threshold enhances motoneurone excitability during fictive locomotion in the cat

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1. Experiments were conducted on decerebrate adult cats to examine the effect of brainstem-evoked fictive locomotion on the threshold voltage (V_{th}) at which action potentials were initiated in hindlimb motoneurones. Measurements of the voltage threshold of the first spike evoked by intracellular injection of depolarizing ramp currents or square pulses were compared during control and fictive locomotor conditions. The sample of motoneurones included flexor and extensor motoneurones, and motoneurones with low and high rheobase currents.

2. In all 38 motoneurones examined, action potentials were initiated at more hyperpolarized membrane potentials during fictive locomotion than in control conditions (mean hyperpolarization -8.0 ± 5.5 mV; range -1.8 to -26.6 mV). Hyperpolarization of V_{th} occurred immediately at the onset of fictive locomotion and recovered in seconds (typically < 60 s) following the termination of locomotor activity.

3. The V_{th} of spikes occurring spontaneously without intracellular current injection was also reduced during locomotion.

4. Superimposition of rhythmic depolarizing current pulses on current ramps in the absence of locomotion did not lower V_{th} to the extent seen during fictive locomotion. We suggest that V_{th} hyperpolarization results from an as yet undetermined neuromodulatory process operating during locomotion and is not simply the result of the oscillations in membrane potential occurring during locomotion.The hyperpolarization of V_{th} for action potential initiation during locomotion is a state-dependent increase in motoneurone excitability. This V_{th} hyperpolarization may be a fundamental process in the generation of motoneurone activity during locomotion and perhaps other motor tasks.

Electrical stimulation of the brainstem in paralysed decerebrate cats evokes a centrally generated pattern of motor output (fictive locomotion) that has many of the characteristics of overground locomotion in adult quadripedal mammals (see Rossignol, 1996). During fictive locomotion, motoneurones innervating limb muscles receive alternating excitatory and inhibitory synaptic currents from the central pattern generator (CPG) for locomotion (Jordan, 1983). These result in the rhythmic fluctuations of membrane potential (locomotor drive potentials, LDPs) that underlie the patterned activation of motoneurones during locomotion. The transformation of rhythmic excitatory drive into trains of action potentials is governed by the passive and active membrane properties of motoneurones. It is now known that some of these properties are altered during locomotion. For example, the post-spike after-hyperpolarization (AHP) is reduced in motoneurones during fictive locomotion (Brownstone *et al.* 1992; Schmidt, 1994) and there is the appearance of a voltage-dependent excitatory current (Brownstone *et al.* 1994). This voltage-dependent excitation results in non-linear responses of motoneurones to depolarizing currents, which may facilitate the recruitment of motoneurones, or augment motoneuronal output evoked by reflex or central excitation (Brownstone *et al.* 1994; McCrea *et al.* 1997; Bennett *et al.* 1998). These changes in motoneurone membrane properties result in increased motoneuronal firing in response to intracellular current injection during fictive locomotion (Brownstone *et al.* 1992; Fedirchuk *et al.* 1998). The fictive locomotor state thus appears to include processes that increase the excitability of hindlimb motoneurones.

The membrane potential at which action potentials are initiated in response to sufficient depolarizing currents (the voltage threshold, V_{th}) is not a fixed value in motoneurones. For example, V_{th} tends to be higher (more depolarized) in higher rheobase motoneurones (Gustafsson & Pinter, 1984) and the V_{th} of action potentials occurring

later in a train during repetitive firing increases (accommodation) (Kolmodin & Skoglund, 1958). The present study sought to determine whether the V_{th} of hindlimb motoneurones was altered during fictive locomotion. To this end V_{th} was measured in motoneurones recorded in decerebrate and paralysed adult cats during control conditions and during brainstem-evoked fictive locomotion. A comparison of the V_{th} threshold in each motoneurone during these two states revealed a locomotor-related decrease (i.e. hyperpolarization) in motoneuronal V_{th} during fictive locomotion. We suggest that V_{th} hyperpolarization is another means by which motoneurone properties are re-configured to enhance motoneuronal output in the locomotor state. Parts of these data have been presented in abstract form (Krawitz *et al.* 1997).

METHODS

Surgical procedures

Data were obtained from six cats of either sex weighing 2.0–3.1 kg. All surgical and experimental protocols were in compliance with the guidelines set out by the Canadian Council on Animal Care and the University of Manitoba. Anaesthesia was induced and maintained with halothane (5% and 0.8–1.8%, respectively) delivered in an oxygen/nitrous oxide mixture (40%/60%). A surgical plane of anaesthesia was confirmed by continuous monitoring of the arterial blood pressure via a carotid artery cannula and by repeatedly testing for the lack of pedal withdrawal and corneal reflexes as well as muscle tone. A glucose sodium bicarbonate buffer (5 g glucose, 0.84 g $NaHCO_3$ per 100 ml) was infused intravenously (5–10 ml h^{-1}) throughout the experiment.

The peripheral nerves innervating the following muscles of the left hindlimb were dissected and cut: sartorius (Sart), the posterior biceps mounted with semitendinosus, semimembranosus-anterior biceps (SmAB), lateral gastrocnemius-soleus (LGS), medial gastrocnemius (MG), plantaris (Pl), flexor digitorum or hallucis longus (FDHL), the remaining mixed posterior tibial nerve (Tib) as it enters the foot, and tibialis anterior (TA). The common peroneal nerve could be stimulated to test for antidromic activation, and the nerve innervating the right anterior biceps was dissected to monitor extensor activity of the contralateral hindlimb. Remaining ipsilateral and contralateral branches of the femoral, sciatic and common peroneal nerves, and the tendons attached around the hip joint, were cut.

A dorsal laminectomy of the L4–L6 vertebrae exposed the lower lumbar spinal cord, and the animal was fixed in a stereotaxic recording frame. Mineral oil pools were fashioned for the spinal cord and both hindlimbs, and the dissected nerves were placed on conventional silver hook bipolar electrodes for stimulation or recording. A craniotomy was performed and a mechanical precollicular/postmammillary decerebration was completed. All tissue rostral to the plane of decerebration was removed rendering the animal totally insentient and allowing the anaesthetic to be discontinued. The animal was injected with the neuromuscular blocker Pavulon (pancuronium bromide; 1.2 mg, supplemented with 0.6 mg every 45 min) and artificially ventilated to maintain expired CO_2 at 3–5%. Decreases in blood pressure were countered by the intravenous administration of a blood volume expander (6% Gentran 70). At the termination of the experiments, the animals were killed by the intravenous administration of potassium chloride.

Fictive locomotion

Electrical stimulation (50–220 μA, 0.5 ms pulses at 15–27 Hz) of the mesencephalic locomotor region (MLR) was used to evoke rhythmic and alternating activity in hindlimb flexor and extensor motoneurones. Monopolar stimulating electrodes were placed bilaterally in the brainstem and their positions adjusted to optimize the production of fictive locomotion. Unilateral stimulation was usually sufficient to evoke locomotion but occasionally bilateral stimulation was required. Fictive locomotion was monitored from amplified, rectified and filtered records from peripheral nerves (electroneurograms, ENGs), which were displayed continuously.

Intracellular recordings

Intracellular recordings from lumbar motoneurones were obtained using glass microelectrodes filled with 2 M potassium acetate solution (3–8 MΩ). The primary aim of this study was to compare the membrane potential at which action potentials were initiated (V_{th}) during control and locomotor conditions. Use of the discontinuous current clamp (DCC) mode of an Axoclamp-2A amplifier (Axon Instruments) permitted reliable measurements of membrane potential during injection of large intracellular currents. The ability of the electrode to pass the current without rectification was continuously assessed using a high speed, high gain oscilloscope trace of the electrode voltage (i.e. the unswitched 'monitor' output). Only data where comparisons between control and locomotor conditions were made using the same DCC switching rate are reported. The intracellular recording and a monitor of injected current were digitized (membrane potential at 10 kHz; monitor of injected current at 3.3 kHz), as well as rectified-integrated ENG and cord dorsum recordings (500 Hz and 5 kHz, respectively), and stored on a computer for subsequent analysis using software developed within our group (details at www.scrc.umanitoba.ca/doc/).

Immediately after impalement motoneurones were identified by antidromic activation from one of the peripheral nerves. For the 12 motoneurones not antidromically activated by any of the peripheral nerves available, the presumptive motoneurone was classified as flexor or extensor based on its pattern of activity during fictive locomotion. Hyperpolarizing 50 ms current pulses (typically 2 nA), were injected to determine the motoneuronal input resistance, although in four motoneurones input resistance was estimated from depolarizing current injections of < 5 nA. Rheobase was defined as the minimum amplitude of a depolarizing (50 ms duration) current pulse that evoked an action potential. In two cells rheobase was estimated using a slowly rising current ramp (see Table 1). The intracellular amplifier was then placed in DCC mode and the switching rate for current injection adjusted while monitoring electrode voltage. Under control conditions without MLR stimulation and fictive locomotion, injection of a triangular ramp or pulses of depolarizing current were used to initiate an action potential(s) in the motoneurone. Fictive locomotion was evoked later in the same trial, and the intracellular current injection was repeated (see Fig. 1*A*). The extracellular DC potential recorded immediately after withdrawing the microelectrode was measured and subtracted from the intracellular potential. Recordings in which the intracellular or extracellular DC values were suspected of drifting were discarded.

Voltage threshold measurement

Voltage threshold (V_{th}) was measured for the first spike elicited either from 50 ms depolarizing square pulses or, more commonly, from slow (5–15 s) triangular ramps of intracellularly injected depolarizing current (as in Fig. 1). Measurements are reported only for the first spike evoked to avoid the potential for previous spikes to influence V_{th} by either spike accommodation or inter-spike trajectory. In order to standardize measurements, V_{th} was defined as the membrane potential at which depolarization increased at $\sim$10 V s^{-1} (i.e. the initiation of the action potential; see Brownstone *et al.* 1992). At the 10 kHz sampling rate used, this V_{th} estimate corresponded to the voltage value of the first data point where the following data point was $\geqslant 1$ mV depolarized. The V_{th} of each cell

was measured in the same data file during control conditions (i.e. in the absence of brainstem stimulation) and brainstem-evoked fictive locomotion. Each cell thus served as its own control (see Fig. 1). In some cells, several ramps of current that varied in amplitude and/or duration were injected but comparisons between the individual V_{th} measurements during the locomotor and control states were usually made from identically shaped current injections (as in Fig. 1). In some cases a hyperpolarizing bias current was injected from which the depolarizing ramp was initiated. This procedure permitted measurement of the V_{th} of the first action potential during locomotion when locomotion-related firing would have interfered with the measurement (e.g. Fig. 5).

RESULTS

Locomotor-related changes in V_{th} were assessed in 38 motoneurones innervating a variety of hindlimb muscles and having action potential amplitudes in control

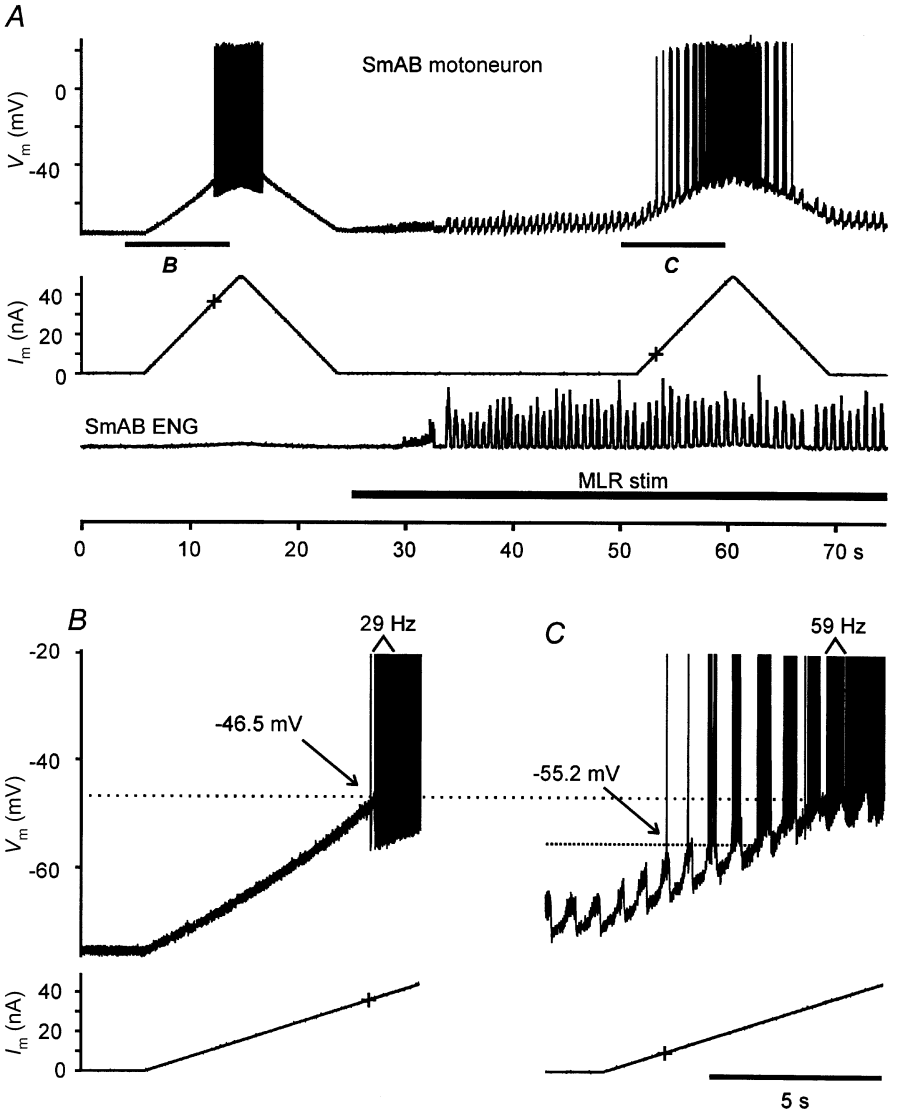

Figure 1. Firing was elicited from antidromically identified lumbar motoneurones by intracellular current injection, prior to and during MLR-evoked fictive locomotion

A shows a trial for a SmAB motoneurone, where a 50 nA ramp of current was injected, after which the brainstem stimulation was started (indicated by the bar under the ENG trace). Fictive locomotion was evident as rhythmic activity that alternated between extensor and flexor ENGs (not illustrated). Discontinuous current clamp recording allowed accurate measurement of the membrane potential during simultaneous current injection. Bars labelled *B* and *C* denote the time periods expanded in panels *B* and *C*. *B* shows that the voltage threshold for production of action potentials (V_{th}) before fictive locomotion was −46.5 mV. *C* shows that during fictive locomotion, less current was required to fire the neurone (compare current at +) and the V_{th} was hyperpolarized compared to *B*. Note that the neurone fired at 29 Hz before locomotion, and at 59 Hz at the same membrane potential during locomotion (see bracketed areas). The *Y*-axes in *B* also apply to *C*, and the time bar shown below *C* also applies to *B*. V_m, membrane potential; I_m, membrane current.

Table 1. Changes in motoneurone V_{th} during fictive locomotion

Motoneurone ($n = 38$)	Rheobase (nA)	R_{in} (MΩ)	V_m control (mV)	V_{th} Control (mV)	V_{th} Locomotion (mV)	V_{th} Difference (mV)
MG	2.0	1.6	−69.3	−64.4	−66.2	−1.8
Tib	2.4	2.6	−61.9	−49.2	−52.0	−2.8
SmAB	2.5	1.1	−63.0	−48.8	−69.7	−21.9
Tib	2.9	1.2	−66.7	−52.4	−55.6	−3.2
E	4.0	1.1	−62.5	−50.0	−53.0	−3.0
E	4.0	0.9	−60.0	−49.2	−51.3	−2.1
MG	4.7	1.2	−75.9	−49.0	−68.1	−19.1
E	5.4	1.4	−63.7	−46.5	−52.2	−5.7
MG	5.9	1.5	−69.4	−49.9	−53.0	−3.1
FDHL	6.0	0.6	−64.0	−47.6	−74.2	−26.6
MG	6.0	1.0	−72.0	−55.1	−63.1	−8.0
E	7.0	1.2	−65.0	−53.3	−63.3	−10.0
SmAB	7.4	1.9†	−65.3	−36.6	−49.3	−12.7
F	7.5	1.4	−72.1	−53.4	−57.7	−4.3
FDHL	7.5	0.8	−58.2	−36.1	−44.1	−8.0
MG	7.9	1.3	−67.1	−38.6	−44.6	−6.0
E	8.0	1.0	−54.0	−38.5	−47.8	−9.3
E	8.2	0.6	−54.0	−33.5	−39.7	−6.2
Tib	8.9	0.7	−84.0	−52.5	−54.5	−2.0
E	9.0	1.1	−69.2	−48.2	−57.9	−9.7
F	9.1	0.9	−65.4	−38.3	−44.0	−5.7
F	11.4	0.8†	−66.6	−49.5	−55.6	−6.1
F	11.6	0.9	−67.1	−47.1	−49.7	−2.6
MG	11.9	0.9	−63.0	−41.4	−43.6	−2.2
SmAB	12.9	0.9	−76.3	−45.6	−50.5	−4.9
MG	12.9	0.9	−75.5	−47.5	−52.7	−5.2
E	14.0	0.5	−60.0	−37.0	−48.8	−11.8
E	15.0	0.5	−74.0	−61.3	−73.7	−12.4
MG	17.5	0.5	−63.2	−43.0	−47.9	−4.9
MG	19.7	0.7	−66.0	−35.2	−45.2	−10.0
Pl	22.5	0.6	−65.0	−42.0	−49.1	−7.1
SmAB	23.7	0.7	−69.6	−32.7	−42.2	−9.5
MG	26.4	0.8	−70.6	−42.9	−46.1	−3.2
E	27.0	0.7	−64.0	−40.1	−52.5	−12.4
SmAB	30.0	0.6	−71.3	−25.2	−33.1	−7.9
SmAB	31.0	0.7	−62.4	−17.4	−29.3	−11.9
SmAB	36.0*	0.6†	−75.6	−46.5	−55.2	−8.7
SmAB	47.0*	0.7†	−64.7	−31.4	−41.7	−10.3
						Mean = −8.0
						S.D. = 5.5

Motoneurones: MG, medial gastrocnemius; Tib, axon projected to the mixed posterior tibial nerve as it enters the foot; SmAB, semimembranosus or anterior biceps; E, presumptive extensor motoneurone; FDHL, flexor digitorum or hallucis longus; F, presumptive flexor motoneurone; Pl, plantaris. R_{in}, input resistance. *Current determined from ramp; † R_{in} determined from ramp.

conditions ranging from 50 to 97 mV (mean 76 mV; 36/38 had spikes of ⩾ 65 mV; 22/38 had spikes of ⩾ 75 mV). Control rheobase and input resistance values are reported in columns 2 and 3 of Table 1. The range of rheobases (2–31 nA) indicates that both motoneurones innervating slow twitch muscle fibres (low rheobase) and those innervating fast twitch muscle fibres were represented in the sample (see Burke, 1981). The 38 motoneurones examined included both flexors and extensors.

The principle aim of the study was to determine the minimum level of membrane depolarization required to evoke an action potential during control and locomotor conditions. Early experiments in this series revealed that the current required to evoke spikes during locomotion was often much less than that required during control conditions. Because of the unpredictability of the change in threshold current during locomotion, V_{th} measurements were most often made from slowly rising current ramps

that began at levels well below threshold. A comparison of the V_{th} of motoneurone spikes elicited from pulse and ramp current injections was made during control (non-locomotor) conditions. In 9 of the 11 motoneurones examined, the V_{th} of the spike evoked by the current ramp was more depolarized than that obtained from the pulse; however, the mean values of V_{th} obtained by the two techniques were not statistically different (Student's paired t test, $P = 0.08$; Wilcoxon signed rank test, $P = 0.07$). All comparisons of V_{th} during locomotor and control conditions are from measurements made using the same technique.

Figure 1*A* shows an intracellular recording from a SmAB motoneurone in which two identical triangular-shaped current injections were delivered. Each went from 0 nA to +50 nA to 0 nA over an 18 s period. The first current injection was delivered in the absence of MLR stimulation and locomotion (control). It evoked an action potential as the current reached 36 nA (marked by a +; see expanded time scale in Fig. 1*B*). The V_{th} was −46.5 mV, as determined by the point at which the change in membrane potential was $\geqslant 10$ V s^{-1}. The second current injection was initiated about 25 s after the onset of electrical stimulation of the MLR (1 ms pulses, 26 Hz). MLR stimulation produced fictive locomotion with a characteristic rhythmic alternation between flexor (not illustrated) and extensor (SmAB illustrated) ENG activity. In this example the rhythmic locomotor-induced depolarizations (i.e. LDPs) were small and well below the amplitude required for recruitment. As can be seen on the expanded time scale of Fig. 1*C*, the current required to initiate an action potential (marked with a +) was reduced during fictive locomotion from the control value of 36 nA to 10 nA. During fictive locomotion the V_{th} was

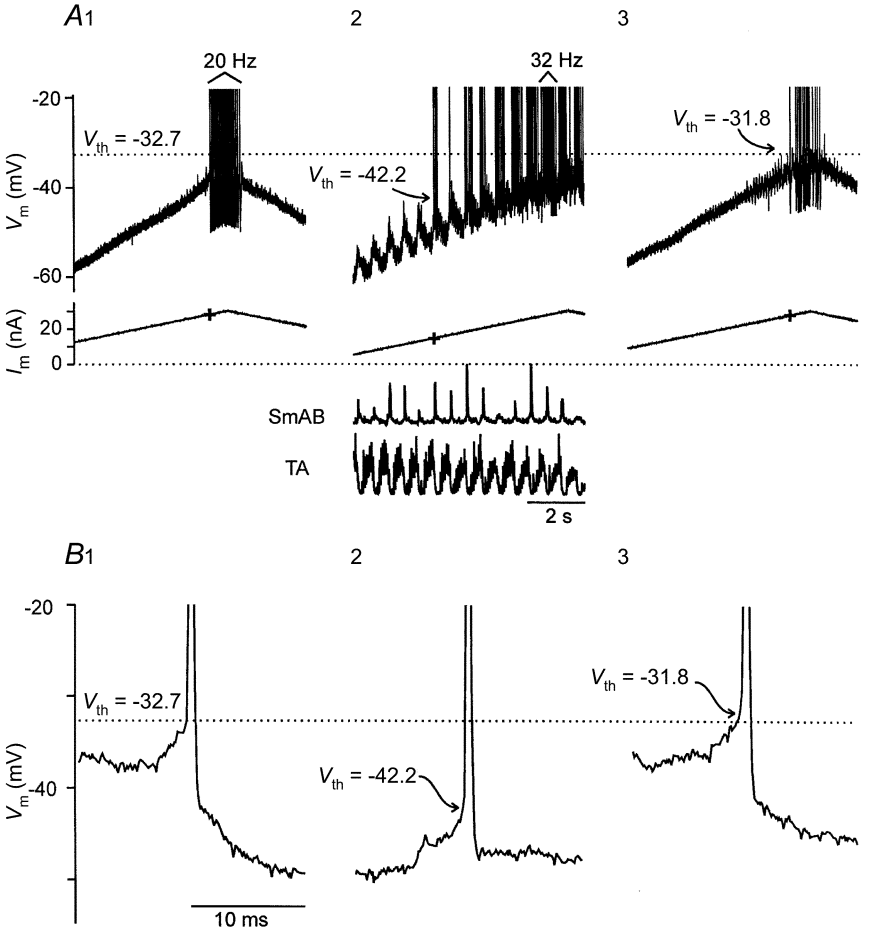

Figure 2. The V_{th} recovers after the cessation of fictive locomotion

This SmAB motoneurone had a V_{th} of −32.7 mV prior to locomotion (*A*1) and −42.2 mV during MLR-evoked fictive locomotion (*A*2; see ENG activity). As in Fig. 1, the current required to elicit firing was reduced during fictive locomotion (compare + in *A*1 and 2) and the neurone fired at a higher rate (32 Hz) during fictive locomotion than at the same membrane potential in the control condition (20 Hz). Within 60 s following the cessation of locomotion the V_{th} had depolarized back to −31.8 mV (*A*3). The time scale shown in *A*2 applies to all traces of *A*1–3. *B*1–3 shows the first action potential of the corresponding firing shown in *A*1–3 on expanded scales to better illustrate the V_{th} value (the point where the V_m $\mathrm{d}V/\mathrm{d}t \geqslant 10$ V s^{-1}). The scale bar in *B*1 also applies to *B*2 and 3.

−55.2 mV, an 8.7 mV hyperpolarization compared to control. Note the increase in the motoneurone firing rate (from 29 to 59 Hz) during fictive locomotion at the same membrane potential (Fig. 1*B* and *C*).

Figure 2 shows a recording from another SmAB motoneurone where the V_{th} was −32.7 mV in the control (Fig. 2*A*1 and *B*1), and −42.2 mV during fictive locomotion (Fig. 2*A*2 and *B*2; i.e. hyperpolarized by 11.5 mV). Note that like the example in Fig. 1, during fictive locomotion less current was needed to evoke an action potential and the motoneurone fired faster (32 *vs.* 20 Hz) at comparable membrane potentials (see Fig. 2*A*1 and 2). A third ramp current injection delivered about 60 s after the cessation of fictive locomotion (Fig. 2*A*3) shows that the V_{th} had returned to −31.8 mV by this time.

The V_{th} of all 38 motoneurones examined during control and locomotor conditions is shown in Table 1. The rightmost column shows that the V_{th} of all 38 hyperpolarized during locomotion (mean -8.0 ± 5.5 mV; median −6.7 mV). Hyperpolarization of V_{th} was not accompanied by a consistent change in the spike overshoot (see Fig. 1) or obvious changes in spike duration (not illustrated). This hyperpolarization of V_{th} was striking both in its incidence (i.e. occurrence in all cells examined) and in the wide range of threshold changes seen during locomotion (−1.8 to −26.6 mV). At present we have no explanation for the differing degrees of threshold lowering in different motoneurones during locomotion. There was no correlation between motoneurone membrane potential recorded before fictive locomotion and V_{th} hyperpolarization during locomotion (linear regression coefficient $r^2 = 0.01$; Pearson product moment correlation, $P = 0.5$). The amount of threshold change was not a function of the particular experiment. In one experiment V_{th} hyperpolarization during fictive locomotion ranged from −1.8 to −19.1 mV in different motoneurones. Figure 3 plots the amount of change in V_{th} against motoneurone rheobase. There was no relationship between motoneurone rheobase and V_{th} hyperpolarization during fictive locomotion (linear regression coefficient $r^2 = 0.01$; Pearson product moment correlation, $P = 0.6$). Thus both high and low rheobase neurones displayed large and small changes in V_{th} during locomotion.

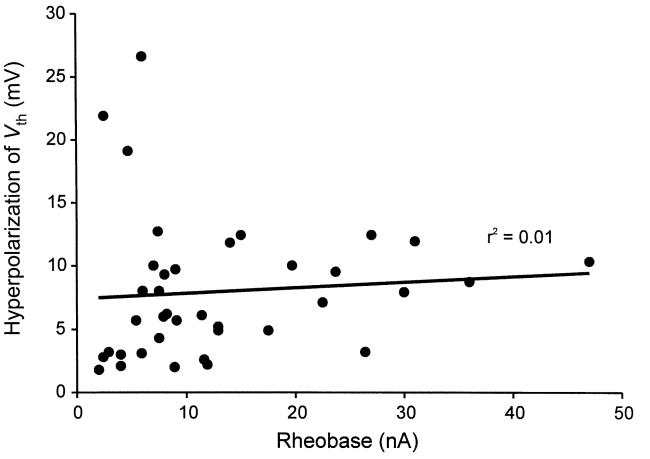

Figure 3. There is no relationship between the amount of V_{th} hyperpolarization during fictive locomotion and the rheobase of the motoneurone

Recovery of V_{th} to within 1 mV of the control value was followed in seven motoneurones. Recovery occurred in three cells in < 30 s, and in 30–145 s in the remaining four cells (see Fig. 2). The use of long duration ramps (typically about 20 s) and the need to wait until all peripheral nerve activity ceased following MLR stimulation precluded an accurate assessment of the minimum time to V_{th} recovery. Nevertheless it is clear that in some cells V_{th} remained hyperpolarized in the period immediately following the bout of fictive locomotion and when rhythmic fluctuations of the membrane potential had ceased.

Figures 1 and 2 show a reduction in the amount of intracellular current needed to evoke an action potential during fictive locomotion compared to control. This increase in motoneurone excitability is, however, difficult to quantify since the depolarization produced by the locomotor circuitry (the depolarizing portion of the LDP) will add to the depolarization produced by current injection. Similarly, estimating changes in the minimum current required to evoke spikes during the hyperpolarizing portion of the LDP is complicated by both the hyperpolarization itself and the synaptic conductances that occur during the hyperpolarizing phase of locomotion. As a result, changes in rheobase current during locomotion are not reported.

Figure 4 illustrates an extensor motoneurone in which the LDPs were large enough to produce rhythmic activation during locomotion in the absence of intracellular current injection. In this cell the control V_{th} was −50 mV (not shown). During locomotion the membrane potential at which spikes were produced on the LDP was −53 mV; i.e. V_{th} became hyperpolarized by 3.0 mV. Current pulses of 4 nA, 50 ms duration were injected into this motoneurone at about 1 Hz with three of these pulse injections shown in the portion of data illustrated in Fig. 4. Since the delivery of current pulses was not synchronized with the fictive step cycle, these pulses occurred at random with respect to the rhythmic depolarization and hyperpolarization of the motoneurone. When these pulses occurred during the hyperpolarized portion of the step cycle it was possible to determine the V_{th} (see the spike produced by the third current pulse injection from the left). The V_{th} of this spike was −53 mV; the same as that produced by locomotor depolarization without current injection. A similar observation was made in one other

cell. Despite the small sample size, these observations are important because they indicate that (1) V_{th} does not vary rhythmically with membrane potential fluctuations during locomotion and (2) V_{th} is reduced to the same extent for spikes produced by the locomotor circuitry and intracellular current injection. The examples in Figs 1 and 2 show a lowering of V_{th} in two motoneurones with small LDPs that were not recruited during fictive locomotion without the addition of intracellular depolarizing current. Thus, the hyperpolarization of V_{th} in these cells during locomotion was not a consequence of motoneurone recruitment.

V_{th} hyperpolarization during locomotion is not the result of rhythmic changes in motoneurone membrane potential

Figure 4 shows that spikes occurring either spontaneously during locomotion or as a result of current injection do so at a hyperpolarized V_{th}. One of the features of locomotion is the rhythmic LDPs in motoneurones. To determine whether rhythmic depolarizations and hyperpolarizations reduce V_{th} in the absence of locomotion, current pulses were superimposed on top of current ramps during control conditions in eight motoneurones. Voltage thresholds were measured for action potentials produced by a current ramp alone (control), those produced by a ramp current with superimposed current pulses without locomotion, and for those occurring on the current ramp during fictive locomotion (as in Figs 1 and 2). Figure 5 illustrates the data from one of these cells. The V_{th} of the first spike produced by ramp current injection alone was −48.2 mV (Fig. 5*A*1 and *B*1). Current pulses (10 nA, ~300 ms) superimposed on the ramp depolarization resulted in ~10 mV membrane potential oscillations (Fig. 5*A*2). The V_{th} of spikes evoked by the combination of ramp and pulse current injection was somewhat hyperpolarized (by 2.3 mV) compared to the spike evoked by ramp current injection alone (Fig. 5*A*2 and *B*2). During locomotion, this motoneurone displayed LDPs with about a 10 mV peak-to-peak amplitude (Fig. 5*A*3). Hyperpolarizing bias current was needed to prevent firing on the LDP during locomotion. During locomotion, the V_{th} of the first spike evoked on the current ramp was −57.9 mV (Fig. 5*A*3 and *B*3). This was a hyperpolarization of 9.7 mV compared to the spike evoked by ramp current during control and a hyperpolarization of 7.4 mV compared to the spike evoked by the combination of ramp and pulse current injection during control conditions. In all eight cells examined in this manner the hyperpolarization of V_{th} during locomotion (9.3 to 26.6 mV) was larger than that produced by the combination of pulse and ramp current injection during control conditions (1.9 to 11.4 mV). The mean V_{th} hyperpolarization during locomotion (14.3 ± 2.1 mV) was significantly larger than the V_{th} hyperpolarization for firing evoked by combining current pulses and ramps (4.7 ± 1.4 mV; paired t test, $P = 0.013$).

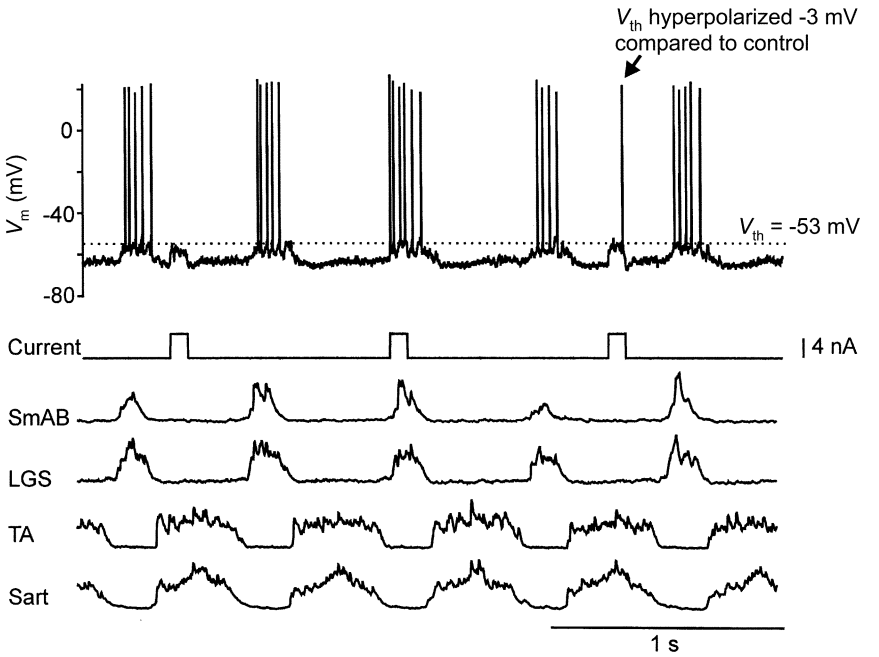

Figure 4. During fictive locomotion the V_{th} is hyperpolarized during both phases of the fictive step cycle

In this extensor motoneurone, current pulses were delivered at approximately 1 Hz during fictive locomotion (lower traces show MLR-evoked ENG activity). One current pulse occurring during the inactive phase of the fictive step cycle elicited an action potential (marked by the arrow) with a V_{th} of −53.0 mV. This was the same as the V_{th} seen during the active phase, and was −3.0 mV hyperpolarized compared to control.

The use of hyperpolarizing bias current in some cells to prevent rhythmic action potential generation during locomotion is potentially a further complication in determining the extent to which V_{th} changes during locomotion. To address the extent of this possible complication, the amount of constant hyperpolarizing current preceding the ramp was varied in six cells in the absence of locomotion. In five cells, changing the hyperpolarizing bias current by 10–20 nA altered V_{th} by $\leqslant$ 2.2 mV. In one cell the change was 7.0 mV. In three of these six cells, V_{th} was increased (i.e. depolarized) by increases in hyperpolarizing bias current, in two cells V_{th} was hyperpolarized with increasing hyperpolarizing bias current, and one cell showed no change in V_{th}. Constant hyperpolarization of the motoneurone preceding the ramp therefore might have small effects on the measured V_{th}. Since this effect is small and often opposite to the hyperpolarization of V_{th} seen during fictive locomotion, it is unlikely to account for the locomotor-related V_{th} hyperpolarization that we have described. In some cases, it may have caused us to underestimate the amount of V_{th} hyperpolarization occurring during locomotion.

Further evidence that the hyperpolarization of V_{th} seen during fictive locomotion is not simply the result of rhythmic fluctuations in membrane potential is presented in Fig. 6. This MG motoneurone was recorded under three conditions. Figure 6*A* shows firing evoked by 15 nA, 250 ms current pulses in the absence of fictive locomotion. The V_{th} during this control condition was

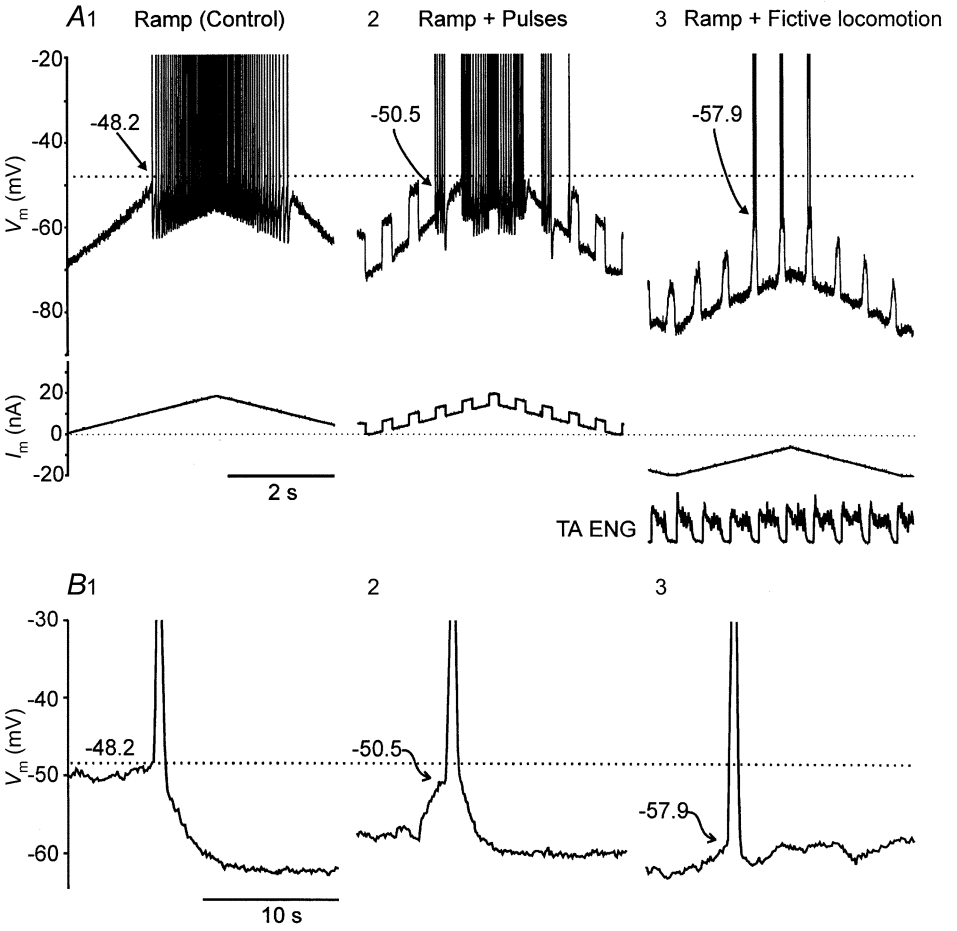

Figure 5. Membrane potential oscillations produced by current pulses can hyperpolarize V_{th}, but not to the same degree as seen during fictive locomotion

*A*1 shows a recording from an extensor motoneurone during a current ramp in the absence of fictive locomotion. The V_{th} in this control condition was −48.2 mV. *A*2 shows that 10 nA, 300 ms current pulses produced approximately 10 mV depolarizations of the membrane potential that were subthreshold for spiking, until they were superimposed on a current ramp. Fictive locomotion was then elicited with MLR stimulation. This neurone required the injection of hyperpolarizing current to be kept from firing spontaneously. As the current ramp was increased, the neurone began firing on the depolarizing portion of the LDPs. The first action potential of the repetitive firing for *A*1–3 is shown in *B*1–3 on expanded time and voltage scales to better illustrate the measured V_{th}. This motoneurone had a control rheobase of 9 nA and a resting V_m of −69.2 mV.

−31.0 mV. Fictive locomotion began soon after MLR stimulation commenced (Fig. 6*B*) but LDPs in this motoneurone were not well developed. The threshold of the spikes evoked by the CPG, without any intracellular current injection, was −37.1 mV (−6.1 mV compared to control). As is common in these preparations, fictive locomotion may change with constant MLR stimulation. After about 3 min prominent LDPs were evident in the motoneurone (Fig. 6*C*). Despite the clear increase in rhythmic drive to this motoneurone, the V_{th} for the LDP-evoked firing (−37.4 mV) was similar to that recorded in Fig. 6*B*. This example illustrates that the extent of threshold lowering in this motoneurone was not related to the size of the LDP. This observation and the prolonged recovery of V_{th} following locomotion suggest that V_{th} is associated with the locomotor state and not the rhythmic changes in motoneurone membrane potential *per se*.

DISCUSSION

This study demonstrates that action potentials in motoneurones are initiated at more negative membrane potentials during fictive locomotion than in the absence of locomotor activity. This locomotor-related hyperpolarization of V_{th} (i.e. lowering of V_{th}) occurred in all 38 motoneurones examined. These included flexors and extensors and motoneurones with either high or low rheobases (see Table 1). Because each motoneurone served as its own control, we could see that V_{th} hyperpolarization occurred immediately at the onset of, and recovered in seconds following, fictive locomotion. Threshold hyperpolarization occurred for spikes recruited during fictive locomotion in the absence of current injection (e.g. Figs 4 and 6), as well for spikes evoked by injection of intracellular depolarizing current (e.g. Figs 1 and 2). In addition, the V_{th} seemed not to be phasically modulated during the depolarizing and hyperpolarizing parts of the fictive step cycle, and the V_{th} hyperpolarization during locomotion was not dependent on the presence of well-developed LDPs. We suggest that V_{th} hyperpolarization is a 'state-dependent' phenomenon associated with the fictive locomotor process.

The present study is the first to demonstrate a reduction in motoneurone V_{th} during locomotion, but not the first report of modulation of neuronal V_{th}. For example, following a classical conditioning paradigm to decrease the amplitude of the H-reflex in monkeys, the mean V_{th} of spinal motoneurones became depolarized (Carp & Wolpaw, 1994). Mean motoneurone threshold potential is also depolarized in chronic spinal cats compared to spinal-intact animals (Hochman & McCrea, 1994). Cleary *et al.* (1998) have shown that the median V_{th} from their sample of motoneurone recordings in *Aplysia* was hyperpolarized the day following long-term sensitization of the siphon withdrawal reflex. Thus the hyperpolarization of V_{th} that we have observed during locomotion may reflect a general means of neuromodulatory control of neuronal excitability in a manner appropriate for a particular behavioural state. The present observations that V_{th} can change within seconds of the onset of brainstem stimulation and before the induction of repetitive firing in motoneurones as well as its recovery following locomotion are consistent with a mechanism that involves release of a neuromodulatory substance. The nature of this neuromodulator and whether spinal or supraspinal sources are involved remains to be determined. It also

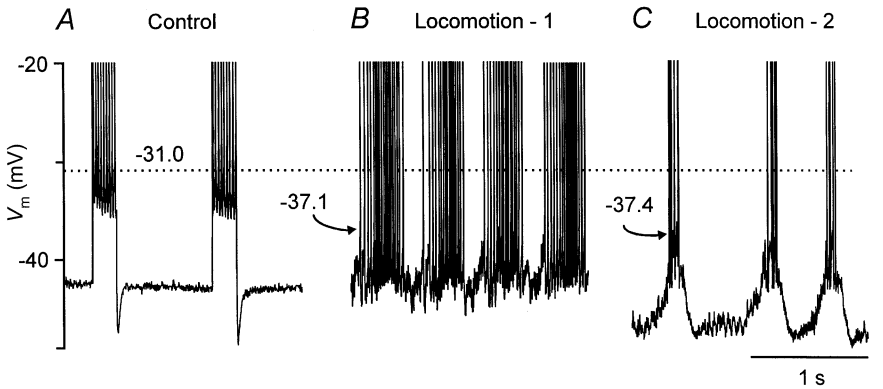

Figure 6. Hyperpolarization of V_{th} during fictive locomotion does not depend on the amplitude of the LDPs in the motoneurone

A shows firing evoked by the injection of current pulses (15 nA, 200 ms) into this MG motoneurone in the absence of fictive locomotion. The V_{th} for this control condition was −31.0 mV and is denoted by the dotted line. *B* shows a recording from this same motoneurone after MLR stimulation had been initiated (Locomotion - 1). The cell exhibited spontaneous firing linked to the fictive step cycle, but had only small LDPs. The V_{th} for this fictive locomotion-induced firing (no current injection) was −37.1 mV. *C* shows the same neurone approximately 3 min later when the fictive locomotion had become more robust (Locomotion - 2). During this period, the cell exhibited approximately 8 mV LDPs and locomotor-related firing that had a V_{th} of −37.4 mV.

remains to be determined whether V_{th} hyperpolarization is a feature of other behaviours or whether different mechanisms contribute to threshold lowering in different species or under different conditions.

Many studies have noted the increase (depolarization) of voltage threshold that occurs during repetitive firing in cat motoneurones. Thus the V_{th} becomes more depolarized for successive action potentials of a spike train induced either by synaptic activation (Kolmodin & Skoglund, 1958) or by intracellular current injection (Granit *et al.* 1963; Barrett *et al.* 1980). This V_{th} depolarization may contribute to the decrease in firing rates seen during long trains of repetitive firing and is thought to be caused by accommodation of sodium channels (Schwindt & Crill, 1982). The locomotor-dependent hyperpolarization of V_{th} described here occurred for the first action potential evoked during fictive locomotion (see Figs 1 and 2) and is, therefore, not a consequence of previous action potentials. This state-dependent hyperpolarization of V_{th} would tend to counter accommodation and the accompanying late adaptation during repetitive firing. The reduction of late adaptation during brainstem-evoked fictive locomotion (Krawitz *et al.* 1996) is consistent with this suggestion. It is also known that motoneurones have biophysical properties related to the type of muscle that they innervate (i.e. slow or fast twitch; see Burke, 1981). In non-locomoting preparations motoneurones innervating fast-type muscle are more likely to show accommodation to current ramps than those innervating slow twitch muscle (Burke & Nelson, 1971). In contrast, the locomotor-related V_{th} hyperpolarization is unrelated to motoneurone type, since large hyperpolarizations of V_{th} occurred in both low and high rheobase motoneurones (Fig. 3 and Table 1).

The hyperpolarization of V_{th} during fictive locomotion is not caused by oscillations of the motoneurone membrane potential underlying the LDPs. V_{th} hyperpolarization produced by superimposing square current pulses on top of current ramps in the absence of locomotion was always smaller than that occurring during locomotion. This is despite the fact that the current pulses caused an even more rapid change in the membrane potential trajectory than the LDPs. Furthermore, the V_{th} hyperpolarization could be large in the absence of substantial LDPs (see Fig. 6*B*) and persisted for seconds after fictive locomotion, when LDPs were absent. Therefore, while the motoneurone membrane potential trajectory during fictive locomotion might contribute to V_{th} hyperpolarization, our results suggest that V_{th} hyperpolarization is caused by a locomotor-dependent modulation of the threshold properties of motoneurones.

To our knowledge, the rapid modulation of V_{th} as a means of enhancing motoneuronal excitability during a motor task has not previously been described in any preparation. Its occurrence in every motoneurone examined indicates that V_{th} lowering, like AHP reduction and the release of voltage-dependent excitation (see Introduction) is another motoneurone membrane property that is regulated during locomotion. Interestingly, these changes in membrane properties would enhance motoneuronal excitability during locomotion and tend to counter the decrease in excitability that could result from the increase in motoneurone conductance that occurs during fictive locomotion (Shefchyk & Jordan, 1985; Gosgnach *et al.* 2000). The large reduction in the current required to evoke firing during locomotion (eg. Figs 1 and 2) suggests that overall the excitability of motoneurones increases during fictive locomotion. This increased excitability would have large ramifications for motoneuronal recruitment and firing since less depolarization from either central or reflex pathways would be required to recruit any given motoneurone. Furthermore, because motoneurone firing properties are different during locomotion from those at rest, predictions of motoneurone firing during locomotion based on their firing properties in the non-locomoting state should be made with caution.

The present study did not examine the mechanism(s) underlying the hyperpolarization of motoneuronal V_{th} during fictive locomotion, nor the direct consequences on repetitive firing. In addition, we have no satisfactory explanation for the wide variation in the degree of V_{th} hyperpolarization seen in different motoneurones (see Table 1). Currently, both physiological studies and computer simulations are being utilized to examine how modulation of motoneuronal sodium and/or potassium conductances might contribute to this phenomenon (Dai *et al.* 1998*a*,*b*, 2000). In addition, a large scale simulation of spinal cord circuitry has been used to show that V_{th} hyperpolarization results in increased output of motoneurone pools in response to an excitatory synaptic input (Dai *et al.* 1999).

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