CHANGES IN MOTONEURONE PROPERTIES DURING FICTIVE LOCOMOTION IN THE DECEREBRATE CAT

BY

SHERRY KRAWITZ

A Thesis submitted to
The Faculty of Graduate Studies
In Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department of Physiology
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

Changes in Motoneurone Properties During Fictive Locomotion in the Decerebrate Cat

BY

Sherry Krawitz

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

This thesis has been made possible only through the guidance, wisdom, and persistent efforts of my supervisor, Dr. L.M. Jordan. Drs. D.A. McCrea, R.M. Brownstone, and B. Fedirchuk have provided critical instruction and assistance. I am also grateful to Dr. B.J. Schmidt. These are understatements. I thank each of you and remain grateful.

Thanks to Mr. Gilles Detillieux for your superb software and assistance. I am pleased to acknowledge the participation of Drs. Y. Dai and B. Noga in some of the experiments. Special thanks to Mrs. Sharon McCartney and Maria Setterbom for unfailing computer skills and help offered with good humour. Mr. Matt Ellis provided excellent technical assistance. Thanks also to Mrs. Gail McIndless.

The work was funded by a Medical Research Council of Canada Studentship.

The gathering of expertise at the Winnipeg Spinal Cord Research Centre is unrivalled. The added advantage of the Winnipeg experience is the pleasure of working with each and every one of the group.

The thesis is dedicated to my parents Gerald and Estelle for your unconditional support. Thank you.

ABSTRACT

Early descriptions of the firing properties of spinal motoneurones were based on the results of intracellular current injection in an animal in a quiescent state, in most cases the barbiturate anesthetised cat. It is now known that some of these properties are altered during locomotion. Changes in motoneurone properties during fictive locomotion evoked by electrical stimulation of the brainstem in paralysed decerebrate cats include the reduction of the post-spike afterhyperpolarisation (Brownstone et al 1992; Schmidt 1994), and the appearance of a voltage-dependent excitatory current (Brownstone et al 1994). This voltage-dependent excitation results in non-linear responses of motoneurones to depolarising 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 and increased excitability of hindlimb motoneurones during fictive locomotion.

These changes provided the impetus to explore the possibility of other changes in spinal motoneurone properties during fictive locomotion in the decerebrate cat. Motoneurone properties examined in this thesis are 1) the membrane potential at which action potentials are initiated (the voltage threshold, Vth), and 2) the tendency of motoneurones to slow their action potential firing rate, over tens of seconds, during sustained or intermittent activation (late adaptation). The thesis consists of two projects:

1) a comparison of the voltage threshold for firing during locomotion and at rest, and
2) a comparison of late adaptation during locomotion and at rest.

The conclusions of the thesis are:

1) The threshold voltage at which action potentials are initiated is reduced (hyperpolarised) during fictive locomotion in the cat. This state-dependent hyperpolarisation of voltage threshold enhances motoneurone excitability during locomotion.
2) Late adaptation is abolished in spinal motoneurones during fictive locomotion.

The underlying mechanisms are not known, and are not explored in this thesis, but it is suggested that the fast sodium channel is modulated during locomotion. Sodium channel modulation is implicated in voltage threshold hyperpolarisation and in the abolition of late adaptation during fictive locomotion.

The demonstrated changes in spinal motoneurone properties during locomotion are further examples of state-dependent changes in "intrinsic" properties of mammalian motoneurones. These changes all contribute to increased excitability of motoneurones during locomotion, and result in firing which is more robust and variable during locomotion than firing evoked by intracellular current injection in an animal in a quiescent state.

GENERAL INTRODUCTION

Part A. Initial descriptions of motoneurone firing based on the results of intracellular current injection

The firing properties of spinal motoneurones have been defined by the results of intracellular current injection in an animal in a quiescent state, in most cases in the barbiturate anesthetised cat. Granit, Kernell and Shortess (Granit et al. 1963) documented with intracellular recordings from rat and cat spinal motoneurones the linear relation between injected current strength and steady firing frequency. This relation delineates the "primary range" of firing (on average 51 Hz), with a corresponding primary frequency-current strength slope [f-I slope; (Kernell 1965a)]. Beyond this range, with stronger stimulating current, the motoneurone is capable of firing at higher frequencies. These discharges at frequencies higher than those compatible with a primary f-I slope, are within a "secondary range" of firing (on average 125 Hz), with an f-I slope 2-6 times steeper than that characterising the primary range. Different firing ranges were thus attributed to cat motoneurones having different repetitive properties when stimulated by weak and strong currents with the proviso that "Results obtained by injected currents should clearly be tested also in situations employing synaptic excitation" (Kernell 1965a). A tertiary range of firing may be produced with even stronger stimulating currents, with an f-I slope generally greater than that of secondary range (Schwindt 1973).

Kernell (1965b) also found corresponding differences in the afterpotentials succeeding the repetitive spikes, the afterhyperpolarisations (AHPs), with progressively shorter AHP duration at higher firing rates. AHP duration was recognised as a determinant of firing

frequency by depressing excitability of the motoneurone (Coombs et al. 1957; Granit et al. 1963), so that as the AHP increases, the firing frequency is reduced.

The augmentation of the AHP was thus considered a contributing factor to the reduction, or adaptation, of firing frequency. The term adaptation was first used to refer to the brief phase of slowing of spike frequency preceding steady state firing (Granit, et al. 1963), more broadly defined as the decrease of firing rate as a function of stimulus duration (Kernell 1965a), or over time. Initial, early, and late phases of adaptation are distinguished (Powers et al. 1999), corresponding to an initial rapid drop in frequency over the first few interspike intervals, followed by a more gradual decline, and subsequent slower process (time constants of 250 ms and 10-20 s, respectively). During repetitive firing of dorsal spinocerebellar tract neurons evoked by constant current injection, AHP depression over the first three interspike intervals is accompanied by a "negative" adaptation, i.e., an initial increase in frequency (Gustafsson et al. 1978). These findings provide direct evidence not only for a role of the AHP in determining spike frequency, and but also for the capability of the nervous system to overcome or reverse adaptation.

Late adaptation, in contrast to initial or early adaptation, refers to the gradual decrease in frequency over tens of seconds of motoneurone firing in response to sustained or intermittent depolarizing current injection in cells in a resting state (Kernell and Monster 1982b; Spielmann et al. 1993; Sawczuk et al. 1995b). Kernell and Monster (1982b) observed late adaptation in motoneurones in the spinal cord of anesthetised cats (steady currents injected for up to four minutes). While the decline in firing frequency occurred over several tens of seconds, most of the late adaptation occurred during the first thirty seconds of firing. The drop in frequency was found to be greater when the firing rate at the beginning of discharge was higher (i.e., with a

stronger intensity of current stimulation). The drop in frequency was also found to be more pronounced for fast-twitch units than for slower twitch units (Kernell and Monster 1982a). The decline in firing rate was hypothesised to be great enough to cause a 60% decrease in force during the first minute of current stimulation, and thus likely a significant factor contributing to fatigue in voluntary or reflex contractions. Slow motoneurones, because their late adaptation is less than that of fast motoneurones, are better suited for maintaining steady postural contractions (Kernell and Monster 1982a). Motoneurones with a small amount of late adaptation would therefore presumably also be better suited for maintaining rhythmic muscle contractions as in locomotion.

Spielmann et al (Spielmann et al. 1993) observed late adaptation in cat spinal motoneurones stimulated by extracellular steady current application over four-minute periods, as well as by intermittent current application. In both instances the reduction in firing frequency was smaller for slow motoneurones than for fast motoneurones, emphasising the advantages of slow motoneurones for maintaining prolonged firing with sustained activation. Sawczuk et al (1995b) recognized three, rather than two, distinct phases of adaptation, and characterised the time course of initial, early, and late adaptation in rat hypoglossal motoneurones (using brainstem slice) in response to 60-second current injections. Sawczuk et al. (1995a) correlated motoneurone firing rates and spike frequency adaptation with recruitment and rate modulation. The match between late adaptation, the progressive decrease in spike frequency, and an increase in motor unit contraction time, was confirmed. As previously suggested (Sawczuk et al. 1995a), this match may be a strategy to optimise force production in fatiguing conditions.

Adaptation is distinguished from "accommodation" which refers to the increase in threshold (firing level) for the initiation of action potentials with increased duration of repetitive

firing. The threshold rises as the rate of rise of the current stimulus decreases, so that the threshold is lower for a rapidly rising depolarising current than for a more slowly depolarising current (e.g., Brownstone 1989; Bradley and Somjen 1960; Schlue et al. 1974). Concomitant phenomena are a decrease in the overshoot/amplitude of the action potential and a decrease in the rate of rise of the action potential. The underlying mechanism is thought to be a combination of partial sodium channel inactivation (by membrane depolarisation) and an increase in potassium conductance.

The voltage threshold, Vth, the membrane potential at which action potentials are initiated in response to depolarising currents, occurs when sodium ion influx exceeds potassium ion efflux, and is thus variable in the quiescent state. The threshold potential is the net result of active conductances in the motoneurone. The conductances in mammalian spinal motoneurone are ten: fast sodium, persistent sodium, delayed rectifier potassium, A-current (transient outward), calcium-dependent potassium, T-type calcium, L-type calcium, N-type calcium, h-current (mixed cation), and potassium leak current (Binder et al. 1996). Threshold properties, i.e., the activation of conductances which is variable even at rest, underlie the phenomena of adaptation and accommodation.

Schwindt and Crill (1980), identified a persistent inward current (PIC) which is non-activating and can last seconds if not deactivated by inhibitory input. Its effect on synaptic integration in the motoneurone is to allow the motoneurone to behave in a bistable manner whereby brief excitatory inputs activate long-lasting plateau potentials, or self-sustained firing, and brief inhibitory inputs return the cell to a resting state (Heckman et al. 2003). The PIC is generated largely by an L-type Ca++ current, with contribution from a persistent Na+ current, and possibly a non-selective cation current (Heckman et al. 2003). The PIC under certain

circumstances can dominate motoneurone firing and is responsible for the phenomena of bistable bahaviour and plateau potentials in the motoneurone (Heckman, et al. 2003) (see further below, part B.

Kolmodin and Skoglund (1958) showed a more depolarised Vth with an increased duration of rhythmic firing in the non-anesthetised spinal cat (accommodation). With increased frequency of firing evoked by afferent stimulation there is a successive lowering of the critical membrane potential for spike initiation. Also observed concurrent with the depolarisation of the firing level was a decrease in spike amplitude. The same variations were found (Sasaki and Otani 1961) in action potentials evoked by current injection as were found during natural activity.

Barrett et al (1980) reproduced these findings by current injection into motoneurones of the anesthetised cat: the somatic voltage threshold is depolarised as the magnitude of the injected current step increases (Schwindt and Crill 1982). Not only does the voltage threshold increase with injected current strength (steady firing rate) but voltage threshold increases over time at a given injected current strength. Spike height also decreases and spike duration increases in parallel with depolarisation of the voltage threshold. These features are described as part of the accommodative process.

Gustafsson and Pinter (1984) also found variability in the depolarisation of the Vth among motoneurone types (in anesthetised cats). Threshold tended to be more depolarised in motoneurones with higher rheobase currents (high-rheobase motoneurones were considered to be fast type; see also Burke and Nelson (1971).

Part B. Motoneurone firing during fictive locomotion differs from firing in the quiescent state

Early reports of motoneurone firing in cats during locomotion report significant differences from firing evoked by intracellular current injection in animals in a quiescent state. First considered are extracellular recordings in the decerebrate cat during treadmill locomotion induced by mesencephalic stimulation:

1) During the burst of motoneurone firing (recorded from ventral root filaments) with each step of treadmill locomotion, initial high frequency firing was followed by adaptation of firing frequency (Zajac and Young 1980). The average firing rate of a motoneurone during a burst of firing did not vary much from step to step (brainstem stimulation for each walk continued for 15-30 seconds, resulting in 15-18 steps by the cat). Zajac and Young also report "doublets" at the beginning of bursts of spikes, initial spikes with a short interspike interval, followed by longer interspike intervals (steady firing rate). Doublet firing would allow rapid development of force to a high level which can then be maintained by subsequent low-frequency stimulation (Zajac and Young 1980; Hoffer et al. 1981). Extracellular recordings during treadmill locomotion in intact adult cats revealed features not observed during firing in decrebrate cats:

2) Hoffer et al (1981) reported continual modulation of firing frequency within a burst of firing (rate modulation) without initial doublets. (In decerebrate cats, further increases in tension

are mediated by recruitment of additional motor units.) Average and peak firing rates increased with faster gaits, also unlike motoneurone firing in decerebrate cats.

Intracellular recordings during fictive locomotion in the decerebrate cat have shown changes in intrinsic membrane properties during the transition from the resting to the locomotor state:

3) The afterhyperpolarisation (AHP) is reduced in motoneurones during the repetitive firing of fictive locomotion in the decerebrate cat, i.e., the AHP amplitude is reduced compared to that following action potentials produced by depolarising current injection (Brownstone et al. 1992; Schmidt 1994). The regulation by the AHP of repetitive firing induced by intracellular current injection is not seen during the repetitive firing of fictive locomotion. In addition, the usual linear relation between the amount of current injected and the frequency of repetitive firing is changed during fictive locomotion. No relation was seen during fictive locomotion, or motoneurone firing may be increased in response to intracellular current injection during fictive locomotion (Brownstone et al. 1992; Fedirchuk et al. 1998).

4) The activation of voltage-dependent conductances during fictive locomotion results in non-linear responses of motoneurones to depolarising currents (Brownstone et al. 1994). The excitatory component of the locomotor drive potential behaves in a voltage-dependent manner, such that its amplitude increases with depolarisation (Brownstone et al. 1994). The non-linear input-output relation is such as that seen with plateau potentials (Hounsgaard et al. 1988).

AHP reduction, the activation of voltage-dependent conductances, and the generation of plateau potentials during locomotion all attest to increased motoneurone excitability, resulting in increased firing frequency during locomotion. Voltage-dependent excitation may facilitate the recruitment of motoneurones, or augment motoneurone output evoked by reflex or central activation (McCrea et al. 1997; Bennett et al. 1998). Schwindt and Crill (1977) suggested that persistent inward currents (PICs) could allow motoneurones to remain depolarised and to fire repetitively after the source of excitatory input was removed. Furthermore, they observed that PICs could allow larger postsynaptic potentials in response to synaptic input as the motoneurone becomes more depolarised.

While there is no direct evidence of PIC activation during locomotion, the PIC is dependent on neuromodulatory input (physiological neurotransmitters, the monoamines) and may therefore influence motoneurone firing during fictive locomotion (Heckman et al. 2003). The PICs result in a long lasting shift in membrane potential, the plateau potential, or self-sustained firing (persisting after the stimulus ceases) by the motoneurone. The L-type Ca++ current plays a large role in generating the PIC, is enhanced by serotonin, and its channels are distributed widely in motoneurone dendrites. As mentioned above, a persistent Na+ current and possibly a non-selective cation current contribute to the PIC. Neuromodulatory control is from several sources in addition to serotonin. The dendritic origin of the PIC situates it ideally for influencing synaptic integration (Heckman et al. 2003). It is tempting to assume activation of the PIC during normal motor behaviour including locomotion.

It is thus well established that fundamental motoneurone properties are altered in the presence of neuromodulators during fictive locomotion, resulting in increased motoneuronal excitability. It is reasonable to ask how other motoneuronal properties which define firing in the

quiescent state (firing induced by intracellular current injection) may be altered with the transition from the resting to the locomotor state.

Bigland-Ritchie (Bigland-Ritchie et al. 1986) suggested that the decline in motoneurone firing rate during fatigue produced by a sustained maximum voluntary contraction may be due to changes in motoneurone excitability. A preliminary observation of spike-frequency adaptation during fictive locomotion (short bouts of locomotion) was that of a lack of adaptation, associated with the decreased AHP (Brownstone 1989). This is consistent with increased motoneuronal excitability during locomotion. In the quiescent state, the Vth is variable, e. g., more depolarised in higher rheobase motoneurones, and also showing accommodation. The voltage threshold is a second property which may be altered in the locomotor state.

The aim of this thesis is to examine possible changes in firing frequency, specifically the presence or absence of late adaptation, and in voltage threshold, during fictive locomotion in the decerebrate cat. Changes in these properties could result in increased motoneurone excitability, and enable and facilitate locomotion.

The following hypotheses are examined:

1) Motoneurone voltage threshold is state-dependent, varying with the transition from the quiescent to the locomotor state.

2) Late adaptation is absent during the locomotor state and may be reversed with the transition from the resting to the locomotor state, resulting in increases in firing frequency during fictive locomotion.

The model is the adult decerebrate cat with MLR-evoked fictive locomotion, presenting the following advantages:

1) Each motoneurone serves as its own control;
2) MLR stimulation obviates the need for exogenous chemical stimulation of the nervous system;
3) The properties described may be readily compared quantitatively with properties originally described in the adult cat preparations of earlier physiological experiments.

REFERENCES

Barrett, E. F., Barrett, J. N., and Crill, W. E. Voltage-sensitive outward currents in cat motoneurones. J Physiol (Lond) 304: 251-276, 1980.

Bennett, D. J., Hultborn, H., Fedirchuk, B., and Gorassini, M. Synaptic activation of plateaus in hindlimb motoneurons of decerebrate cats. J Neurophysiol 80: 2023-2037, 1998.

Bigland-Ritchie, B. R., Dawson, N. J., Johansson, R. S., and Lippold, O. C. J. Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. J Physiol (Lond) 379: 451-459, 1986.

Binder, M., Heckman CJ, and Powers RK The physiological control of motoneuron activity. In Rowell, L. and Shepard, J. eds. Handbook of Physiology. Section 12, Exercise: Regulation and Integration of Multiple Systems. American Physiological Society. 1996, 3-53.

Bradley, K. and Somjen, G. G. Accommodation in motoneurones of the rat and the cat. J Physiol 156: 75-92, 1961.

Brownstone, R. M., Gossard, J.-P., and Hultborn, H. Voltage-dependent excitation of motoneurones from spinal locomotor centres in the cat. Exp Brain Res 102: 34-44, 1994.

Brownstone, R. M., Jordan, L. M., Kriellaars, D. J., Noga, B. R., and Shefchyk, S. On the regulation of repetitive firing in lumbar motoneurones during fictive locomotion in the cat. Exp Brain Res 90: 441-445, 1992.

Brownstone, R. On the regulation of repetitive firing in lumbar motoneurones during fictive locomotion in the cat . Winnipeg, Canada, University of Manitoba, Ph.D. Thesis. 1989.

Burke, R. E. and Nelson, P. G. Accommodation to current ramps in motoneurons of fast and slow twitch motor units. Int J Neurosci 1: 347-56, 1971.

Coombs, J. S., Curtis, D. R., and Eccles, J. C. The interpretation of spike potentials of motoneurones. J Physiol (Lond) 139: 198-231, 1957.

Fedirchuk, B, McCrea, DA, Dai, Y, Jones, KE, and Jordan, LM. Motoneuron frequency/current relationships during fictive locomotion in the cat. Society for Neuroscience Abstracts 24, 652. 98.

Granit, R., Kernell, D., and Shortess, G. K. Quantitative aspects of repetitive firing of mammalian motoneurons caused by injected currents. J Physiol (Lond) 168: 911-931, 1963.

Gustafsson, B., Linstrom, S., and Zangger, P. Firing behaviour of dorsal spinocerebellar tract neurones. J Physiol 275: 321-43, 1978.

Gustafsson, B. and Pinter, M. J. An investigation of threshold properties among cat spinal alpha-motoneurones. J Physiol (Lond) 357: 453-483, 1984.

Heckman, C. J., Lee, R. H., and Brownstone, R. M. Hyperexcitable dendrites in motoneurons and their neuromodulatory control during motor behavior. Trends Neurosci 26: 688-95, 2003.

Hoffer, J. A., O'Donovan, M. J., Pratt, C. A., and Loeb, G. E. Discharge patterns of hindlimb motoneurons during normal cat locomotion. Science 213: 466-467, 1981.

Hounsgaard, J., Hultborn, H., Jespersen, B., and Kiehn, O. Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. J Physiol 405: 345-67, 1988.

Kernell, D. The adaptation and the relation between discharge frequency and current strength of cat lumbosacral motoneurones stimulated by long-lasting injected currents. Acta Physiol Scand 65, 65-73. 1965a.

Kernell, D. The limits of firing frequency in cat lumbosacral motoneurones possessing different time course of afterhyperpolarization. Acta Physiol Scand 65: 87-100, 1965b.

Kernell, D. and Monster, A. W. Motoneurone properties and motor fatigue. An intracellular study of gastrocnemius motoneurones of the cat. Exp Brain Res 46: 197-204, 1982a.

Kernell, D. and Monster, A. W. Time course and properties of late adaption in spinal motoneurones of the cat. Exp Brain Res 46: 191-196, 1982b.

Kolmodin, G. M. and Skoglund, C. R. Slow membrane potential changes accompanying excitation and inhibition in spinal moto- and interneurons in the cat during natural activation. Acta Physiol Scand 44: 11-54, 1958.

McCrea, DA, Krawitz, S, Fedirchuk B, and Jordan, LM. Group I-evoked extensor motoneurone activity is amplified by voltage-dependent depolarizations during locomotion. Soc. Neurosci. Abst. 23, 298.3. 97.

Powers, R. K., Sawczuk, A., Musick, J. R., and Binder, M. D. Multiple mechanisms of spike-frequency adaptation in motoneurones. J Physiol Paris 93: 101-14, 1999.

Sasaki, K. and Otani, T. Accommodation in spinal motoneurons of the cat. Jap. J. Physiol. 11: 443-456, 1961.

Sawczuk, A., Powers, R. K., and Binder, M. D. Intrinsic properties of motoneurons. Implications

for muscle fatigue. Adv Exp Med Biol 384: 123-34, 1995a.

Sawczuk, A., Powers, R. K., and Binder, M. D. Spike frequency adaptation studied in hypoglossal motoneurons of the rat. J Neurophysiol 73: 1799-1810, 1995b.

Schlue, W. R., Richter, D. W., Mauritz, K. H., and Nacimiento, A. C. Mechanisms of accommodation to linearly rising currents in cat spinal motoneurons. J Neurophysiol 37: 310-5, 1974.

Schmidt, B. J. Afterhyperpolarization modulation in lumbar motoneurons during locomotor-like rhythmic activity in the neonatal rat spinal cord *in vitro*. Exp Brain Res 99: 214-222, 1994.

Schwindt, P. and Crill, W. Role of a persistent inward current in motoneuron bursting during spinal seizures. J Neurophysiol 43: 1296-1318, 1980.

Schwindt, P. and Crill, W. E. A persistent negative resistance in cat lumbar motoneurons. Brain Res 120: 173-178, 1977.

Schwindt, P. C. Membrane potential trajectories underlying motoneuron rhythmic firing at high rates. J Neurophysiol 36: 434-449, 1973.

Schwindt, P. C. and Crill, W. E. Properites of a peristent inward current in normal and TEA-injected motoneurons. J Neurophysiol 43: 1700-1724, 1980.

Schwindt, P. C. and Crill, W. E. Factors influencing motoneuron rhythmic firing: results from a voltage-clamp study. J Neurophysiol 48: 875-890, 1982.

Spielmann, J. M., Laouris, Y., Nordstrom, M. A., Robinson, G. A., Reinking, R. M., and Stuart,

D. G. Adaptation of cat motoneurons to sustained and intermittent extracellular activation. J Physiol 464: 75-120, 1993.

Zajac, F. E. and Young, J. L. Discharge properties of hindlimb motoneurons in decerebrate cats during locomotion induced by mesencephalic stimulation. J Neurophysiol 43: 1221-1235, 1980.

Published in Journal of Physiology (2001), 532.1, pp.271-281

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

S. Krawitz, B. Fedirchuk, Y. Dai, L. M. Jordan & D.A. McCrea

Department of Physiology, University of Manitoba, 730 William Avenue, Winnipeg, Manitoba, Canada, R3E 3J7

Corresponding Author:

L.M. Jordan
Department of Physiology,
University of Manitoba,
730 William Avenue,
Winnipeg, MB
Canada R3E 3J7

tel: (204) 789-3694
fax: (204) 789-3930
email: larry@scrc.umanitoba.ca

24 text pages, 1 Table, 6 Figures

Running title:
"hyperpolarization of Vth during locomotion"

Keywords:
Action potential, Locomotion, Motoneurone

SUMMARY

1. Experiments were conducted on decerebrate adult cats to examine the effect of brainstem-evoked fictive locomotion on the threshold voltage (Vth) 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 control conditions (mean hyperpolarization -8.0 ± 5.5 mV; range -1.8 to -26.6 mV). Hyperpolarization of Vth occurred immediately at the onset of fictive locomotion and recovered in seconds (typically < 60s) following the termination of locomotor activity.
3. The Vth 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 Vth to the extent seen during fictive locomotion. We suggest that Vth 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.
5. The hyperpolarization of Vth for action potential initiation during locomotion is a state-dependent increase in motoneurone excitability. This Vth hyperpolarization may be a fundamental process in the generation of motoneurone activity during locomotion and perhaps other motor tasks.

INTRODUCTION

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) which 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 afterhyperpolarization (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 that 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, Vth) is not a fixed value in motoneurones. For example, Vth tends to be higher (more depolarised) in higher rheobase motoneurones (Gustafsson and Pinter, 1984) and the Vth of action potentials occurring later in a train during repetitive firing increases (accommodation) (Kolmodin and Skoglund, 1958). The present study sought to determine whether the Vth of hindlimb motoneurones was altered during fictive

locomotion. To this end Vth was measured in motoneurones recorded in decerebrate and paralysed adult cats during control conditions and during brainstem evoked fictive locomotion. A comparison of the Vth threshold in each motoneurone during these two states reveals a locomotor-related ***decrease*** (i.e. hyperpolarization) in motoneuronal Vth during fictive locomotion. We suggest that Vth hyperpolarization is another means by which motoneurone properties are re-configured to enhance motoneuronal output in the locomotor state. Portions of this data have been presented in abstract form (Krawitz et al. 1997).

METHODS

Surgical Procedures:

Data was obtained from 6 cats of either sex weighing from 2.0 to 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%, 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 (5g glucose; 0.84g $NaHCO_3$ per 100mL) was infused intravenously (5-10 ml/hour) 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 insensate 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 minutes) 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 msec 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 2M potassium acetate solution (3-8 M□). The primary aim of this study was to compare the membrane potential at which action potentials were initiated (Vth) during control and locomotor conditions. Use of the discontinuous current clamp (DCC) mode of an Axoclamp 2-A amplifier 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 those twelve 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 4 motoneurones input resistance was estimated from depolarizing current injections of < 5nA. Rheobase was defined as the minimum amplitude of a depolarizing (50 ms duration) current pulse that evoked an action potential. In 2 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 was used to initiate action potential(s) in the motoneurone. Fictive locomotion was evoked later in the same trial, and the intracellular current injection was repeated (see Fig. 1A). 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 (Vth) measurement:

Voltage threshold (Vth) was measured for the first spike elicited from either 50 ms depolarizing square pulses or, more commonly, from slow (5-15 s) triangular ramps of

intracellularly injected depolarizing current (as in Figure 1). Measurements are reported only for the first spike evoked to avoid potential for previous spikes to influence Vth by either spike accommodation or inter-spike trajectory. In order to standardize measurements, Vth was defined as the membrane potential at which depolarization increased at 10 V/s (i.e. the initiation of the action potential; see Brownstone et al. 1992). At the 10 kHz sampling rate used, this Vth estimate corresponded to the voltage value of the first data point where the following data point was 1 mV depolarized. The Vth 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 Vth measurements during the locomotor and non-locomoting 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 Vth 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 Vth were assessed in 38 motoneurones innervating a variety of hindlimb muscles and having action potential amplitudes in control conditions ranging from 50 to 97 mV (mean 76 mV; 36/38 had spikes $\geq$ 65 mV; 22/38 had spikes $\geq$ 75 mV). Control rheobase and input resistance values are reported in the first two columns of Table 1 on the left. The range of rheobases (2 to 31 nA) indicates that both motoneurones innervating slow (low rheobase) and fast twitch muscle fibre 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, Vth measurements were most often made from slowly rising current ramps that began at levels well below threshold. A comparison of the Vth 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 Vth of spike evoked by the current ramp was more depolarized than that obtained from the pulse, however the mean values of Vth 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 Vth during locomotor and control conditions are from measurements made using the same technique.

Figure 1 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 second 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. 1B). The Vth was -46.5 mV, as determined by the point at which the change in membrane potential was $\geq$ 10 V/s. The second current injection

was initiated about 25s after the onset of electrical stimulation of the MLR (1 ms pulses, 26 Hz). MLR stimulation produced fictive locomotion with 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 Figure 1C, the current required to initiate an action potential (marked with a "+") was reduced during fictive locomotion from 36 to 10 nA. During fictive locomotion the Vth was -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 (1B & 1C).

Figure 2 shows a recording from another SmAB motoneurone where the control Vth was -32.7 mV (Fig. 2; A1, B1), and -44.2 mV during fictive locomotion (Fig. 2; A2, B2; i.e. hyperpolarized by 11.5 mV). Note that like the example in Figure 1, during fictive locomotion less current was needed to evoke an action potential and the motoneurone fired faster (32 Hz vs 20 Hz) at comparable membrane potentials (see Fig. 2 A1, A2). A third ramp current injection delivered about 60 seconds after the cessation of fictive locomotion (Panel A3) shows that the Vth had returned to -31.8 mV by this time.

The Vth of all 38 motoneurones examined during control and locomotor conditions is shown in Table 1. The right most column shows that the Vth of all 38 hyperpolarized during locomotion (mean -8.0 ± 5.5 mV; median -6.7 mV). Hyperpolarization of Vth 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 Vth was striking both in its incidence (i.e. occurred in all cells examined) and the wide range of threshold change 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 Vth 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 Vth hyperpolarization during fictive locomotion ranged from -1.8 mV to -19.1 mV in different motoneurones. Figure 3 plots the amount of change in Vth and motoneurone rheobase. There was no relationship between motoneurone rheobase and Vth hyperpolarization during fictive locomotion (linear regression coefficient r^2=0.01, Pearson product moment correlation, p=0.6). Thus both high and low rheobase neurons displayed large and small changes in Vth during locomotion.

Recovery of Vth to within 1 mV of the control value was followed in 7 motoneurones. Recovery occurred in 3 cells in < 30 seconds, and in 30 to 145 seconds in the remaining 4 cells (see Fig. 2). The use of long duration ramps (typically about 20s) and the need to wait until all peripheral nerve activity ceased following MLR stimulation precluded an accurate assessment of the minimum time to Vth recovery. Nevertheless it is clear that in some cells Vth 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. 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 Vth was -50 mV (not shown). During locomotion the membrane potential at which spikes were produced on the LDP was -53 mV; i.e. Vth became hyperpolarized by 3.0

mV. Fifty ms duration, 4 nA pulses of current were injected into this motoneurone at about 1 Hz with 4 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 Vth (see the spike produced by the third current pulse injection from the left). The Vth 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) Vth does not vary rhythmically with membrane potential fluctuations during locomotion and 2) Vth is reduced and to the same extent for spikes produced by the locomotor circuitry and intracellular current injection. The examples in Figures 1 & 2 show Vth lowering in two motoneurones with small LDPs that were not recruited during fictive locomotion without the addition of intracellular depolarizing current. Thus, the hyperpolarization of Vth in these cells during locomotion was not a consequence of motoneurone recruitment.

Vth 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 occur at a hyperpolarized Vth. One of the features of locomotion is the rhythmic LDPs in motoneurones. To determine if rhythmic depolarizations and hyperpolarizations reduce Vth in the absence of locomotion, current pulses were superimposed on top of current ramps during control conditions in 8 motoneurones. Voltage thresholds were measured for action potentials produced by a current ramp alone (control), action potentials produced by a ramp current with superimposed current pulses without locomotion, and for spikes 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 Vth of the first spike produced by ramp current injection alone was -48.2 mV (Fig. 5 A1, B1). Current pulses (10 nA, ~300 ms) superimposed on the ramp depolarization resulted in ~10 mV membrane potential oscillations (Fig. 5, A2). The Vth of spikes evoked by the combination of ramp and pulse current injection was somewhat hyperpolarized (2.3 mV) compared to the spike evoked by ramp current injection alone (A2, B2). During locomotion, this motoneurone displayed LDPs with about a 10 mV peak to peak amplitude (A3). Hyperpolarizing bias current was needed to prevent firing on the LDP during locomotion. During locomotion, the Vth of the first spike evoked on the current ramp was -57.9 mV (A3, B3). This was a hyperpolarization of 9.7 mV compared to the spike evoked by ramp current during control and a hyperpolarization of 6.4 mV compared to the spike evoked by the combination of ramp and pulse current injection during control conditions. In all 8 cells examined in this manner the hyperpolarization of Vth 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 (1.8 to 11.4 mV). The mean Vth hyperpolarization during locomotion (14.3 ± 2.1 mV) was significantly larger than the Vth hyperpolarization for firing evoked by combining current pulses and ramps (4.7 ± 1.4 mV; paired t-test, $p=0.013$).

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 Vth changes during locomotion. To address the extent of this possible complication, the amount of constant hyperpolarizing current preceding the ramp was varied in 6 cells in the absence of locomotion. In 5 cells changing the hyperpolarizing bias current by 10 to 20 nA altered Vth by 2.2 mV. In one cell the change was 7.0 mV. In 3 of these 6 cells, Vth was increased (i.e. depolarized) by increases in hyperpolarizing bias current, in 2 cells Vth was hyperpolarized with increasing hyperpolarizing bias current, and 1 cell showed no change in Vth. Constant hyperpolarization of the motoneurone preceding the ramp therefore might have small effects on the measured Vth. Since this effect is small and often opposite to the hyperpolarization

of Vth seen during fictive locomotion, it is unlikely to account for the locomotor-related Vth hyperpolarization that we have described. In some cases, it may have caused us to underestimate the amount of Vth hyperpolarization occurring during locomotion.

Further evidence that the hyperpolarization of Vth seen during fictive locomotion is not simply the result of rhythmic fluctuations in membrane potential is presented in Figure 6. This MG motoneurone was recorded under 3 conditions. Panel A shows firing evoked by 15 nA, 250 ms current pulses in the absence of fictive locomotion. The Vth during this control condition was -31.0 mV. Fictive locomotion began soon after MLR stimulation commenced (Fig. 6, Panel 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 minutes prominent LDPs were evident in the motoneurone (Fig. 6, Panel C). Despite the clear increase in rhythmic drive to this motoneurone, the Vth for the LDP evoked firing (-37.4 mV) was similar to that recorded in panel B. This example illustrates that the extent of threshold lowering in this motoneurone is not related to the size of the LDP. This observation and the prolonged recovery of Vth following locomotion, suggests that Vth 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 Vth (i.e. *lowering* of Vth) 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 Vth hyperpolarization occurred immediately at the onset, 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 & 6), as well for spikes evoked by injection of intracellular depolarizing current (e.g. Figs 1 & 2). In addition, the Vth seemed not to be phasically modulated during the depolarizing and hyperpolarizing parts of the fictive step cycle, and the Vth hyperpolarization during locomotion was not dependent on the presence of well developed LDPs. We suggest that Vth hyperpolarization is a "state-dependent" phenomenon associated with the fictive locomotor process.

The present study is the first to demonstrate a reduction in motoneurone Vth during locomotion, but not the first report of modulation of neuronal Vth. For example, following a classical conditioning paradigm to decrease the amplitude of the H-reflex in monkeys, the mean Vth of spinal motoneurones becomes depolarized (Carp and Wolpaw, 1994). Mean motoneurone threshold potential is also depolarized in chronic spinal cats compared to spinal-intact animals (Hochman and McCrea, 1994). Cleary et al. (1998) have shown that the median Vth from their sample of motor neurone recordings in *Aplysia* was hyperpolarized the day following long-term sensitization of the siphon withdrawal reflex. Thus the hyperpolarization of Vth 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 Vth 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 remains to be determined whether Vth 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 Vth becomes more depolarized for successive action potentials of a spike train induced either by synaptic activation (Kolmodin and Skoglund, 1958) or by intracellular current injection (Granit et al. 1963; Barrett et al. 1980). This Vth depolarization may contribute to the decrease in firing rates seen during long trains of repetitive firing and thought to be caused by accommodation of sodium channels (Schwindt and Crill, 1982). The locomotor dependent hyperpolarization of Vth described here occurred for the first action potential evoked during fictive locomotion (see Figs. 1 & 2) and is, therefore, not a consequence of previous action potentials. This state-dependent hyperpolarization of Vth 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 and Nelson, 1971). In contrast, the locomotor-related Vth hyperpolarization is unrelated to motoneurone type, since large hyperpolarizations of Vth occurred in both low and high rheobase motoneurones (Fig. 3, Table 1).

The hyperpolarization of Vth during fictive locomotion is not caused by oscillations of the motoneurone membrane potential underlying the LDPs. Vth 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 Vth hyperpolarization could be large in the absence of substantial LDPs (see Fig. 6B) and persisted for seconds after fictive locomotion, when LDPs were absent. Therefore, while the motoneurone membrane potential trajectory during fictive locomotion might contribute to Vth hyperpolarization, our results suggest that Vth hyperpolarization is caused by a locomotor dependent modulation of the threshold properties of motoneurones.

To our knowledge, the rapid modulation of Vth 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 Vth 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 and Jordan, 1985; Gosgnach et al. 2000). The large reduction in the current required to evoke firing during locomotion (eg. Fig 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 than at 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 Vth 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 Vth 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. 1998a,b; Dai et al. 2000a,b). In addition, a large scale simulation of spinal cord circuitry has been used to show that Vth hyperpolarization results in increased output of motoneurone pools in response to an excitatory synaptic input (Dai et al. 1999).

REFERENCES

Barrett EF, Barrett JN, Crill WE (1980) Voltage-sensitive outward currents in cat motoneurones. *Journal of Physiology* **304**, 251-276.

Bennett DJ, Hultborn H, Fedirchuk B, Gorassini M (1998) Synaptic activation of plateaus in hindlimb motoneurons of decerebrate cats. *Journal of Neurophysiology* **80**, 2023-2037.

Brownstone RM, Jordan LM, Kriellaars DJ, Noga BR, Shefchyk SJ (1992) On the regulation of repetitive firing in lumbar motoneurones during fictive locomotion in the cat. *Experimental Brain Research* **90**, 441-445.

Brownstone RM, Gossard JP, Hultborn H (1994) Voltage-dependent excitation of motoneurones from spinal locomotor centres in the cat. *Experimental Brain Research* 102, 34-44.

Burke RE (1981) Motor units: anatomy, physiology, and functional organization. In Handbook of physiology, Section I The nervous system. American Physiological Society, Vol II, Part I, 345-422.

Burke RE, Nelson PG (1971) Accommodation to current ramps in motoneurons of fast and slow twitch motor units. International Journal of Neuroscience 1, 347-356.

Carp JS, Wolpaw JR (1994) Motoneuron plasticity underlying operantly conditioned decrease in primate H-reflex. *Journal of Neurophysiology* **72**, 431-442.

Cleary LJ, Lee WL, Byrne JH (1998) Cellular correlates of long-term sensitization in Aplysia. Journal of Neuroscience 18, 5988-5998.

Dai Y, Jones KE, Fedirchuk B, Jordan LM (1998a) Computer simulation: a study of the excitability of cat lumbar motoneurons during fictive locomotion. *Society for Neuroscience Abstracts* **24**, 427.11.

Dai Y, Jones KE, Fedirchuk B, Krawitz S, Jordan LM (1998b) Modeling the lowering of motoneuron voltage threshold during locomotion. In *Annals of the New York Academy of Sciences, "Neuronal Mechanisms for Generating Locomotor Activity"* **860**, 492-495.

Dai Y, Bashor D, Fedirchuk B, Jordan LM (1999) Motoneuron threshold hyperpolarization: alteration of population output predicted by a large scale simulation. *Society for Neuroscience Abstracts* **25**, 664.10.

Dai Y, Jones KE, Fedirchuk B, Jordan LM (2000a) Effects of voltage trajectory on action potential voltage threshold in simulations of cat spinal motoneurons. Neurocomputing 32-33, 105-111.

Dai Y, Jones KE, Fedirchuk B, McCrea DA, Jordan LM (2000b) A modelling study of locomotion-induced hyperpolarization of voltage threshold in cat lumbar motoneurones. *Journal of Physiology* Submitted

Fedirchuk B, McCrea DA, Dai Y, Jones KE, Jordan LM (1998) Motoneuron frequency/current relationships during fictive locomotion in the cat. *Society for Neuroscience Abstracts* **24**, 652.15.

Granit R, Kernell D, Shortess GK (1963) Quantitative aspects of repetitive firing of mammalian motoneurones, caused by injected currents. *Journal of Physiology* **168**, 911-931.

Gosgnach S, Quevedo J, Fedirchuk B, McCrea DA (2000) Depression of group Ia monosynaptic EPSPs in cat hindlimb motoneurones during fictive locomotion. *Journal of Physiology* **526**, 639-652.

Gustafsson B, Pinter MJ (1984) An investigation of threshold properties among cat spinal - motoneurones. *Journal of Physiology* **357**, 453-483.

Hochman S, McCrea DA (1994) Effects of chronic spinalization on ankle extensor motoneurons II. Motoneuron electrical properties. *Journal of Neurophysiology* **71**, 1468-1479.

Jordan LM (1983) Factors determining motoneuron rhythmicity during fictive locomotion. In *Neural origin of rhythmic movements.* A. Roberts and B. Roberts eds. Society for Experimental Biology Symposium **37**, 423-444.

Kolmodin GM, Skoglund CR (1958) Slow membrane potential changes accompanying excitation and inhibition in spinal moto- and interneurons in the cat during natural activation. *Acta Physiologica Scandinavica* **44**, 11-54.

Krawitz S, Brownstone RM, Noga BR, Jordan LM (1996) Can the nervous system overcome a possible central fatigue process - late adaptation? *Muscle and Nerve* **Supp 4**, S52.

Krawitz S, Fedirchuk B, Dai Y, Jordan LM, McCrea DA (1997) Locomotion hyperpolarizes the voltage threshold of cat lumbar motoneurones. *Society for Neuroscience Abstracts* **23**, 298.4.

McCrea DA, Krawitz S, Fedirchuk B, Jordan LM (1997) Group I-evoked extensor motoneurone activity is amplified by voltage-dependent depolarizations during locomotion. Society for Neuroscience Abstracts 23, 298.3.

Rossignol S (1996) Neural control of stereotypic limb movements. In *Handbook of Physiology, Section 12. Exercise: Regulation and Integration of Multiple Systems, LB Rowell and JT Sheperd Eds. American Physiological Society*, 173-216.

Schmidt BJ (1994) Afterhyperpolarization modulation in lumbar motoneurons during locomotor-like rhythmic activity in the neonatal rat spinal cord in vitro. Experimental Brain Research 99, 214-222.

Schwindt PC, Crill WE (1982) Factors influencing motoneuron rhythmic firing: results from a voltage-clamp study. Journal of Neurophysiology 48, 875-890.

Shefchyk SJ, Jordan LM (1985) Motoneuron input-resistance changes during fictive locomotion produced by stimulation of the mesencephalic locomotor region. Journal of Neurophysiology 54, 1101-1108.

Acknowledgements:

The authors gratefully acknowledge the excellent technical assistance provided by Kim Madec, Maria Setterbom and Matt Ellis, and the participation of Dr. Kelvin Jones in some of these experiments. SK was the recipient of a Medical Research Council (MRC) of Canada Studentship; BF thanks the Manitoba Division of the Canadian Paraplegic Association for salary support. This work was funded by a MRC of Canada program grant to LMJ and DAM.

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	0.8	−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.

FIGURE LEGENDS

Figure 1: Firing was elicited from antidromically identified lumbar motoneurones by intracellular current injection, prior to and during MLR-evoked fictive locomotion. Panel A shows a trial for a SmAB motoneurone, where a 50 nA ramp of current was injected, after which the brainstem stimulation was started (bar under ENG). Fictive locomotion was evident as rhythmic activity that was alternating between extensor and flexor ENGs (not illustrated). Discontinuous current clamp recording allowed accurate measurement of the membrane potential during simultaneous current injection. The bars labelled "B" and "C" denote the time periods which are expanded below as Panels B and C. Panel B shows that the voltage threshold for production of action potentials (Vth) before fictive locomotion was -46.5 mV. Panel C shows that during fictive locomotion, less current is required to fire the neurone (compare current at "+") and the Vth is hyperpolarized compared to B. Note that the neurone fires at 29 Hz before locomotion, and at 59 Hz at the same membrane potential during locomotion (see bracketed areas). The Y-axes in Panel B also apply to C, and the time bar shown below Panel C also applies to B.

Figure 2: The Vth recovers after the cessation of fictive locomotion. This SmAB motoneurone had a Vth of -32.7 mV prior to locomotion (A1) and -44.2 mV during MLR-evoked fictive locomotion (A2). As in Figure 1, the current required to elicit firing is reduced during fictive locomotion (compare "+") and the neurone fires at a higher rate (32 Hz) during fictive locomotion than at the same membrane potential in the control condition (20 Hz). Within 60 seconds following the cessation of locomotion the Vth had depolarized back to -31.8 mV (A3). The time scale shown in A2 is the same for all traces of Panels A1, A2 and A3. Panels B1, B2 and B3 show the first action potential of the firing shown in the corresponding panel above (A1, A2 or A3) at expanded scales to

better illustrate the Vth value (the point where the Em dV/dt 10 V/s). The scale bar in B1 also applies to B2 and B3.

Figure 3: There is no relationship between the amount of Vth hyperpolarization during fictive locomotion and the rheobase of the motoneurone.

Figure 4: During fictive locomotion the Vth 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. One current pulse occurring during the inactive phase of the fictive step cycle elicited an action potential (marked by the arrow) with a Vth of -53.0 mV. This was the same as the Vth seen during the active phase, and -3.0 mV hyperpolarized compared to control.

Figure 5: Membrane potential oscillations produced by current pulses can hyperpolarize the Vth, but not to the same degree as seen during fictive locomotion. Panel A1 shows a recording from an extensor motoneurone during a current ramp in the absence of fictive locomotion. The Vth in this "control" condition was -48.2 mV. A2 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 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 each panel A1-A3 is shown below that panel (Panels B1-B3) at expanded time and voltage scales to better illustrate the measured Vth. This motoneurone had a control rheobase of 9 nA and a resting Em of -69.2 mV.

Figure 6: Hyperpolarization of Vth during fictive locomotion does not depend on the amplitude of the LDPs in the motoneurone. Panel 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 Vth for this control condition was -31.0 mV and is denoted by the dotted line. Panel B shows the 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 Vth for this fictive locomotion induced firing (no current injection) was -37.1 mV. Panel C shows the same neurone approximately 3 minutes 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 Vth of -37.4 mV.

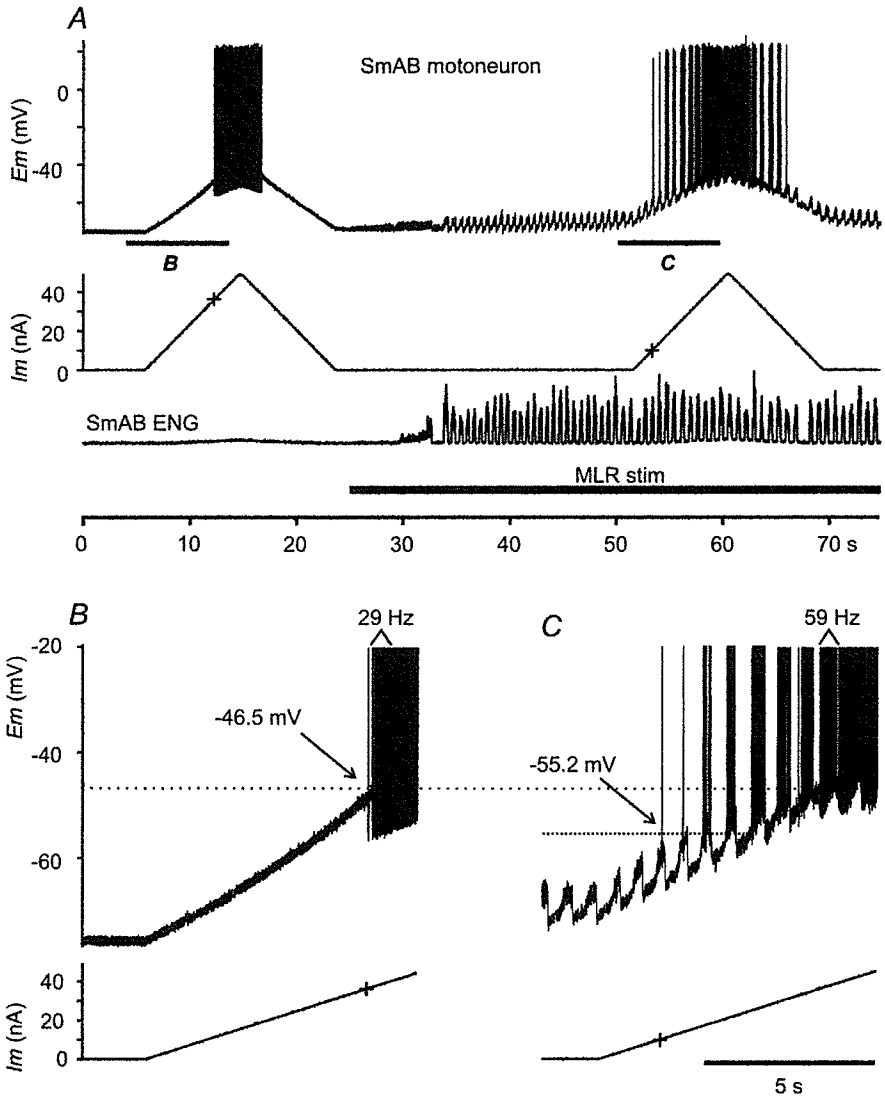


Figure 1, Krawitz et al, J. Physiol. (2001), 532.1, pp.271-281

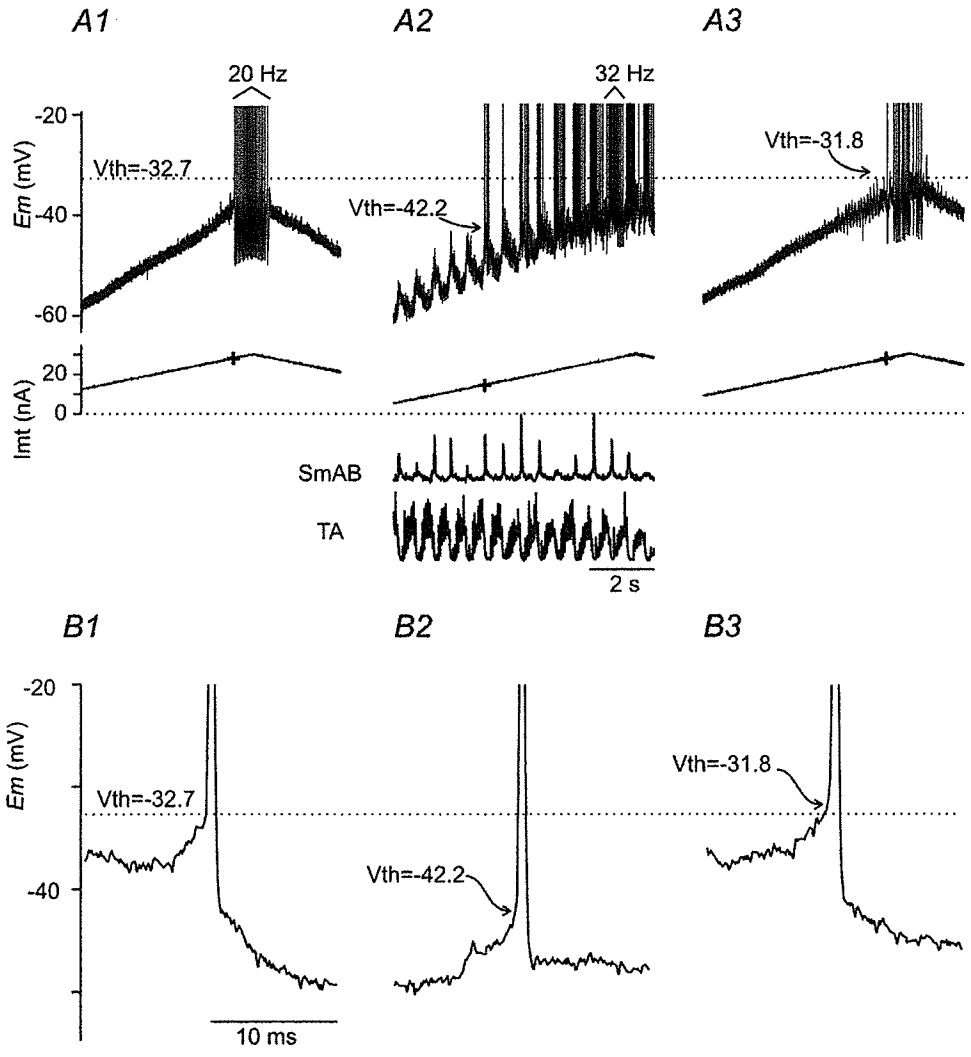


Figure 2, Krawitz et al, J. Physiol. (2001), 532.1, pp.271-281

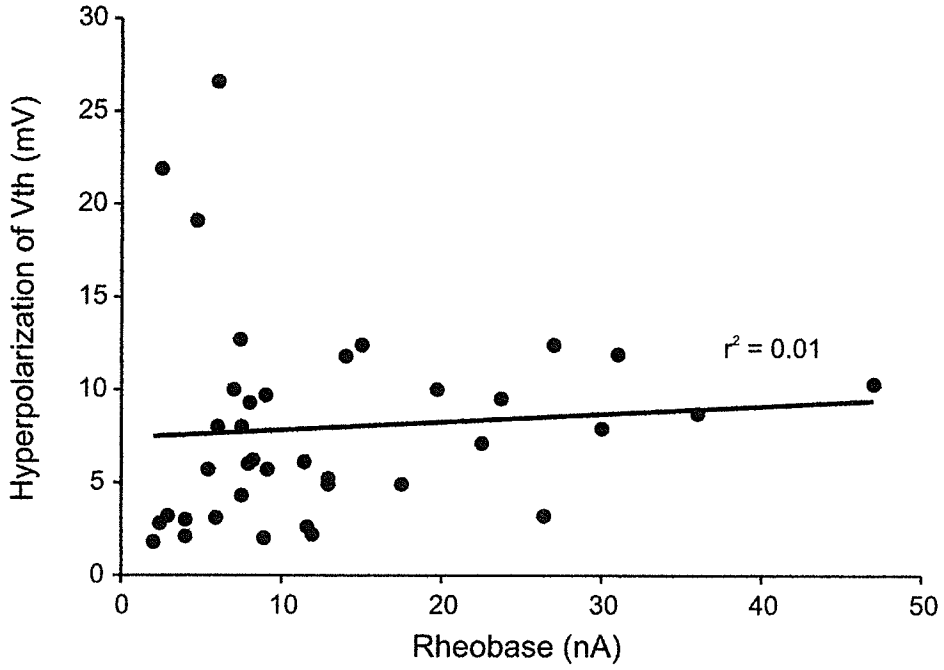


Figure 3, Krawitz et al, J. Physiol. (2001), 532.1, pp.271-281

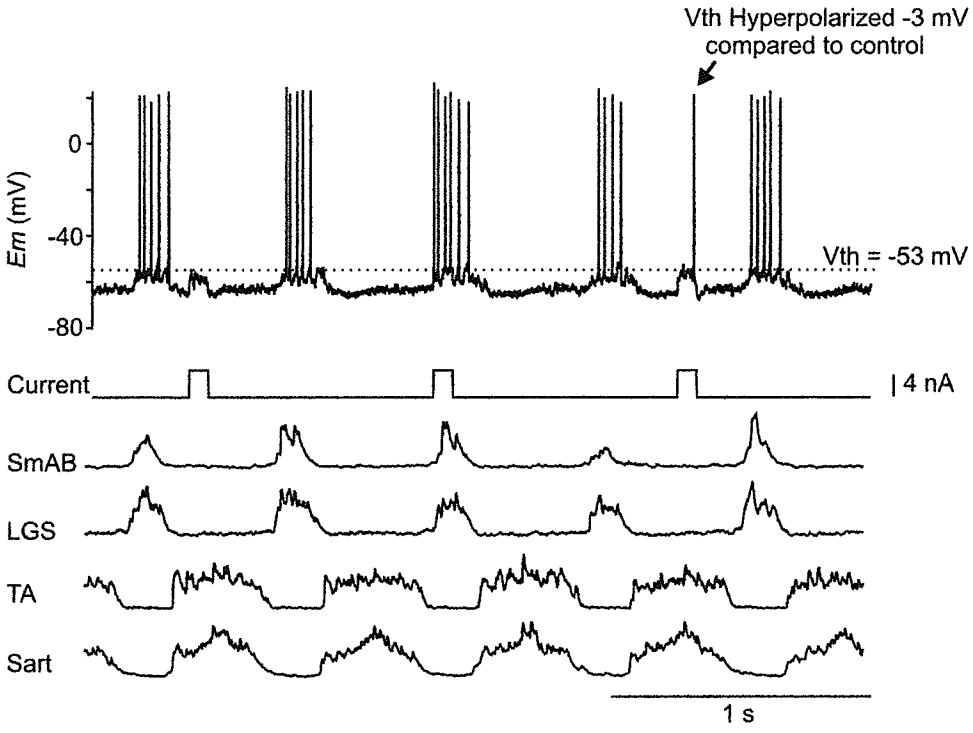


Figure 4, Krawitz et al, J. Physiol. (2001), 532.1, pp.271-281

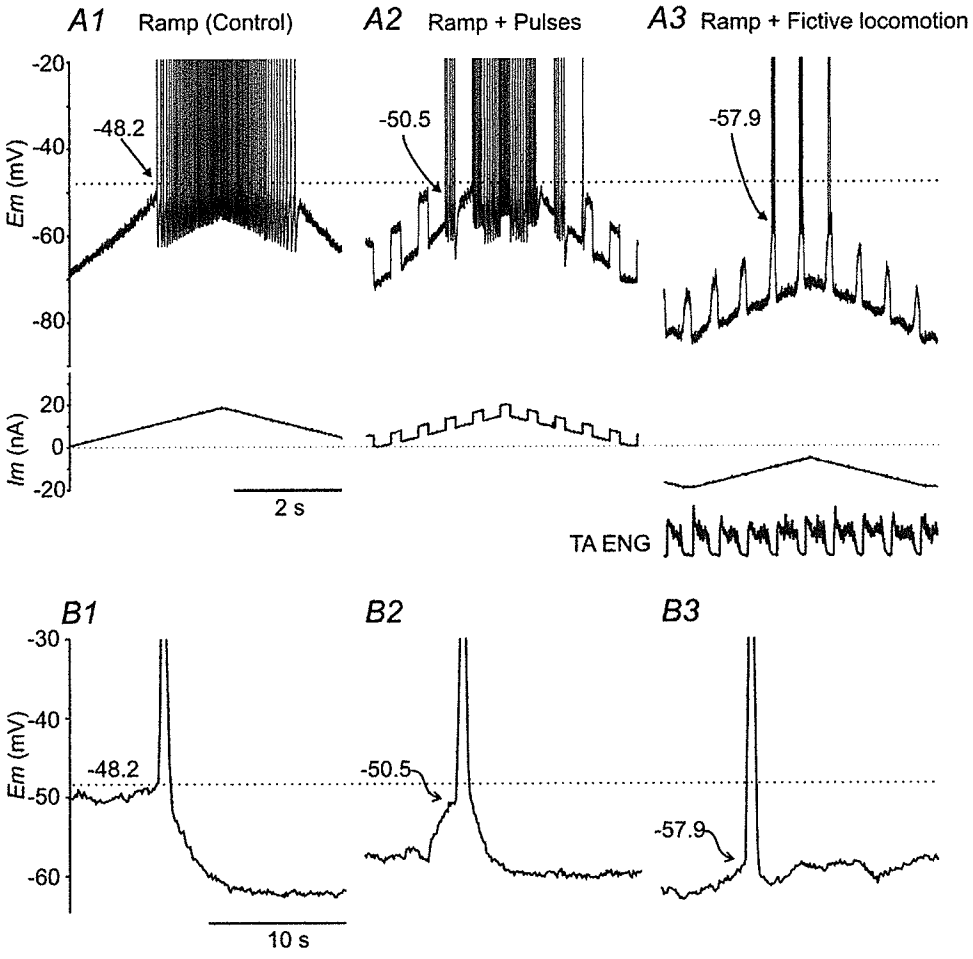


Figure 5, Krawitz et al, J. Physiol. (2001), 532.1, pp.271-281

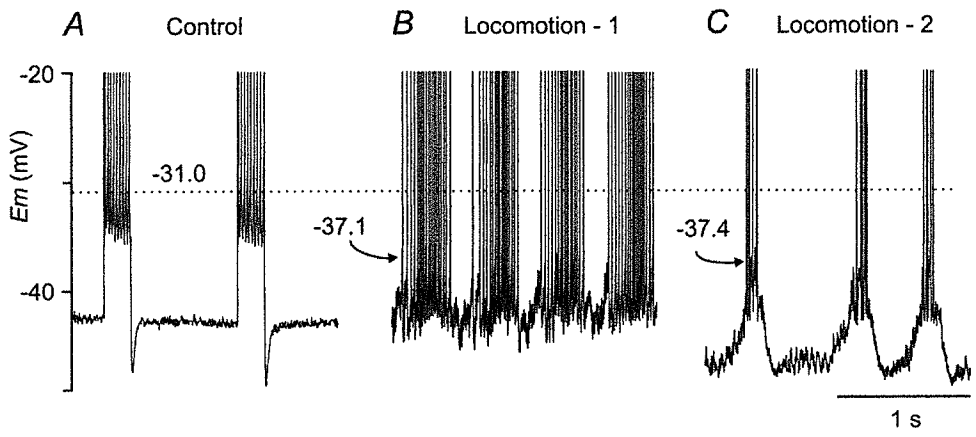


Figure 6, Krawitz et al, J. Physiol. (2001), 532.1, pp.271-281

Late adaptation in spinal motoneurones is abolished during fictive locomotion in the cat

S. Krawitz, R.M. Brownstone & L.M. Jordan

Department of Physiology
University of Manitoba
730 William Avenue
Winnipeg, Manitoba
Canada R3E 3J7

ABSTRACT

1. Experiments were conducted on decerebrate cats to examine whether the slowing of action potential firing rate in spinal motoneurones, occurring over tens of seconds during sustained or intermittent activation (late adaptation), is reduced during brainstem-evoked fictive locomotion. Intracellular recordings from lumbar spinal motoneurones during brainstem-evoked fictive locomotion were compared with similar recordings during control conditions designed to simulate locomotor firing conditions as closely as possible without brainstem stimulation. Firing in control conditions was evoked by intracellular depolarizing current injection.
2. During four-minute trials of locomotor firing, none of eight lumbar motoneurones showed a decrease in motoneuron firing, i.e., no late adaptation was observed. By four minutes in each trial of locomotion, motoneuronal firing reached frequencies ranging from around 35 Hz to 100 Hz. During locomotion, the amplitude of the post-spike afterhyperpolarisation was reduced. In control conditions, the firing frequency continually decreased.
3. No correlation was found between the absence or presence of accommodation (shown by concomitant decrease in both action potential rate of rise and overshoot) and locomotion. During the control trials, there was ongoing accommodation (concomitant with late adaptation).
4. Experiments were also conducted to assess the recovery of motoneurones following trials of firing evoked both by current injection and during fictive

locomotion. Following the control trials, the firing frequency (as assessed by test pulses of current injection) was decreased before recovering to the pre-trial firing frequency. Following the trials of fictive locomotion, the firing frequency (as assessed by similar test pulses of current injection) surpassed the pre-trial firing frequency. Motoneuronal firing frequency was thus enhanced both during and immediately following fictive locomotion.

5. The abolition of late adaptation in spinal motoneurones during fictive locomotion is an example of a state-dependent change in the "intrinsic" properties of mammalian motoneurones. This change contributes to increased excitability of motoneurones during locomotion, and results in more robust and variable firing during locomotion than firing evoked by intracellular current injection in an animal in a quiescent state.

INTRODUCTION

Spike-frequency adaptation (SFA) refers to the slowing of action potential firing rate during sustained or intermittent activation under a variety of conditions. SFA has been well-defined in spinal motoneurons, where there is a period of early adaptation, occurring over the first several action potentials (Granit et al. 1963a), and a period of late adaptation occurring over tens of seconds (Kernell and Monster 1982b). When one studies the time constant of the rate of decay of the instantaneous firing frequency, there may be an intermediate period of adaptation. This led Powers et al. (1999) to define three phases of spike frequency adaptation: initial, occurring over the first few intervals; early, occurring over the next few seconds; and late, occurring over tens of seconds. We will use these definitions here. Despite these phenomenological definitions, the mechanisms underlying SFA are not entirely clear. The functional significance of SFA in motoneurones is also not known, but it is thought to play an important part in limiting the capacity of motoneurones to participate in the development of muscle force during posture and movement. Direct evaluation of the role of adaptation in movements such as locomotion, respiration, and other natural movements has not been carried out. Based upon the work of Kernell and his colleagues (Kernell 1965a; Kernell 1965b; Kernell and Monster 1982a; Kernell and Monster 1982b) using intracellular current injection, it has been suggested that adaptation gives rise to the decline in motoneurone discharge rate during fatigue associated with a sustained maximal voluntary contraction (Bigland-Ritchie et al. 1986). In anaesthetized cats, intermittent and sustained activation of spinal motoneurones with extracellular electrical stimulation revealed that both types of activation resulted in adaptation (Spielmann et al. 1993).

The term adaptation was first used to refer to the brief phase of slowing of spike frequency preceding steady state firing (Granit et al. 1963b), more broadly defined as the decrease of firing rate as a function of stimulus duration (Kernell 1965a). Initial, early, and late phases of adaptation are distinguished (Powers, et al 1999), corresponding to an initial rapid drop in frequency over the first few interspike intervals, followed by a more gradual decline, and subsequent slower process (time constants of 250 ms and 10-20 s, respectively – see Powers, et al (1999). Late adaptation refers to the gradual decrease in frequency over tens of seconds of motoneurone firing in response to sustained or intermittent depolarizing current injection in cells in a resting state (Kernell and Monster 1982b; Sawczuk et al. 1995; Spielmann, et al 1993).

Most investigations of spinal motoneurones, including those on SFA, have been in anaesthetised animals. It is now clear, however, that 'intrinsic' neuronal properties are affected by general anaesthesia. For example, the well-studied phenomenon of plateau potentials (Hultborn 1999) was not recognised in the previous decades of study, as motoneurones do not express plateau potentials in anaesthetised animals. It was through the use of decerebrate animals that these plateaux were found. Furthermore, it is now well recognised that the 'intrinsic' properties of neurones change dependent on the state of the nervous system. This has not only been demonstrated in the crustacean stomatogastric ganglion (Harris-Warrick and Marder 1991) and other invertebrates, but also in the adult cat. For example, during fictive locomotion, there is a reduction in the post-spike afterhyperpolarisation (Brownstone et al. 1992), a change in the frequency-current relation (Brownstone, et al 1992), and a hyperpolarisation of action potential voltage

threshold (Krawitz et al. 2001). These changes are all thought to be important for the regulation of motoneurone output by the central nervous system.

This study was undertaken to examine the hypothesis that, since late adaptation would be counter-productive to sustaining robust locomotor activity over even tens of seconds, this intrinsic property should be reduced during locomotion. These studies were undertaken in the adult decerebrate cat in which fictive locomotor activity was produced by stimulation of the mesencephalic locomotor region (Shik et al. 1966) during intracellular recordings from lumbar spinal motoneurones. We demonstrate that late adaptation is, in fact, completely abolished during fictive locomotion. Furthermore, the intrinsic properties most correlated with late adaptation are those produced by fast sodium channels. We therefore suggest that late adaptation results from slow inactivation of sodium channels. Further, we suggest that this property can be regulated by the central nervous system: during fictive locomotion, this longer time-constant of inactivation is removed, resulting in the abolition of late adaptation.

METHODS

Preparation: The data were obtained from lumbar motoneurones of five cats of either sex weighing 2-3 kg. All surgical and experimental protocols were approved and performed in accordance with the Canadian Council on Animal Care guidelines. Each animal was anaesthetised with halothane in a mixture of oxygen and nitrous oxide. Blood pressure was monitored via a carotid artery cannula. A tracheostomy was performed. The cephalic or brachial vein was cannulated for infusion of drugs and sodium bicarbonate buffer. Intravenous dexamethasone (4 mg) was given to prevent brain stem swelling following decerebration later in the preparation. An adequate surgical level of anaesthesia was assured by repeated testing for lack of pedal withdrawal, corneal reflexes and muscle tone.

Branches of the sciatic nerve bilaterally were dissected free and cut for mounting on biplolar electrodes for stimulating or recording. Left hindlimb nerves dissected included muscle branches to semimembranosus mounted with anterior biceps (SmAB), posterior biceps mounted with semitendinosus (PBSt), gastrocnemius with soleus (GS), flexor digitorum and /or hallucis longus (FDHL), plantaris (Plant), tibialis anterior (TA), superficial peroneal (SP). Right hindlimb nerves dissected included SmAB, PBSt. Branches of the femoral nerve on one side were also dissected free and cut, including branches to the three vasti and sartorius (Sart). Bipolar nerve cuffs were placed around these branches for stimulating or recording. The contralateral femoral nerve was cut.

A laminectomy of L4-L7 exposed the lower lumbar spinal cord dorsally. The animal was fixed in a stereotaxic frame with all limbs pendent. The skin over the lumbar

area was suspended so as to form a pool over the exposed lumbar spinal cord. Mineral oil filled the pool and was heated to 38 degrees Celsius by a heating lamp. The sciatic nerves were extended horizontally in specially designed plastic trays also filled with mineral oil. Within these mineral oil pools the dissected nerve branches were mounted on bipolar electrodes for stimulating or recording.

A craniectomy was performed and the animal was decerebrated by sectioning of the brainstem at the precollicular postmamillary level (Shik, et al 1966; Steeves et al. 1975). All tissue rostral to the transection was removed, allowing the anaesthesia to be discontinued. The animal was paralysed with an intravenous injection of pancuronium bromide (Pavulon) supplemented periodically throughout the experiment to maintain a state of flaccid paralysis. The animal was artificially ventilated to maintain expired CO2 at 3-5%. Intravascular volume was maintained with intravenous Dextran. Norepinephrine was also infused intravenously when necessary to counter hypotension.

Fictive locomotion: Monopolar stimulating electrodes were placed bilaterally in the brainstem for stimulation of the mesencephalic lcomotor region (MLR) Electrical stimulation (50-220 μA, 0.5-1.0 ms rectangular pulses at 10-25 Hz) of the MLR evoked rhythmic and alternating activity in hindlimb flexor and extensor motoneurones. Fictive locomotion was monitored by electroneurogram (ENG) activity in the peripheral nerves. The activity was amplified, rectified and filtered, and the ENGs were displayed continuously.

Data collection: Intracellular recordings from lumbar motoneurones were obtained with single-barrelled glass microelectrodes filled with 2 M potassium citrate (resistance 3-10 MΩ, tip diameter less than 2 μm). Recordings were made using either a

bridge circuit or discontinuous current clamp using an Axoclamp 2-A® amplifier.. Data were recorded on analogue tape (Vetter®) and digitised using rates of 10 kHz for the intracellular signal.

Upon impalement of a cell, the motoneurone was immediately identified by antidromic stimulation of one of the peripheral nerves. Rheobase was defined as the minimum current required to evoke an action potential during injection of a 50 ms depolarising current pulse. A hyperpolarising pulse (3 nA, 50 ms duration) was injected to measure motoneuronal input resistance.

As the aim of this study was to describe firing frequencies during and after a period of fictive locomotion, appropriate control conditions were to simulate firing conditions during locomotion as closely as possible without stimulation of the MLR. Firing in control conditions was to be evoked by intracellular depolarizing current injection delivered at a strength estimated to produce repetitive firing at a frequency which would reasonably match frequencies expected during locomotion. In order to determine the required current strength, a single depolarizing pulse (500 ms duration) was injected, of approximately 20 nA. The strength of this pulse was then adjusted in order to elicit repetitive firing of a frequency comparable to that typically achieved during fictive locomotion. This target was 30-40 Hz, based on previous experiments (Brownstone, et al 1992). Many motoneurones were later found to exceed this frequency during locomotion so that a "perfect match" was not achieved for all cells, although frequencies did match reasonably well. This pulse (referred to below as the "control pulse") was also critical because the frequency of the firing it evoked was used to monitor cell health. Once the strength of the control pulse was determined for a cell, that

strength was consistently used for all subsequent depolarising current injections in the same cell.

Control conditions were also designed to simulate the pattern of locomotor firing which occurs only during the depolarised phase (the excitatory component) of the locomotor drive potential (LDP). The depolarised and hyperpolarised phases of the LDP alternate at a rate of about one Hz. The depolarised phases were therefore simulated in control conditions by repetitive (approximately 1.7 Hz) rectangular depolarising pulses (500 ms duration) The injected current was approximately 20 nA but adjusted as explained above to evoke repetitive firing with a frequency that would match typical locomotor frequencies. The depolarising pulses were delivered for four minutes producing a four minute control trial of repetitive firing.

Fictive locomotion was evoked by stimulation of the MLR for a four minute trial of locomotor firing. Intracellular recordings during each four minute trial allowed frequencies in control conditions and during locomotion to be compared. Four minutes was decided as a suitable duration for comparing frequencies in order to evaluate late adaptation (Spielmann et al 1993 used 4 min.). Trials of shorter duration - less than one minute - from previous experiments were inadequate for comparison as it was found that locomotion often establishes itself over tens of seconds. At the same time maintaining cell health was a priority and required monitoring at regular intervals.

Cell health was monitored by means of depolarizing pulses injected following each four minute trial of firing (both control and locomotor). A series of single pulses (500 ms duration; approximately 20 nA as determined for each cell as described above) was delivered at 30 second intervals over 5 minutes (approximately ten pulses; each pulse

rectangular shaped and identical to the "control pulse"). Membrane potentials were measured as well as firing frequencies during the current injections. If a cell became inappropriately depolarised (i.e., more depolarised than –40 mV) or if the firing frequency during the five minute interval between trials failed to recover to the level attained before firing trials, the cell was rejected. A cell was also rejected if the height of its action potential was reduced more than 10%. Only cells recorded during well-developed periods of fictive locomotion were used in the analysis.

The firing protocol in summary consisted of the "control pulse", a four minute trial of firing in control conditions, a series of depolarising pulses to monitor cell health, a four minute trial of locomotor firing, another series of depolarising pulses, and so on.

The protocol was varied so that in some cells the four minute trial of fictive locomotion immediately followed the "control pulse" and preceded the control firing while in other cells the order of control and locomotor trials was reversed. Once trials of control and locomotor firing had each been recorded the sequence of control and locomotor trials was varied arbitrarily and continued as long as cell health was maintained.

Data analysis: Intracellular recordings were analysed using software designed for the Spinal Cord

Research Centre at the University of Manitoba (see www.scrc.umanitoba.ca/doc/ for details). The instantaneous firing frequency during the LDPs of the locomotor trials and during the depolarising current injections of the control trials were measured, as were the average instantaneous firing frequencies. (The latter appear in the figures.) Averages were calculated on the basis of units of 100 action potentials (arbitrarily determined).

Action potential amplitude was measured from the voltage threshold, the membrane potential at which the action potential was initiated. This was defined as the membrane potential at which the rate of rise of the action potential reaches 10 V/s (the intracellular signal was digitally differentiated; see Brownstone et al, 1992). At the sampling rate of 10 kHz this corresponded to the voltage value of the first data point where the following data point was at least 1 mV depolarised (i.e. depolarised 1 mV in one 100 ms sampling interval). The action potential overshoot (the membrane potential depolarization beyond 0 mV) and the rate of rise of the action potential were also measured. The amplitude of the afterhyperpolarisation (AHP) was measured as the difference between the voltage threshold of the action potential and the peak of the AHP.

RESULTS

In all control trials, and at the onset of MLR stimulation, prior to the establishment of well-coordinated locomotion, adaptation was evident. When locomotor activity became well-established, the adaptation disappeared. This point is illustrated in Figure 1. Figure 1A shows the typical onset of locomotion with electrical stimulation of the MLR and how it improves with time as locomotion is established. No intracellular current was injected during the ensuing four minute trial of locomotion. The top traces are a series of four intracellular recordings from a left sartorius motoneurone, each trace depicting a five second period of firing extracted from the 220 second trial. The lower traces show ENG recordings of peripheral nerves (left quadriceps, left sartorius and right sartorius) during five second periods corresponding to the intracellular recordings. Together the traces provide evidence of locomotor activity with the characteristic rhythmic alternation between flexor (sartorius) and extensor (quadriceps) ENG activity as well as between left and right limb activity. Activity progressed throughout the trial in the following manner: initial tonic firing of the cell with irregular firing in the ENGs (Figure1A:a) subsequently began to show phases of firing interspersed by silent periods as the ENGs began to show regular periods of activity, alternating right-left and flexor-extensor (Figure 1A:b). With time, the motoneurone firing became more regular, alternating between phases of membrane depolarisation when the cell fired and hyperpolarisation when the cell was silent, corresponding to regular phases of the step cycle. This phasic activity is reflected in the ENGs (Figure 1A:c and 1A:d).

Figure 1B depicts data from the same motoneurone and the same trial of locomotion as in Figure 1A. It is typical of those cells (five of eight cells) in which the firing frequency initially decreased over 60 to 90 seconds with the onset of MLR stimulation and subsequently increased with the establishment of locomotion. The data covers the entire 220 second trial from which the five second samples were extracted in Figure 1A. Averaged instantaneous firing frequency of the cell throughout the trial is plotted against time. The letters along the abscissa correspond to those in Figure 1A, showing where the five second extracts fit in the context of the entire trial. When the MLR is first stimulated and the cells starts to fire, the firing frequency initially falls over 70 seconds, but as the motoneurone progresses towards firing in regular locomotor phases, the firing frequency gradually increases and overcomes the adaptation previously displayed. The late adaptation which occurred with the onset of motoneurone firing *reversed* as locomotor activity was established. Intracellular recordings from eight lumbar motoneurones all showed an absence of late adaptation during well-developed fictive locomotion. Action potential amplitudes ranged from 66 to 89 mV in control conditions.

An attempt was made initially to analyse previously recorded bouts of locomotor activity. These bouts were of less than one minute duration. As it appeared that firing during locomotion would often not stabilise for a number of seconds before locomotion was well established, no conclusions could be drawn regarding the presence or extent of a decline in firing frequency over tens of seconds (late adaptation) during such brief bouts of locomotion. Nevertheless, questions were raised about a possible trend: seven of nine cells examined showed a slowing of firing frequency (adaptation), for example, from 45

Hz to 20 Hz (not shown). This occurred despite reduced afterhyperpolarisations during locomotion. In four of these cells, however, the firing frequency started to increase after initially slowing. It remained unclear to what extent the initial "stabilising phase" of locomotion confounded or revealed a sequence of firing frequency which consisted of first decreasing then increasing to recover and in some cases exceed frequencies recorded at the outset of firing. As data such as that illustrated in Figure 1 shows, the nervous system is capable of overcoming adaptation during locomotion. For this to be detected, a protocol that allows examination of motoneurone firing over extended periods of time such as used in Figure 1 is necessary. In subsequent experiments, firing frequency during bouts of locomotion of four minutes duration were compared with firing during control trials (MLR not stimulated and firing induced by intracellular injection of depolarising current) of the same duration (see Methods).

The data from eight cells met the strict criteria for detailed analysis of late adaptation. During fictive locomotion, the firing rates in none of these 8 cells decreased over the four minute trial period. That is, no late adaptation was observed. In five of the eight cells the firing frequency initially decreased over the first 25 to 90 seconds following onset of MLR stimulation as locomotor activity was beginning. With the establishment of steady locomotion, the firing frequency then increased and there was no subsequent adaptation. The frequencies of the remaining three cells gradually increased from the onset of firing. By four minutes in each trial of locomotion the motoneurones reached steady frequencies around 35 Hz (four cells), 45 Hz (one cell), 60 Hz (two cells), and 100 Hz (one cell).

The data in Figure 2 are from intracellular recordings of a gastrocnemius/soleus motoneurone. Motoneurone firing induced by intracellular current injection in the absence of MLR stimulation (control conditions) is contrasted with firing during locomotion. In control conditions, the firing frequency decreases over tens of seconds and continues to decrease (Figure 2A). This is the late adaptation well known to occur in motoneurones in response to suprathreshold current injection. However, with the establishment of locomotion, there is a reversal of adaptation (Figure 2A, bottom). In a similar fashion to the cell illustrated in Figure 1, the firing frequency initially decreases but subsequently increases so that within one minute from the onset of MLR stimulation, the firing frequency has surpassed the initial frequency and continues to fire at even higher frequencies. This is in stark contrast to the adaptation seen with current injection in the absence of locomotion.

Data from the same control and locomotor trials as in Figure 2A are shown in Figure 2B and Figure 2C. Figure 2B plots the action potential rate of rise (ROR) against time, measured during the control (left graphs) and locomotor (right graphs) trials. During the control trial there is ongoing accommodation, as evident by the decreasing action potential ROR. It has not been documented whether or not accommodation is characteristic of locomotor firing, but in this trial of locomotion the action potential ROR did not decrease but instead increased slightly, providing no evidence of accommodation.

In the same trials, decreases and increases of action potential overshoot were measured and found to correlate with decreases and increases, respectively, in action potential ROR. This correlation between the action potential overshoot and its rate of rise was consistently found throughout all the other trials in the remaining cells, whether

firing in response to current injection or during locomotion. This is consistent with current injection studies (Richter and Heyde 1975) showing concomitant decreases in both action potential ROR and overshoot. Simultaneous decreases in both parameters signal the presence of accommodation. For this reason the overshoot is not illustrated in Figure 2.

The correlation of increasing AHP amplitude and ongoing accommodation with late adaptation when a motoneurone fires repetitively in response to current injection was consistently seen in our control trials (Richter and Heyde 1975). During locomotion, a reduction of AHP amplitude (not shown; see (Brownstone, et al 1992) and the absence of adaptation were consistent findings in all cells examined. No correlation, however, was found between the absence or presence of accommodation and locomotion. Four of eight cells showed accommodation in the absence of adaptation whereas three cells similarly in the locomotor state did not (accommodation in the remaining cell was unclear), providing no correlation between the suppression of adaptation and the presence or absence of accommodation during locomotion.

Experiments were done to assess the recovery of motoneurones following trials of firing evoked both by current injection and during locomotion. The motoneurones' firing frequencies (instantaneous firing frequency of the first interval and average firing frequency) were plotted, in response to rectangular depolarising pulses (~20 nA, 500 ms duration) given at 30 second intervals over five minutes following each four-minute trial of locomotion and current injections (control conditions). Identical current pulses were injected, first a single depolarising pulse before any of the four-minute trials and then every 30 seconds over five minutes following each trial of locomotor firing and control

firing. The order of locomotor firing trials and control firing trials was random; the trials were repeated to eliminate possible confounding effects. During the five minutes subsequent to both locomotor and control trials, the firing frequency (both instantaneous firing frequency of the first interval and average firing frequency) attained pre-trial levels, indicating full recovery of the cell after each trial of firing. Repetitive firing evoked by the single pulse before any of the trials was critical as it was this pre-trial firing frequency to which firing evoked by pulses after the trials were compared. The cell's recovery after each trial to at least the pre-trial firing frequency is evidence of its good health throughout the successive trials. The firing frequency following both locomotor trials surpassed the pre-trial frequency. In contrast the firing frequency evoked by the first few pulses following both control trials was decreased before recovering to the pre-trial frequency. The decreased frequency following the control trials is consistent with late adaptation occurring with firing produced by intracellular current injection, unlike the enhanced firing frequency during and immediately following fictive locomotion.

Motoneurones during fictive locomotion fire at higher and more variable frequencies than during firing induced by intracellular current injection in the absence of MLR stimulation and locomotion ("at rest"). This is consistent with the absence of spike frequency adaptation during locomotion, as show in this study, and with our previous finding that locomotion is accompanied by a lowering of voltage threshold (Krawitz et al. 2001). Figure 4 compares the firing frequencies of a MG motoneurone in the absence of MLR stimulation and locomotion (control conditions) with those occurring during MLR stimulation and fictive locomotion. Intracellular recordings were obtained from a MG

motoneurone during a sequence of identical triangular-shaped current injections (see (Krawitz, et al 2001) for details). Firing is evoked at various voltage thresholds. The more hyperpolarised voltage thresholds occur during fictive locomotion, as shown previously (Krawitz, et al 2001) , and the mean firing frequencies of 35 Hz and 45 Hz recorded during fictive locomotion at Vth of –47 mV and –45 mV respectively, exceed all frequencies recorded in control conditions. Firing could not be evoked at such hyperpolarised membrane potentials (Vth of –47 mV and –45 mV) during control conditions. At voltage thresholds comparable in control conditions and during locomotion, for example at Vth of –43 mV (measured during current injections of identical slopes in all trials), the mean firing frequency was 30 Hz with a maximum firing frequency of 32 Hz in control conditions whereas during locomotion the mean was 56 Hz, reaching a maximum instantaneous firing frequency of 89 Hz.

These firing rates also point to the variability in instantaneous firing frequency during locomotion which is much greater than in its absence (control conditions). In the trials quoted above (with Vth of –43 mV), the motoneurone fired at frequencies varying throughout a range of 77 Hz during the locomotor drive potential (LDP), from a minimum instantaneous firing frequency of 22 Hz to a maximum of 89 Hz. The same motoneurone in control conditions at the same Vth of –43 Hz fired relatively uniformly (with regularly spaced interspike intervals) over a very tight range, with instantaneous firing frequencies varying only 4 Hz, from 28 Hz to 32 Hz. There were also more action potentials produced during locomotion (25) than in control conditions (17).

Repetitive firing during locomotion attains high frequencies relative to typical firing induced by currrent injection in the absence of locomotion. The high firing

frequencies during locomotion are maintained with varying interspike intervals. These observations do not include an attempt to describe or explain the highly variable pattern of motoneurone firing during the LDP. The contrast, however, between locomotor firing and the regularity of motoneurone firing evoked solely by current injection was seen consistently. This comparison was completed for one cell but similar data have been collected from at least nine other motoneurones.

Sudden increases in both the firing frequency and the number of action potentials during a LDP were seen concurrent with increases in amplitude of the LDP with depolarisation (not shown). This supports the suggestion that the previously described voltage-dependent non-linear increases in the excitatory component of the LDP contribute to the "high rates of repetitive firing typically seen during locomotion" (Brownstone et al. 1994).

DISCUSSION

These results demonstrate that late adaptation is, in fact, completely abolished during fictive locomotion, as we hypothesized. Furthermore, the motoneurone intrinsic properties that appear to be associated with late adaptation are those produced by fast sodium channels, as evidenced by the changes in rate of rise of the action potential and peak amplitude of the action potential. We therefore suggest that late adaptation results from slow inactivation of sodium channels. Further, we suggest that this property can be regulated during fictive locomotion; such that this longer time-constant of inactivation is removed, resulting in the abolition of late adaptation.

The mechanism that underlies late adaptation is not known. Here, we have studied the same MNs in two states, one where they adapt (current injection during our control condition) and one where adaptation does not occur (locomotion). Of all the properties we examined, the most likely underlying factor is the fast Na channel. We suggest that perhaps there is a very slow time constant of inactivation (10s of seconds) for this channel. Our data also shows that the absence of late adaptation does not correlate with the AHP, which diminishes during locomotion at the same time that late adaptation disappears (Brownstone, et al. 1992).

There is no indication that the changes in late adaptation during locomotion are related to early adaptation. During locomotion, early adaptation is not apparent. There is a high degree of variability of firing rate (Figure 4), which is consistent with the reduction of the AHP (Person and Kudina 1972). The AHP is classically thought to be responsible for early adaptation (Baldissera et al. 1978; Baldissera, et al 1978; Barrett et al. 1980; Kernell and Sjoholm 1973; Sawczuk et al. 1997). Therefore, it is possible that

the lack of measurable early adaptation during locomotion is because the AHP is decreased. However, the role of the AHP in early adaptation is now questionable (Powers, et al 1999), Miles and Brownstone, unpublished.

It is plausible that Na channel inactivation underlies early adaptation (Miles and Brownstone, unpublished). Perhaps late adaptation is produced by a similar mechanism with a longer time constant. For this to be true, Na channel inactivation would require multiple time constants of inactivation.

Clearly Na channels can be modulated (Cantrell et al. 1997; Astman et al. 1998; Cantrell and Catterall 2001; Cantrell et al. 1999). Na channel modulation is implicated as a likely mechanism for voltage threshold hyperpolarization associated with locomotion (Dai et al. 2002; Krawitz, et al 2001).

The "intrinsic" properties of mammalian motoneurones change dependent on the state of the animal, and the present demonstration that late adaptation is abolished in spinal motoneurones during locomotion is an example of this. Together, the alterations in spinal motoneurone properties that are know to occur during locomotion (reduced AHP, the appearance of plateau properties, the hyperpolarization of the voltage threshold, and the suppression of late adaptation) all contribute to increased excitability of motoneurons during locomotion, and account for the fact that motoneurone firing is more robust and more variable than would be predicted based upon results obtained with intracellular current injection alone.

REFERENCES

Astman, N., Gutnick, M. J., and Fleidervish, I. A. Activation of protein kinase C increases neuronal excitability by regulating persistent Na+ current in mouse neocortical slices. J Neurophysiol 80: 1547-51, 1998.

Baldissera, F., Gustafsson, B., and Parmiggiani, F. Saturating summation of the afterhyperpolarization conductance in spinal motoneurones: a mechanism for 'secondary range' repetitive firing. Brain Res 146: 69-82, 1978.

Barrett, E. F., Barrett, J. N., and Crill, W. E. Voltage-sensitive outward currents in cat motoneurones. J Physiol (Lond) 304: 251-276, 1980.

Bigland-Ritchie, B. R., Dawson, N. J., Johansson, R. S., and Lippold, O. C. J. Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. J Physiol (Lond) 379: 451-459, 1986.

Brownstone, R. M., Gossard, J.-P., and Hultborn, H. Voltage-dependent excitation of motoneurones from spinal locomotor centres in the cat. Exp Brain Res 102: 34-44, 1994.

Brownstone, R. M., Jordan, L. M., Kriellaars, D. J., Noga, B. R., and Shefchyk, S. On the regulation of repetitive firing in lumbar motoneurones during fictive locomotion in the cat. Exp Brain Res 90: 441-445, 1992.

Cantrell, A. R. and Catterall, W. A. Neuromodulation of Na+ channels: an unexpected form of cellular plasticity. Nat Rev Neurosci 2: 397-407, 2001.

Cantrell, A. R., Smith, R. D., Goldin, A. L., Scheuer, T., and Catterall, W. A.

Dopaminergic modulation of sodium current in hippocampal neurons via cAMP-dependent phosphorylation of specific sites in the sodium channel alpha subunit. J Neurosci 17: 7330-8, 1997.

Cantrell, A. R., Tibbs, V. C., Westenbroek, R. E., Scheuer, T., and Catterall, W. A. Dopaminergic modulation of voltage-gated Na+ current in rat hippocampal neurons requires anchoring of cAMP-dependent protein kinase. J Neurosci 19: RC21, 1999.

Dai, Y., Jones, K. E., Fedirchuk, B., McCrea, D. A., and Jordan, L. M. A modelling study of locomotion-induced hyperpolarization of voltage threshold in cat lumbar motoneurones. J Physiol 544: 521-36, 2002.

Granit, R., Kernell, D., and Shortess, G. K. The behaviour of mammalian motoneurones during long-lasting orthodromic, antidromic and trans-membrane stimulation. J Physiol (Lond) 169: 743-754, 1963a.

Granit, R., Kernell, D., and Shortess, G. K. Quantitative aspects of repetitive firing of mammalian motoneurons caused by injected currents. J Physiol (Lond) 168: 911-931, 1963b.

Harris-Warrick, R. M. and Marder, E. Modulation of Neural Networks for Behavior. Ann Rev Neurosci 14: 39-57, 1991.

Hultborn, H. Plateau potentials and their role in regulating motoneuronal firing. Prog Brain Res 123: 39-48, 1999.

Kernell, D. The adaptation and the relation between discharge frequency and current

strength of cat lumbosacral motoneurones stimulated by long-lasting injected currents. Acta Physiol Scand 65, 65-73. 1965a.

Kernell, D. The limits of firing frequency in cat lumbosacral motoneurones possessing different time course of afterhyperpolarization. Acta Physiol Scand 65: 87-100, 1965b.

Kernell, D. and Monster, A. W. Motoneurone properties and motor fatigue. An intracellular study of gastrocnemius motoneurones of the cat. Exp Brain Res 46: 197-204, 1982a.

Kernell, D. and Monster, A. W. Time course and properties of late adaption in spinal motoneurones of the cat. Exp Brain Res 46: 191-196, 1982b.

Kernell, D. and Sjoholm, H. Repetitive impulse firing: comparisons between neurone models based on 'voltage clamp equations' and spinal motoneurones. Acta Physiol Scand 87: 40-56, 1973.

Krawitz, S., Fedirchuk, B., Dai, Y., Jordan, L. M., and McCrea, D. A. State-dependent hyperpolarization of voltage threshold enhances motoneurone excitability during fictive locomotion in the cat. J Physiol 532: 271-281, 2001.

Person, R. S. and Kudina, L. P. Discharge frequency and discharge pattern of human motor units during voluntary contraction of muscle. Electroencephalogr Clin Neurophysiol 32: 471-83, 1972.

Powers, R. K., Sawczuk, A., Musick, J. R., and Binder, M. D. Multiple mechanisms of spike-frequency adaptation in motoneurones. J Physiol Paris 93: 101-14, 1999.

Richter, D. W. and Heyde, F. Accommodative reactions of medullary respiratory neurons of the cat. J Neurophysiol 38: 1172-80, 1975.

Sawczuk, A., Powers, R. K., and Binder, M. D. Contribution of outward currents to spike-frequency adaptation in hypoglossal motoneurons of the rat. J Neurophysiol 78: 2246-2253, 1997.

Sawczuk, A., Powers, R. K., and Binder, M. D. Spike frequency adaptation studied in hypoglossal motoneurons of the rat. J Neurophysiol 73: 1799-1810, 1995.

Shik, M. L., Severin, F. V., and Orlovsky, G. N. Control of walking and running by means of electrical stimulation of the mid-brain. Biophysics 11: 756-765, 1966.

Spielmann, J. M., Laouris, Y., Nordstrom, M. A., Robinson, G. A., Reinking, R. M., and Stuart, D. G. Adaptation of cat motoneurons to sustained and intermittent extracellular activation. J Physiol 464: 75-120, 1993.

Steeves, J. D., Jordan, L. M., and Lake, N. The close proximity of catecholamine-containing cells to the 'mesencephalic locomotor region' (MLR). Brain Res 100: 663-670, 1975.

West, J. W., Numann, R., Murphy, B. J., Scheuer, T., and Catterall, W. A. A phosphorylation site in the Na+ channel required for modulation by protein kinase C. Science 254: 866-8, 1991.

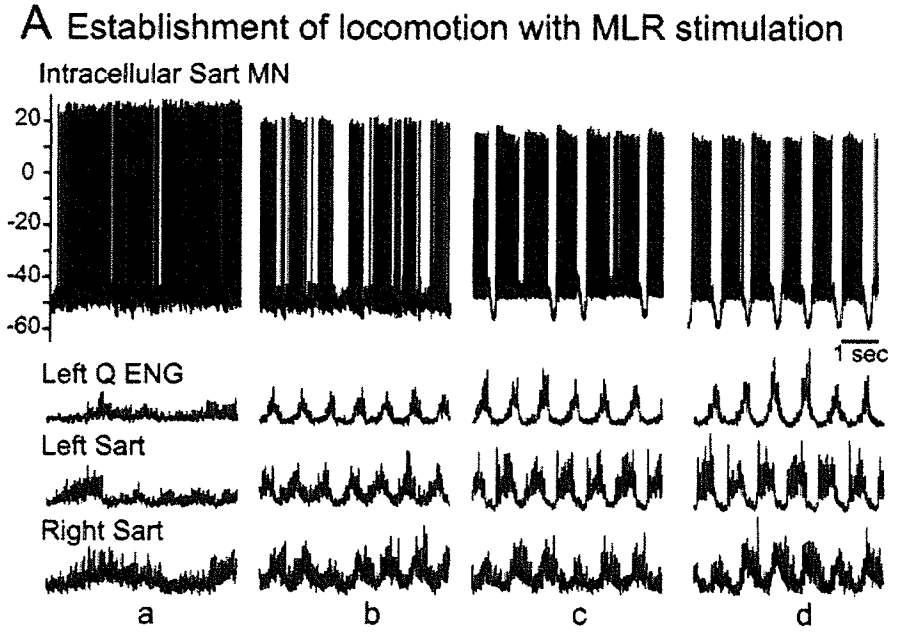
FIGURE LEGENDS

Figure 1: The development of locomotion is associated with a reversal of late adaptation. A illustrates the motoneuron intracellular recording (upper panel and electroneurograms (ENGs) from left quadriceps (Q), and left and right sartorius (Sart). With the onset of MLR stimulation, tonic activity (a) appears, followed by irregular stepping (b), then more robust stepping with some deletions of the hyperpolarizing component of the locomotor drive potential (c) and finally well-developed fictive locomotion (d). B shows the reversal of adaptation as fictive locomotion develops. At the onset of tonic activity (a) firing gradually increases in frequency, then after approximately 20 seconds the average instantaneous frequency begins to decrease. This is the onset of adaptation, that continues through the onset of irregular stepping (b). When the locomotion becomes more robust (c) the adaptation reverses and this reversal continues throughout the period of fictive locomotion.

Figure 2: Late adaptation occurs in the control condition (intracellular current injection, consisting of rectangular depolarizing pulses of 500 ms duration at 1.0 – 1.7 Hz, shown in A, upper panel), but is absent throughout a prolonged period of fictive locomotion (A, lower panel). B illustrates the rate of rise of the action potentials in the Control (upper panel) and locomotion (lower panel) conditions. A decrease in the rate of rise of the action potential (accommodation) is associated with late adaptation in the control condition, but there is no evidence of accommodation during the firing produced by fictive locomotion.

Figure 3: An increase in instantaneous frequency due to intracellular current injection occurs after a period of fictive locomotion (fic. loco), and this recovers to baseline levels (dotted lines) for the instantaneous frequency of the first interval (filled circles) and the averaged firing frequency (open circles). Late adaptation is apparent following comparable periods of control activity, produced by rectangular depolarizing pulses of 500 ms duration at 1.0 – 1.7 Hz. Late adaptation is abolished after a subsequent bout of fictive locomotion.

Figure 4: During locomotion, motoneurones produce more action potentials at higher and more variable frequencies than at rest (upper panel), and these higher firing frequencies during locomotion (lower panel) occur at more hyperpolarized voltage thresholds than at rest.


A Establishment of locomotion with MLR stimulation
Intracellular Sart MN
20
0
-20
-40
-60
1 sec
Left Q ENG
Left Sart
Right Sart
a
b
c
d

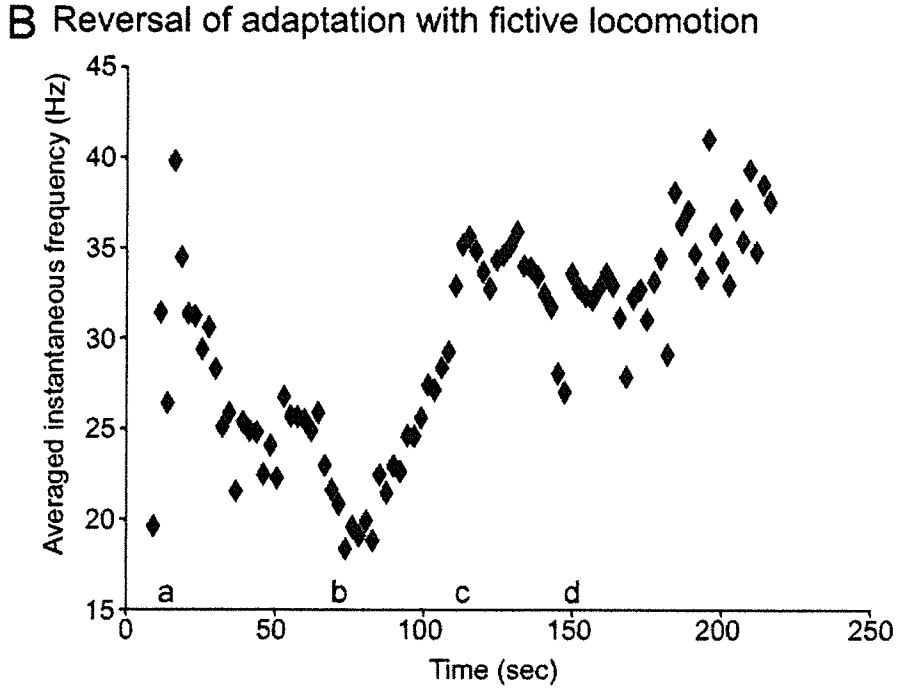

B Reversal of adaptation with fictive locomotion
Averaged instantaneous frequency (Hz)
45
40
35
30
25
20
15
a
b
c
d
0
50
100
150
200
250
Time (sec)

Figure 1

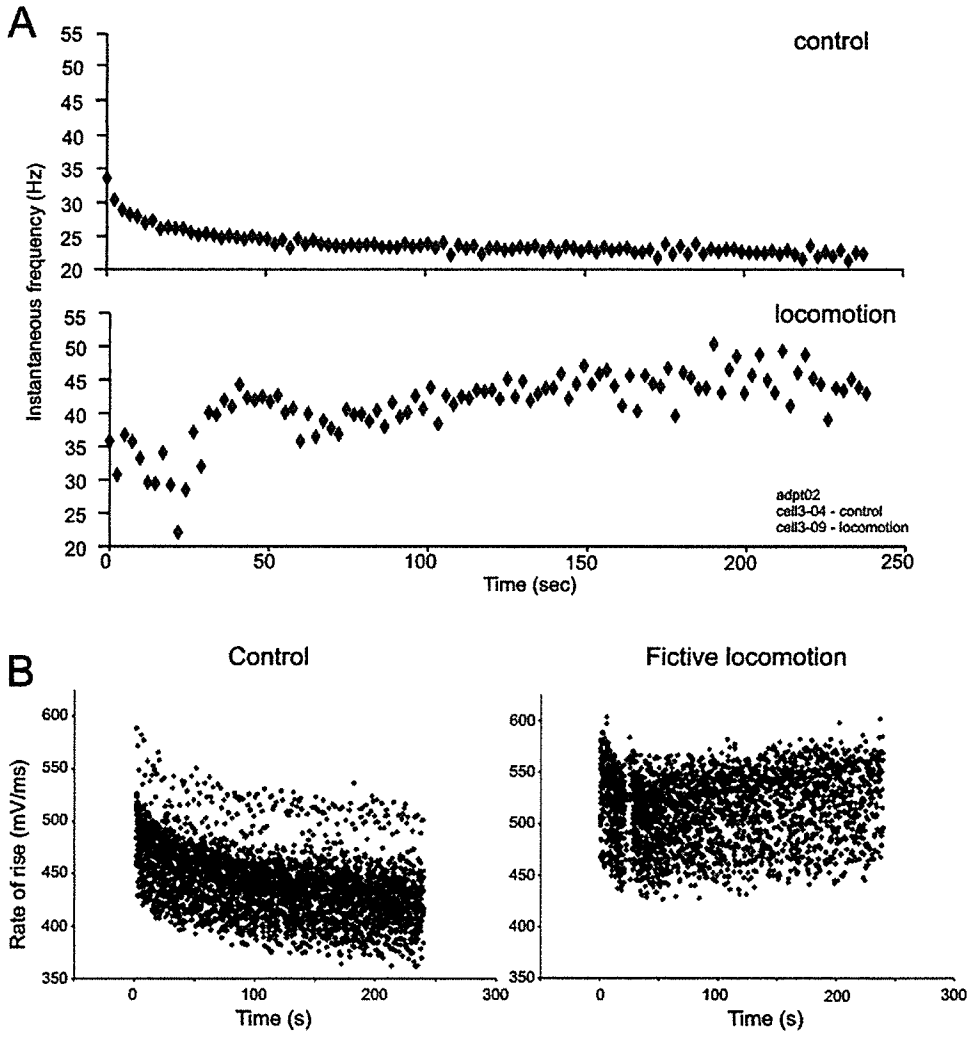

A
control
locomotion
Instantaneous frequency (Hz)
Time (sec)
adpt02
cell3-04 - control
cell3-09 - locomotion
B
Control
Fictive locomotion
Rate of rise (mV/ms)
Time (s)
Time (s)

Figure 2

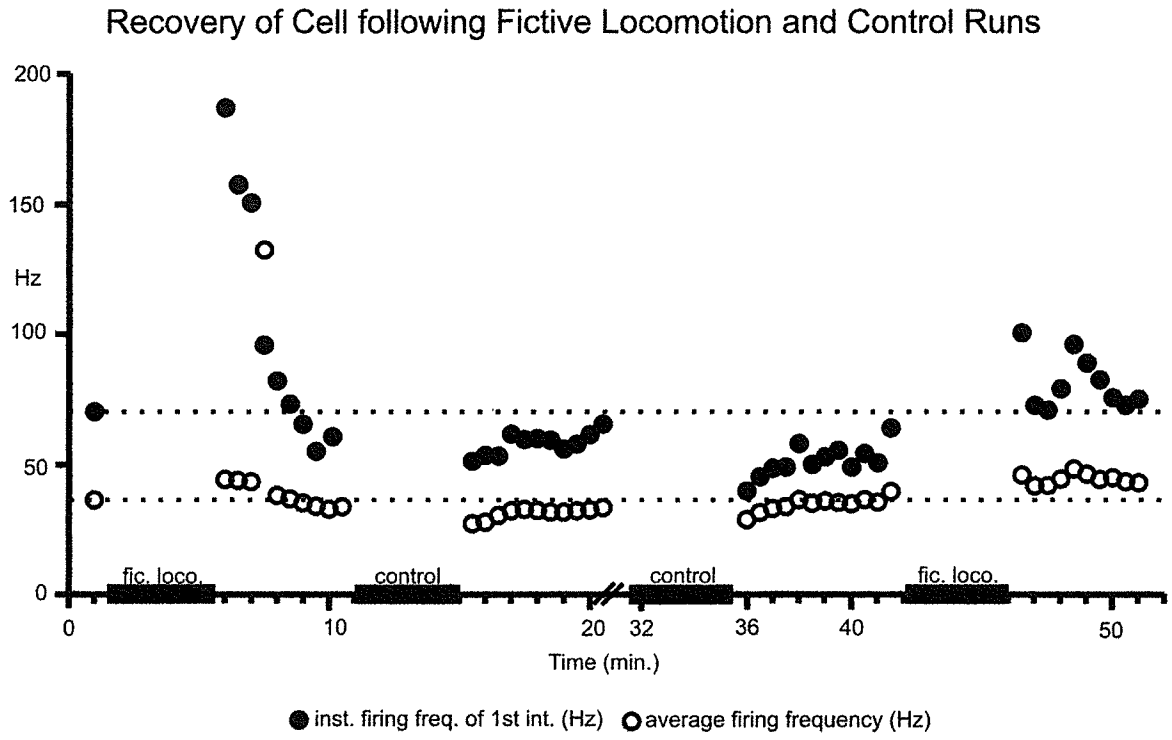

Recovery of Cell following Fictive Locomotion and Control Runs
200
150
Hz
100
50
0
fic. loco.
control
control
fic. loco.
0
10
20
32
36
40
50
Time (min.)
inst. firing freq. of 1st int. (Hz)
average firing frequency (Hz)

Figure 3

During locomotion motoneurones produce more action potentials at higher and more variable frequencies than at rest. The higher firing frequencies during locomotion occur at more hyperpolarized voltage thresholds than at rest.

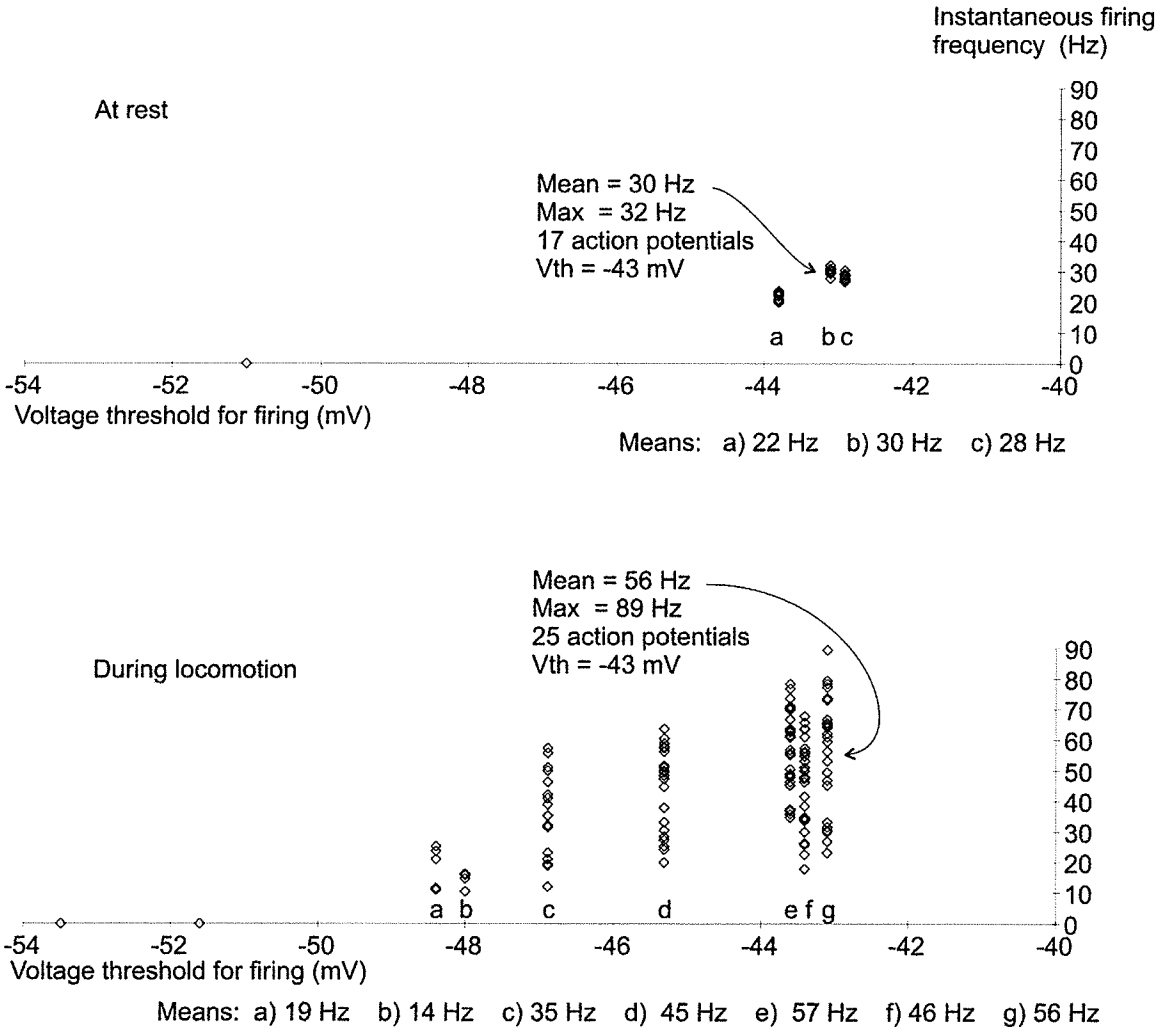


Figure 4

GENERAL DISCUSSION

Summary of results

Two studies comprise the thesis. The most important observations of the first study are: a) the voltage threshold for action potential initiation in lumbar motoneurones is hyperpolarised during fictive locomotion, and b) this hyperpolarisation enhances motoneurone excitability during fictive locomotion. The most important observation of the second study is the absence and reversal of late adaptation of spike frequency during fictive locomotion.

Experimental limitations

A number of technical considerations or limitations of the experiments are recognised. The experiments were designed to describe possible changes in a) voltage threshold, and b) firing frequency, specifically the presence or absence of late adaptation, during fictive locomotion. Such changes might also reveal possible associations or relations of such changes to previously reported state-dependent phenomena, viz., AHP reduction (Brownstone et al 1992), alteration of the frequency-current relation (ibid.), the activation of voltage-dependent conductances (Brownstone et al 1994), and plateau potentials. Full evaluation would entail an examination of the mechanisms underlying the different phenomena, and is therefore beyond the scope of this thesis. The thesis remains descriptive of the observed phenomena, and does not demonstrate or examine the underlying mechanisms. Nor are the consequences of Vth reduction on repetitive firing demonstrated, but implications are considered (below).

The definition of the voltage threshold as the membrane potential at which depolarisation increases at 10 V/s may be considered a technical limitation, but a definition in such terms is appropriate in the absence of a definition in terms of mechanism (i.e., conductances activated). The value of 10 V/s is not arbitrary but consistent with previous reports (Brownstone et al 1992). It is recognised that the threshold is passed when the net result of conductances induces an action potential (the net inward current outbalances net outward current).

Another limitation of the thesis concerns fatigue. The implication for the nervous and neuromuscular systems of the abolition of late adaptation and the maintenance of firing frequency may be to permit locomotion to continue while minimising the effects of fatigue. The central nervous system mechanism(s) of fatigue is not explored in this thesis.

Possible mechanisms underlying Vth reduction and abolition of late adaptation during locomotion

During fictive locomotion, in both flexor and extensor motoneurones, and in motoneurones with both low and high rheobase currents, the voltage threshold for action potential initiation is hyperpolarised. The Vth is reduced during fictive locomotion when action potentials are evoked by intracellular injection of depolarising current, and when locomotor firing occurs spontaneously without intracellular current injection. Reduction

of the Vth occurs during both the hyperpolarised and depolarised phases of the step cycle. Possible mechanisms underlying Vth reduction during locomotion are not explored in this study. The data from this study, however, forms the basis of a modelling study (Dai et al 2002) which extended the examination of the data, confirmed that the reduction in Vth is accompanied by only small changes in action potential height and width, and confirmed that Vth reduction is present to a similar degree in both the hyperpolarised and depolarised phases of the step cycle. Ionic mechanisms possibly mediating the hyperpolarising shift were examined within models of cat lumbar motoneurones (Dai et al 2002). The models incorporated the ten active conductances [gNa, gK(DR), gK(A), gK(AHP), gCa-T, gCa-L, gCa-N, g-h, g-leak, and a persistent sodium conductance, gNa-P] in mammalian spinal motoneurones and used to detect changes in active properties during locomotion that could result in Vth reduction (Dai et al 2002). Simulation results suggest that the ionic mechanisms likely responsible for Vth reduction is modulation of sodium and/or delayed rectifier channels in the initial segment.

Four conductance changes in the motoneurone initial segment which could result in Vth hyperpolarisation are: 1) increasing gNA, 2) shifting gNa voltage dependency in the hyperpolarising direction, 3) reducing gK(DR), and 4) shifting gK(DR) voltage dependency in the depolarising direction (Dai et al 2002). These changes would at subthreshold membrane potentials increase the net inward current and hyperpolarise Vth. The result of Vth hyperpolarisation is an increase in motoneurone excitability, and thus a reduction of the amount of both current and membrane potential depolarisation required to reach threshold.

These conductance changes are dependent on the locomotor state, and as such, would facilitate recruitment of motoneurones during locomotion. The neuromodulatory substances mediating this increase in motoneurone excitability with the onset of locomotion, with the particular action of Vth hyperpolarisation, have been studied in the neonatal rat (Fedirchuk and Dai 2004). The monoamines serotonin and noradrenaline induced Vth hyperpolarisation which was not due to a reduction in accommodation, and occurred without changes in membrane potential or membrane resistance. In addition to facilitation of activation of fast sodium channels and reduction of a potassium conductance, persistent inward currents mediated by calcium and sodium channels (Fedirchuk and Dai 2004, Lee and Heckman 2001, Li et al 2004) or the NMDA current (Maclean and Schmidt 2001) may be facilitated, and contribute to Vth hyperpolarisation.

Both sodium channel inactivation and an increased potassium conductance have been found to underlie accommodation in cat spinal motoneurones (e.g., Schlue et al. 1974). While the mechanism(s) underlying adaptation is not explored in this thesis, an attempt to distinguish the mechanisms underlying adaptation and accommodation was made by measuring accommodative changes throughout the control and locomotor trials.

Evidence of accommodation (decreased action potential amplitude and decreased rate of rise of the action potential; Bradley and Somjen 1961) accompanied the decline in firing rate during the intracellular current injection of the control trials. This confirms the correlation of adaptation with accommodation during current injection (control trials). During fictive locomotion these accommodative changes occurred in most trials in the absence of late adaptation, i.e., ongoing accommodation occurred despite increases in firing frequency. No clear relation therefore emerged between the lack of adaptation and

accommodation during locomotion. The conclusion of these findings is that the cell continues to fire at high rates by a process which appears to be unrelated to accommodative changes.

Several possible mechanisms underlying initial, early, and late adaptation have been proposed. Sawczuk et al 1997 summarises possible mechanisms underlying initial adaptation as medium-duration AHP, fast sodium channel inactivation, and inward rectifier (I-h) deactivation. Those underlying early adaptation are inactivation of persistent sodium current, slow AHP, m-type potassium current, slow sodium channel inactivation, delayed rectifier inactivation, sodium-potassium ATPase activity, and saturation of calcium sequestering systems (for references, see Sawczuk et al. 1997, and further, Powers et al. 1999). Mechanisms underlying early adaptation are also postulated to underlie late adaptation (Sawczuk et al. 1997), but remain unknown. If the process correlates with inactivation of a sodium channel, it would be a slow inactivation of the channel, over tens of seconds. This is slower than a correlation with any AHP. Medium-duration and slow AHP's are correlated with initial and early adaptation. Slow inactivation of sodium current is associated with slow adaptation in mouse and guinea-pig neocortical neurons: inactivation with a time constant of approximately 2 seconds (Fleidervish et al. 1996, Fleidervish and Gutnick 1996). Powers et al (1999) suggest that progressive activation of an inward current may oppose the decline in frequency caused by slow sodium inactivation.

It is possible that an inward current is activated during locomotion. It is also possible that modulation of sodium channels may underlie the hyperpolarisation of Vth

with the onset of locomotion (as mentioned above, Fedirchuk and Dai 2004). There is also a possibility that modulation of sodium channels underlies the abolition of late adaptation with the onset of locomotion (see Astman et al. 1998 re: increased neuronal excitability by regulation of persistent sodium current). Powers et al. (1999) suggest that the magnitude of late adaptation may depend on the interplay between slow inactivation of sodium currents which decreases discharge rate, and slow activation or facilitation of a Ca current that increases discharge rate (Powers et al. 1999).

Implications of Vth reduction and abolition of late adaptation during locomotion

Previous reports have documented state-dependent changes in the intrinsic properties of motoneurones: the reduction of the AHP during fictive locomotion (Brownstone et al. 1992), and the appearance of a voltage-dependent excitatory current (Brownstone et al. 1994). Voltage-dependent excitation results in non-linear responses of motoneurones to depolarising currents, which may facilitate the recruitment of motoneurones, or enhance motoneuronal output evoked by reflex or central excitation (Brownstone et al. 1994; McCrea et al. 1997; Bennet et al. 1998).

Vth reduction is another motoneurone membrane property altered during locomotion. Such changes in membrane properties could enhance motoneurone excitability during locomotion and counter the decrease in excitability that might result from an increase in motoneurone conductance that occurs during locomotion (Shefchyk and Jordan 1985; Gosgnach et al. 2000). The increased excitability of motoneurones during fictive locomotion, implied by the large reduction in current required to evoke

firing during locomotion, has important consequences for motoneurone recruitment and firing. Less depolarisation from either central or reflex pathways would be needed to recruit motoneurones. Easier motoneurone recruitment in turn implies fewer interneurons required to stimulate the motoneurones. In terms of motoneurone output, easier recruitment implies easier (or greater) force production by the muscle.

Because of the critical range of membrane potential depolarisation in which motoneurone excitability is enhanced, just as testing for motoneurone property changes requires testing through a wide range of membrane potentials, so testing in other models would require testing through a full range of force.

Fedirchuk and Dai (2004) point out that Vth reduction in both larger and smaller ventral horn cells may be accompanied by Vth reduction of interneurons. Facilitation of interneuron activation as well as increased motoneurone excitability imply that fewer interneurons are required for motoneurone firing. Consideration of the regulation of motoneurone firing during locomotion would then include, in addition to facilitation of motoneurone firing, the contribution of increased interneuronal excitability, and furthermore, mechanisms required for turning off motoneurone firing.

Modulation of motoneurone properties during locomotion enables the motoneurone to overcome spike-frequency adaptation. During locomotion, the cell continues to fire at high rates by a process which appears unrelated to accommodative changes. The maintenance of firing frequency may help to permit locomotion to continue while minimising the effects of fatigue. While this thesis has not explored the underlying mechanisms, it confirms changes in motoneurone properties (Vth reduction and the

abolition of spike-frequency adaptation) which are dependent on the state of the animal, namely locomotion.

REFERENCES

Astman, N, Gutnick, MJ, and Fleidervish, IA. 1998 Activation of protein kinase C increases neuronal excitability by regulating persistent Na+ current in mouse neocortical slices. J Neurophysiol 80: 1547-1551

Bennett, DJ, Hultborn, H, Fedirchuk, B and Gorassini,M. 1998 Synaptic activation of plateaus in hindlimb motoneurones of decerebrate cats. J Neurophysiol 80: 2023-2037

Bradley, K and Somjen, GG 1961 Accommodation in motoneurones of the rat and cat. J Physiol 156: 75-92

Brownstone, R. M., Jordan, L. M., Kriellaars, D. J., Noga, B. R., and Shefchyk, S. On the regulation of repetitive firing in lumbar motoneurones during fictive locomotion in the cat. Exp Brain Res 90: 441-445, 1992.

Brownstone, R.M., Gossard, J.-P., and Hultborn, H. Voltage-dependent excitation of motoneurones from spinal locomotor centres in the cat. Exp Brain Res 102: 34-44, 1994.

Dai, Y, Jones, KE, Fedirchuk, B, McCrea, DA, and Jordan, LM. A modelling study of locomotion-induced hyperpolarization of voltage threshold in cat lumbar motoneurones. J. Physiol. 544.2:521-536, 2002.

Fedirchuk, B and Dai, Y 2004 Monoamines increase the excitability of spinal neurones in the neonatal rat by hyperpolarising the voltage threshold for action potential threshold. J Physiol 557:355-361

Fleidervish, IA., Friedman, A, and Gutnick, M.J. 1996 Slow inactivation of Na+ current and slow cumulative spike adaptation in mouse and guinea-pig neocortical neurones in slices. J Physiol 493: 83-97

Fleidervish, IA, and Gutnick, MJ. 1996 Kinetics of slow inactivation of persistent sodium current in layer V neurons of mouse neocortical slices. J Neurophysiol 76: 2125-2130

Gosgnach, S, Quevedo, J, Fedirchuk, B and McCrea, D. 2000 Depression of group Ia monosynaptic EPSPs in cat hindlimb motoneurones during fictive locomotion. Zj Physiol 526: 639-652

Lee, RH, and Heckman, CJ. 2001 Essential role of a fast persistent inward current in action potential initiation and control of rhythmic firing. J Neurophysiol 85: 472-475

Li, Y, Gorassini, MA, and Bennett, DJ. 2004 Role of persistent sodium and calcium currents in motoneuron firing and spasticity in chronic spinal cats. J Neurophysiol 91: 767-783

Maclean, JN and Schmidt, BJ. 2001 Voltage-sensitivity of motoneuron NMDA receptor channels is modulated by serotonin in the neonatal rat spinal cord. J Neurophysiol 86: 1131-1138

McCrea, D, Krawitz, S, Fedirchuk, B, and Jordan, L. 1997 Group-I evoked extensor motoneurone activity is amplified by voltage-dependent depolarizations during locomotion. Society for Neuroscience 23, 298.3

Powers, RK, Sawczuk, A, Musick, JR and Binder, MD. 1999 Multiple mechanisms of spike-frequency adaptation in motoneurones. J Physiol (Paris) 93: 101-114

Sawczuk, A, Powers, RK, and Binder, MD 1997 Contribution of outward currents to spike-frequency adaptation in hypoglossal motoneurones of the rat. J Neurophysiol 78: 2246-2253

Schlue WR, Richter DW, Mauritz KH, Nacimiento AC Mechanisms of accommodation to linearly rising currents in cat spinal motoneurones 1974 J Neurophysiol 37: 310-315

Shefchyk, S and Jordan, L. 1985 Motoneuron input-resistance changes during fictive locomotion produced by stimulation of the mesencephalic locomotor region. J Neurophysiol 54: 1101-1108