VILNIUS UNIVERSITY

ROKAS BUIŠAS

THE GAIN OF SPINAL CORD MOTONEURONS AND ITS MODIFICATION

Summary of doctoral dissertation
Biomedical sciences, biophysics (02 B)

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NUGAROS SMEGENŲ MOTONEURONŲ PERDAVIMO FUNKCIJA IR JOS MODIFIKAVIMAS

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CONTENTS

ABBREVIATIONS

5-HT - serotonin

AHP - afterhyperpolarization

AP - action potential

f -I - frequency current relationship

$GABA_A$ - ionotropic receptor (type A) of gamma-aminobutyric acid

I - current

I_{NaP} - persistent inward sodium current

I_{NaT} - transient inward sodium current

MN - motoneurons

TTX - tetrodotoxin

V - potential

1. INTRODUCTION

An ability to make precise and goal-directed movements of their body parts is one of the common features of animals. Spinal cord circuits play an essential role in sensory information processing and execution of coordinated movements.

Motoneurons (MN) are the spinal neurons that directly control the muscle contraction. The gain characterizes how the synaptic input to MN is converted in to action potential firing and subsequent muscle contraction. The high gain allows a high force and fast contraction, while low gain is essential for a fine control of movements.

The firing pattern of MN differs during ballistic and slow voluntary movements also. Tonic low frequency firing is observed during slow voluntary movement, while phasic high frequency firing characterize ballistic movements (Desmedt and Godaux, 1977a,b; Duchateau and Enoka, 2011). During suprathreshold depolarizing current pulse MNs adapt from a higher frequency at the unset to a lower steady state frequency (Granit et al., 1963a; Kernell, 1965; Kernell and Monster, 1982; Sawczuk et al., 1995; Sawczuk et al., 1997). More importantly, adaptation affects not only frequency but also the gain of MNs (Powers et al., 1999; Sawczuk et al., 1995). Gain measured at the beginning of a stimulus (transient gain) is usually higher than gain measured in late state adaptation (steady gain). Adaptation decreased the gain of rat MNs (Granit et al., 1963a; Sawczuk et al., 1995), cat MNs (Kernell, 1965) and turtle spinal cord MNs (Hounsgaard et al., 1988b).

The gain of MN is determined experimentally by injecting suprathreshold currents through microelectrode, measuring the neuron's firing rate and estimating steepness of frequency–current (f-I) relation obtained (Granit et al., 1963a; Kernell, 1965). MNs can be stimulated with square current pulses of increasing amplitude (Granit et al., 1963a; Ito and Oshima, 1965) or with slow triangular current ramps (Bennett et al., 2001b; Button et al., 2006; Hounsgaard et al., 1984; Hounsgaard et al., 1988a; Lee and Heckman, 1998). The ramp stimulus is more convenient to use, however the gain estimated may be affected by adaptation. Thus, it is difficult to compare experimental data of different investigators using various stimulation methods.

The pattern of APs generation in spinal MNs that controls movements depends on synaptic input (Berg et al., 2008; Calvin and Stevens, 1968; Granit et al., 1963b) and

intrinsic response properties (Harvey et al., 2006b; Hounsgaard and Mintz, 1988). Apart from spike frequency adaptation (Powers et al., 1999; Sawczuk et al., 1995), signal processing in MNs is influenced by a number of another nonlinearities mediated by ion conductances described as afterhyperpolarization (Granit et al., 1963a; Kernell, 1965; Vervaeke et al., 2006; Vogalis et al., 2003) and the persistent inward currents (Heckman et al., 2008; Rekling et al., 2000; Schwindt, 1973). Response properties of the MNs can be dramatically altered during spinal network activity by activation of metabotropic receptors for glutamate (Svirskis and Hounsgaard, 1998), serotonin (5-HT) (Heckman et al., 2003; Hounsgaard et al., 1988a; Hultborn et al., 2004; Perrier et al., 2003; Perrier and Cotel, 2008; Perrier and Hounsgaard, 2003) acetylcholine (Alaburda et al., 2002; Miles et al., 2007) or dopamine (Clemens and Hochman, 2004). This may alter the gain by adjusting the response properties of MNs to the behavioural needs (Button et al., 2006; Harvey et al., 2006b; Hounsgaard and Mintz, 1988; Hultborn et al., 2004; Kernell, 1965; Lee and Heckman, 2001; Powers and Binder, 2001).

During spinal network activity MN receives a massive balanced synaptic excitation and inhibition (Berg et al., 2007) therefore their membrane conductances dramatically increase (Alaburda et al., 2005). It is not known how the transient, early and steady gain values of the MNs are affected by increased membrane conductance.

Ion conductances activating close to the threshold for action potentials play a particularly important role in synaptic integration. Among them is the persistent sodium current (I_{NaP}) first demonstrated in nodes of Ranvier of myelinated fibres of *Rana esculenta* (Dubois and Bergman, 1975). I_{NaP} activates at voltages of about 10 mV more negative than transient sodium current (I_{NaT}) (Brown et al., 1994; Magistretti et al., 2006; Urbani and Belluzzi, 2000) and is relatively small. It was found that I_{NaP} corresponds to only about 1 % of I_{NaT} (Crill, 1996; Magistretti et al., 2006; Urbani and Belluzzi, 2000). However, paying attention to the large total conductance of I_{NaT}, the absolute conductance of I_{NaP} is large enough to cause significant depolarization at subthreshold levels when total membrane conductance is low. I_{NaP} increases excitability (Kuo et al., 2006; Lee and Heckman, 2001; Li and Bennett, 2003), participates in locomotor rhythm generation (Tazerart et al., 2008; Zhong et al., 2007) and amplifies synaptic input in MNs (Manuel et al., 2007). The importance of I_{NaP} in functional synaptic integration manifests in modulation of this current by serotonin and norepinephrine (Harvey et al.,

2006a). It would be straight-forward to assume that increased excitability due to I_{NaP} (Kuo et al., 2006; Lee and Heckman, 2001; Li and Bennett, 2003) leads to increased gain in MNs firing (Kuo et al., 2006). Moreover, direct experimental investigation of influence of I_{NaP} on MNs firing properties is difficult to accomplish. TTX, the common blocker of sodium channels, eliminates not only I_{NaP} but also APs. Riluzole is considered to be I_{NaP} blocker, however, it also inhibits transient sodium current (I_{NaT}) (Urbani and Belluzzi, 2000) and delayed rectifier K^+ channels (Ahn et al., 2005). Because of complications related to pharmacological block of I_{NaP}, the way to investigate how I_{NaP} affects the firing properties and the gain of spinal MNs may be the dynamic clamp technique (Sharp et al., 1993; Vervaeke et al., 2006).

1.1. Aim and objectives

The aim of the study is to evaluate the gain of spinal cord motoneurons and to identify its modification.

Objectives:

1. To evaluate and compare the gain of spinal cord motoneurons obtained from triangular ramp and square step current stimulus;
2. To assess the influence of triangular current ramp rising speed on the estimation of the gain of motoneurons;
3. To estimate the influence of increased membrane conductance on the gain of spinal motoneurons;
4. To evaluate the influence of persistent sodium current on the gain of spinal motoneurons.

1.2. Actuality and scientific novelty

For the first time:

- The gain of the same motoneuron was estimated using triangular and square current stimulus;
- The influence of increased membrane conductance on the gain of spinal motoneurons was evaluated.
- It was experimentally demonstrated persistent sodium current in turtle spinal cord motoneurons and estimated its influence to the gain of motoneurons.

1.3. Practical application of the study results

Methodical recommendations for electrical stimulation of neurons: stimulation with square current pulses provides more information about the gain than triangular current ramps.

1.4. Defended statements

1. The gain of motoneurons estimated using triangular current ramps is the same as steady gain of motoneurons obtained using square current steps;

2. The transient and early gain of motoneurons can be estimated only by using square current steps;

3. The increased membrane conductance does not change the gain of motoneurons;

4. Persistent sodium current increases excitability of motoneurons, reduces the transient gain, but does not affect the steady gain.

2. METHODS

2.1. Slice preparation

Red-eared turtles (*Chrysemys scripta elegans*; n=30) placed on crushed ice 2 hr before surgery to induce hypothermic anesthesia (Melby and Altman, 1974) were killed by decapitation. Transverse slices (1.5-2 mm thick) were obtained from the lumbar enlargement. All animals were treated according to the guidelines described by EU regulations and Lithuanian legislation. Experiments were performed at room temperature in a normal Ringer solution containing (mM): 120 NaCl; 5 KCl; 15 $NaHCO_3$; 2 $MgCl_2$; 3 $CaCl_2$; 20 glucose saturated with 98 % O_2 and 2 % CO_2 to obtain pH 7.6.

2.2. Recordings

Intracellular recordings in current-clamp mode were performed with an Axoclamp-2B amplifier (Molecular Devices). Glass pipettes were pulled with puller (PUL100, WPI) and filled with a mixture of 0.9 M potassium acetate and 0.1 M KCl. Recordings were performed in motoneurons if they had a stable membrane potential more negative than -50 mV, if they did not fire spontaneously and amplitude of action potentials was higher than 70 mV (McDonagh et al., 1999). Data were sampled at 10 kHz with a 16-bit analog-to-digital converter (Digidata 1440A, Molecular Devices), displayed by means of Clampex software (Molecular Devices) and stored on a computer hard disk for later analysis. Sodium channels were blocked with tetrodotoxin (TTX, 1 μM; Alomone labs, Jerusalem, Israel). Conductance of MN membrane was increased pharmacologically by applying the muscimol ($GABA_A$ receptor agonist, 2 μM (SIGMA)).

2.3. Data quantification and representation

Input conductance was estimated from responses of MNs to a 0.2-0.6 nA subthreshold positive and negative square current pulses, inducing voltage deflection < 10 mV from resting membrane potential. The threshold current was the minimal current required for action potential generation when stimulated with square pulse and the current value at the moment when first action potential was generated during ramp stimulus. Voltage threshold was measured from the same recordings as threshold current,

when membrane potential starts to depolarize faster than 20 V/s (Munoz and Fuentealba, 2012; Yu et al., 2008). Instantaneous firing frequency was calculated as the reciprocal value of interspike intervals. Frequency-current plots (f-I) were obtained by using the frequency of the first two spikes, the first four spikes and average during 0.5 s starting 1.5 s after stimulus onset (steady state) as a function of the injected current. The slope of the f-I relation was fitted to the steepest (secondary range) linear part of f-I by the least squares method (Microcal Origin software). The MNs were tested with triangular current ramps with rising speed from 0.5 to 20 nA/s and square current pulses of increasing amplitude. The minimal amplitude of square current pulse was adjusted for each MN to induce sustained action potential generation during 2 s stimulus duration (1 s for I_{NaP}). The data were analyzed statistically by using two population (paired) t-test (Microcal Origin software). Significance was accepted when $p<0.05$. Data are presented as a means value ± standard error (SE).

2.4. Dynamic clamp

Dynamic clamp method (Sharp et al., 1993) was used for I_{NaP} compensation. Time-varying stimulus current was calculated in real time at 5 kHz from membrane potential recorded using the programming package LabView (National Instruments). The LabView program interfaced a module written in C++ programming language for computing stimulus according to the calculated conductance value and the measured instantaneous membrane potential. The value of injected current was inversed to I_{NaP} current estimated from its stationary characteristics determined in the subthreshold region. Since we were interested only in influence of I_{NaP} before full-blown spike generation, we assumed that I_{NaP} saturates at the threshold.

3. RESULTS

3.1. Influence of motoneurons stimulation type and spike frequency adaptation on the gain of spinal motoneurons

3.1.1. Gain of spinal motoneurons measured from responses to square current pulses

We measured the gain of spinal MNs from response to square current pulses (Fig. 3.1.A) of increasing amplitude. The frequency of action potentials decreased due to adaptation during square current pulses in all MNs tested (Fig. 3.1.A, upper trace). The transient firing frequency (101.1±13.8 Hz) (first two APs) was significantly higher by 24.7±2.9 % (n=9) than early firing frequency (77.2±12.3 Hz) (first four APs) and significantly higher by 71.2±3.2 % than the steady state frequency (29.5±5.3 Hz) during a depolarizing square current pulse with an amplitude of 2.9 nA. To estimate the influence of adaptation on the gain of MNs we measured the steepness of f-I relation at different stages of adaptation. An example is shown in figure 3.1.C. The steepness of transient f-I (transient gain) was 56.3±6.9 Hz/nA, the steepness of early f-I (early gain) was 43.0±2.8 Hz/nA and the steepness in steady state (steady gain) was 16.5±1.1 Hz/nA (n=9) (Fig. 3.1.D). The transient gain was 19.2±5.9 % higher than early gain, and 68.1±3.7 % higher than the gain in steady state. The steepness of early gain was 60.9±2.9 % higher than in steady state.

3.1.2. Gain of spinal motoneurons measured from responses to current ramps

Current ramps (0.4-6 nA/s) is also used for estimation of the gain of MNs (Bennett et al., 2001b; Button et al., 2006; Harvey et al., 2006b; Hounsgaard et al., 1984; Hounsgaard et al., 1988a; Lee and Heckman, 1998; Lee and Heckman, 2000). We compared the gain measured by square current pulses and slow (1nA/s) ramp from the same MNs (Fig. 3.1.C.D). The steepness of f-I relation measured from responses to a slow current ramp (1 nA/s) was 15.9±1.5 Hz/nA (n=9) and did not differ significantly ($p<0.05$) from steepness of the f-I relation in steady state (16.5±1.1 Hz/nA) determined with square current pulses (Fig. 3.1.D). The transient and the early gain were 68.1±4.9 %

and 62.2±3.8 % respectively higher than the gain measured from slow (1 nA/s) current ramp (n=9) (Fig. 1.D). This shows that slow current ramps provide the steady state gain but not the transient or the early gain in MNs.

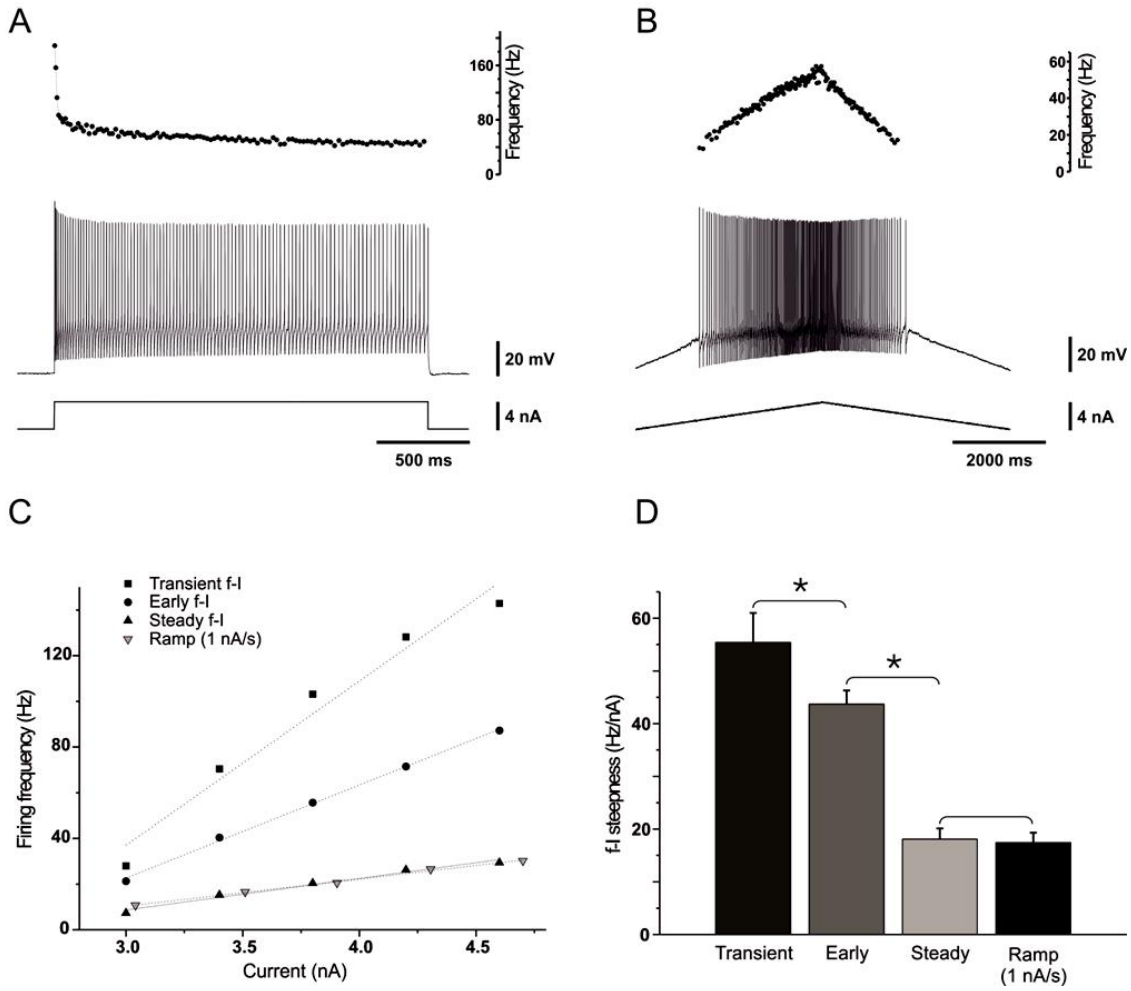

Figure 3.1. Gain of spinal motoneurons estimated from responses to square and ramp current pulses. (**A**) response of motoneuron to square current pulse (bottom) and associated instantaneous firing frequency (top); (**B**) response of motoneurons to a current ramp (bottom) and associated instantaneous firing frequency (top); (**C**) an example of frequency-current (f-I) relation of motoneuron measured from response to square current pulses (at different time intervals) and slow current ramp; recordings in (A) and (B) are from the same motoneuron, example in (C) is from another motoneuron; (**D**) the steepness of f-I relation of motoneurons measured from response to square current pulses (at different time intervals) and slow (1 nA/s) current ramp (n=9); significant differences ($p<0.05$) are marked with *.

3.1.3. Influence of ramp speed on the gain estimation

The gain of spinal MNs decreases with adaptation (Powers et al., 1999; Sawczuk et al., 1995). Therefore the speed of ramp could be an important factor influencing the estimated gain. We measured the gain of the same spinal MNs using current ramps of five different speeds: 0.5; 1; 5; 10; 20 nA/s (n=9). The lowest gain 13.4±1.2 Hz/nA was obtained from slow 0.5 nA/s current ramp while the highest one was 19.8±1.2 Hz/nA from 10 nA/s current ramp (Fig. 3.2.A). The steady gain obtained from square current pulses was 23.7±8.6 % lower (n=9) than the gain obtained from 10 nA/s ramp, while the transient and the early gain were 60.9±4.8 % and 52.9±3.3 % respectively higher than the highest gain obtained from current ramps.

The gain significantly increased with ramp speed using ramps from 0.5 to 5 nA/s, but there were no significant differences of gain using ramps from 5 to 20 nA/s (Fig. 3.2.A). The differences of gain obtained from stimulation by various speed current ramps could be explained by different level of adaptation. The number of action potentials generated during the same amplitude at different ramp speeds can differ dramatically (Fig. 3.2.B.C). We quantified the number of action potentials generated during ramps of different speeds (n=9) (Fig. 3.2.B). We found that MNs produce more spikes during slow ramps than during fast ramps (Fig. 3.2.B.C).

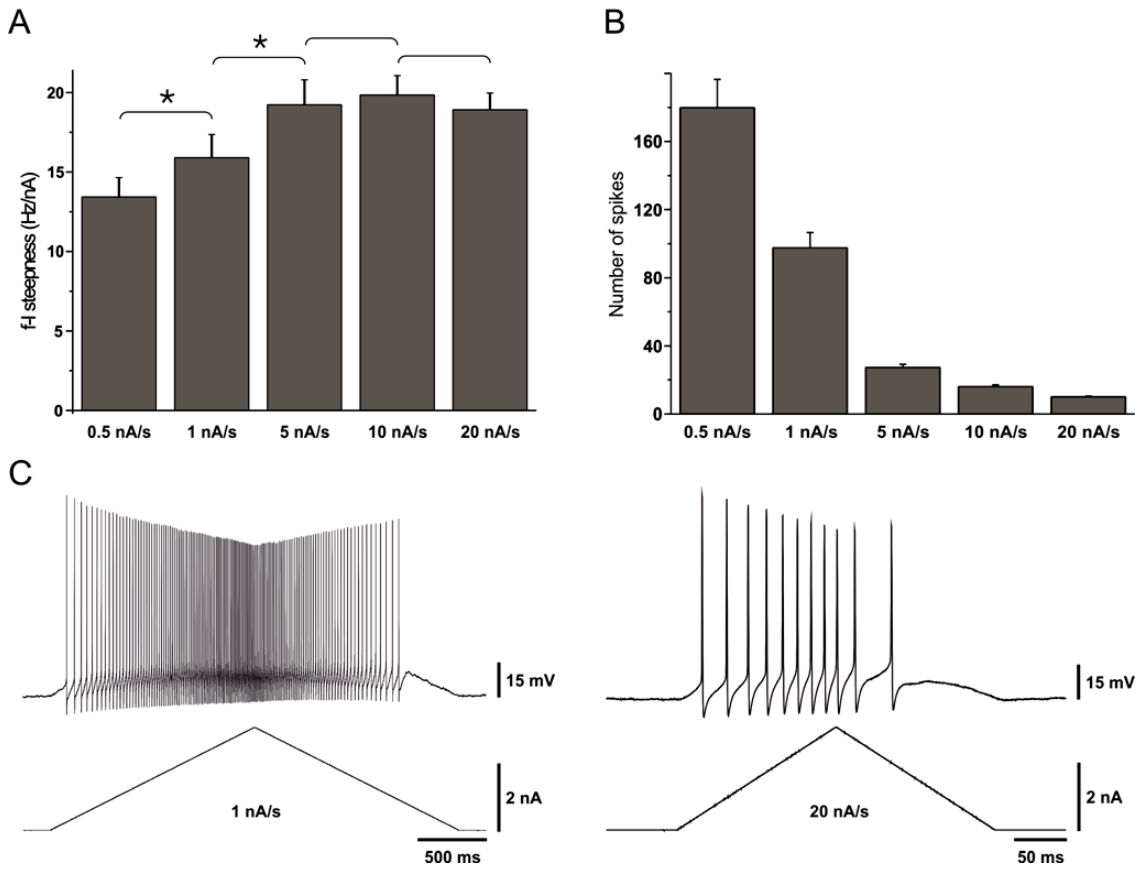

Figure 3.2. Influence of ramp speed on motoneuron gain estimated. (**A**) the steepness of frequency-current (f-I) relation of motoneurons (gain) measured from response to current ramps with different speed (n=9); significant differences ($p<0.05$) are marked with *; (**B**) the number of spikes of motoneurons measured from response to current ramps of different speed (n=9); (**C**) response of motoneuron to slow (1 nA/s) and fast (20 nA/s) current ramp – note difference in number of action potentials during ramps (recordings in (C) are from the same motoneuron).

3.2. Influence of membrane conductance changes on the gain of motoneurons

Pharmacologically, using muscimol (2 μM), MNs input conduction was increased by 38.6±4.9 % from 113.7±11.5 nS (control) to 154.3±12 nS (+muscimol) (n=10) (Fig. 3.4.A). Increased membrane conductance decreased the level of MN excitability (Fig. 3.3). Increased membrane input conductance increased the rheobase by 56.4±9.1 % from 1.2±0.2 nA (control) to 1.9±0.2 nA (+muscimol) (n=10) (Fig. 3.4.B). The transient gain of MNs using square current pulses in control was 66.6±7.6 Hz/nA (n=10), the early gain was 47.4±6.6 Hz/nA and the steady gain - 23.8±4.9 Hz/nA (Fig. 3.4.D).

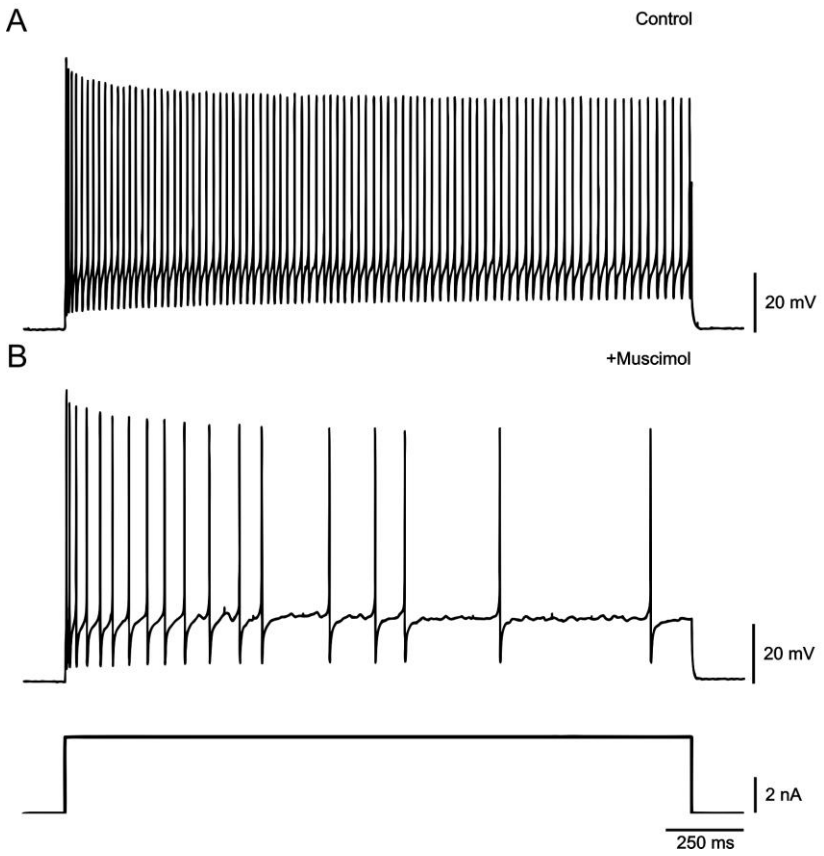

Figure 3.3. Influence of membrane input conductance on motoneuron excitability. (**A**) continuous firing of motoneuron in control during 2 sec current step stimulation; (**B**) reduced continuous firing of motoneuron after incensement of membrane conductance during 2 sec current step stimulation (recordings in (A) and (B) are from the same motoneuron; current step values are the same in control and using pharmacology).

The transient gain of MNs after muscimol was 71.5±6.7 Hz/nA (n=10), the early gain was 47.6±4.0 Hz/nA and the steady gain - 17.3±2.5 Hz/nA. Increased MN membrane conductance did not affect the steepness of f-I relation (Fig. 3.4.C.D).

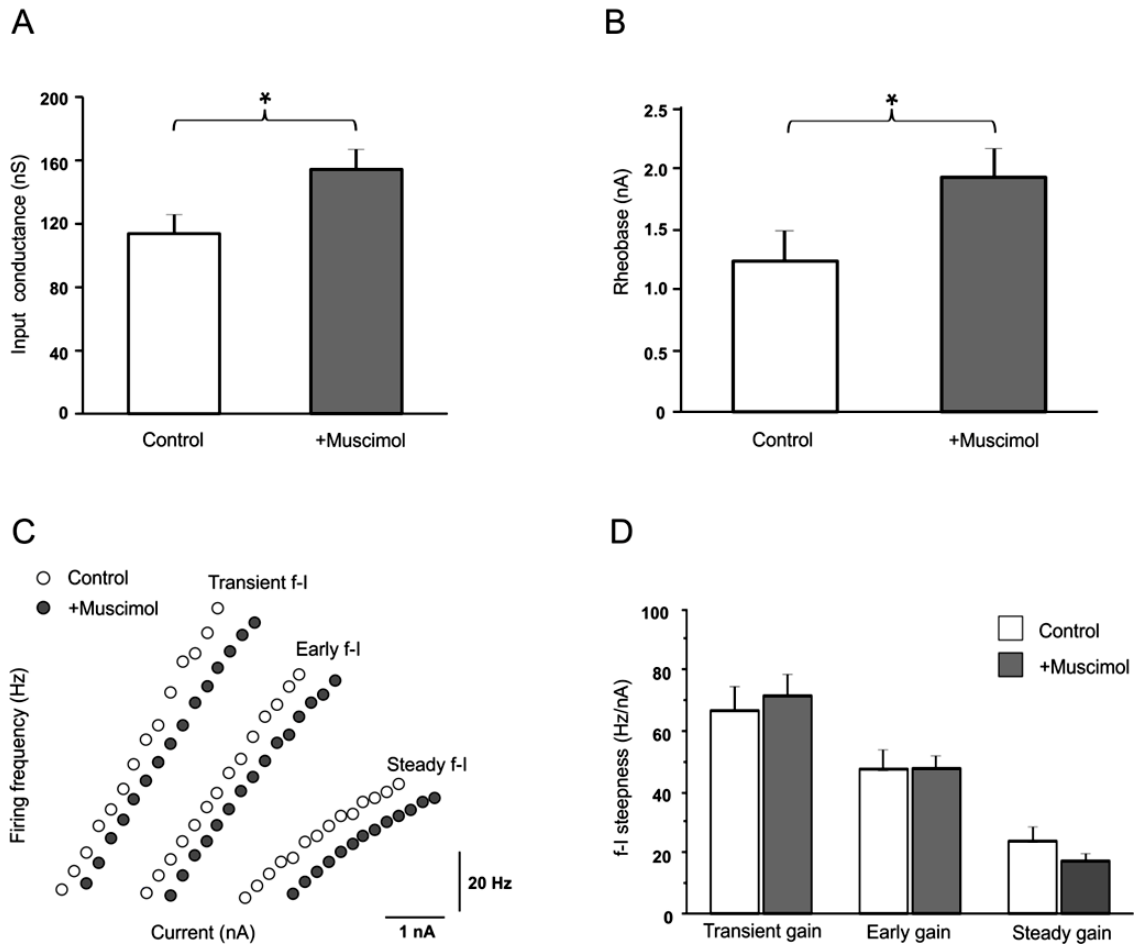

Figure 3.4. Influence of membrane input conductance incensement on the gain of motoneurons. (**A**) muscimol increased membrane input conductance by 38.6±4.9 % (n=10); (**B**) increased membrane input conductance increased the rheobase by 56.4±9.1 % (n=10); (**C**) an example of gain curves from one motoneuron (transient, early and steady) under control (white circles) and using pharmacology (dark circles); (**D**) increased membrane conductance (dark columns) did not reduce the gain of motoneurons (n=10); significant differences ($p<0.05$) are marked with *.

3.3. Influence of persistent sodium current on the gain of spinal motoneurons

3.3.1. TTX decreases amplitude of the response to depolarizing current pulses

Bath applied TTX blocked action potentials and reduced voltage responses to subthreshold current pulses (Fig. 3.5) in spinal motoneurons from adult turtles. The response to hyperpolarizing current pulses was unaffected. This indicates that TTX eliminates a voltage dependent sodium conductance which activates at potentials more positive than resting membrane potential and below the threshold for action potential

generation. This is compatible with persistent sodium current, I_{NaP}, widely described in motoneurons and other types of neurons (Crill, 1996).

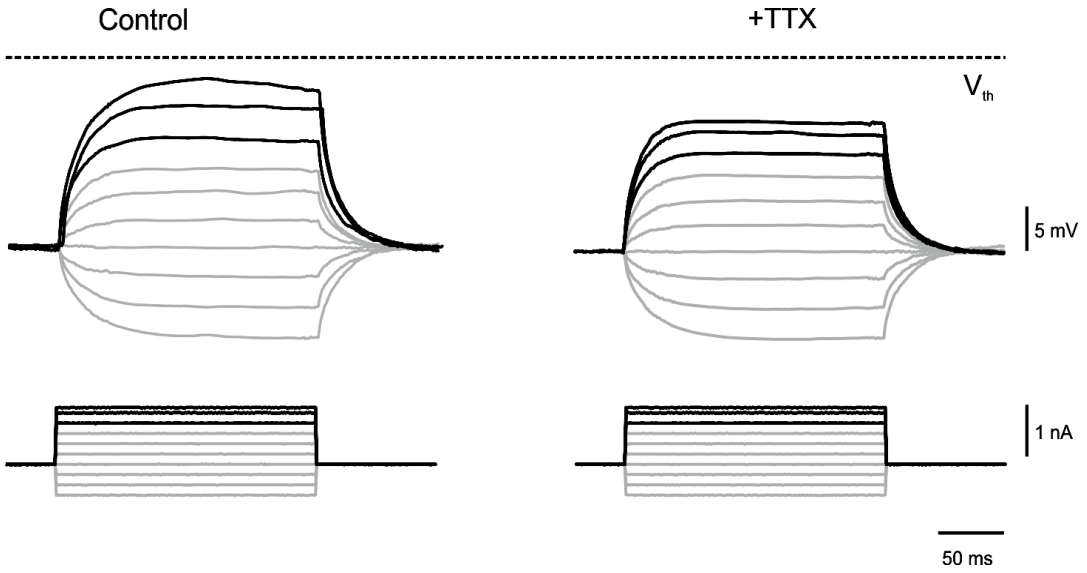

Figure 3.5. TTX application reduced subthreshold excitability due to block of I_{NaP}. Responses to depolarizing current pulses under control conditions were larger (left) comparing to responses after application of TTX (right) in the same motoneuron; dotted line marks the threshold for action potential (V_{th}).

3.3.2. Stationary current-voltage relation characteristics of persistent sodium current

The responses of MNs to current pulses in control and after TTX (Fig. 3.5) were used to evaluate I_{NaP} quantitatively. In all recordings (n=11) the membrane potential equilibrated and no significant change was observed 50 milliseconds after the onset of a 200 ms length current step. This is in agreement with the fact that I_{NaP} inactivates with a characteristic time constant of a few seconds (Kay et al., 1998; Magistretti and Alonso, 1999). We therefore assumed that I_{NaP} activated and did not inactivate during the test pulses we used in our experimental protocol.

The stationary current-voltage relation in MNs was obtained from the voltage deviations in response to the injected current pulses. The current-voltage relation in control (black circles in Fig. 3.6) and after application of TTX (open circles in Fig. 3.6) overlap at hyperpolarized levels and bifurcates at subthreshold levels of depolarization (Fig. 3.6). This confirms that I_{NaP} is generated by a TTX sensitive and voltage dependent

conductance in turtle spinal MNs. Therefore, the difference in current-voltage relation in control and after TTX is attributed to I_{NaP} (grey circles in Fig. 3.6). In all tested MNs (n=11) the stationary current-voltage relation of I_{NaP} in the subthreshold region was approximated quite well by the threshold linear model (Fig. 3.6) expressed quantitatively as follows:

$$\left.\begin{array}{l} I_{NaP}(V) = k(V_{akt} - V_{th}); V \geq V_{th} \\ I_{NaP}(V) = k(V_{akt} - V); V \geq V_{akt} \geq V_{th} \\ I_{NaP}(V) = 0; V < V_{akt} \end{array}\right\}$$

Equation 1.

Here, V_{akt} corresponds to the membrane potential where the abscissa is crossed by the line in the fitted data and, thus, can be interpreted as the effective voltage at which I_{NaP} activates, V_{th} - threshold for generation of action potential. Our experiments indicated V_{akt}=-60.97±0.93 mV. In synaptic integration, the important factors are the conductance activating close to the threshold for generation of action potential. Therefore, we estimated V_{akt} in respect to the threshold for spike generation. We found that V_{akt} is 10.88±0.78 mV negative to the threshold of an action potential. The parameter *k* defines steepness of the current-voltage relation and can be interpreted as the effective conductance of I_{NaP} in the subthreshold region. As it follows from our measurements, *k*=0.054±0.009 μS. Clearly, this quantity depends on the size of a neuron, distribution and density of sodium channels and other modulating factors. The ratio of effective I_{NaP} conductance k with input conductance g_{in} of the MNs was 0.53±0.04. This number indicates that I_{NaP} significantly contributes to the shaping of membrane potentials in spinal MNs at subthreshold levels.

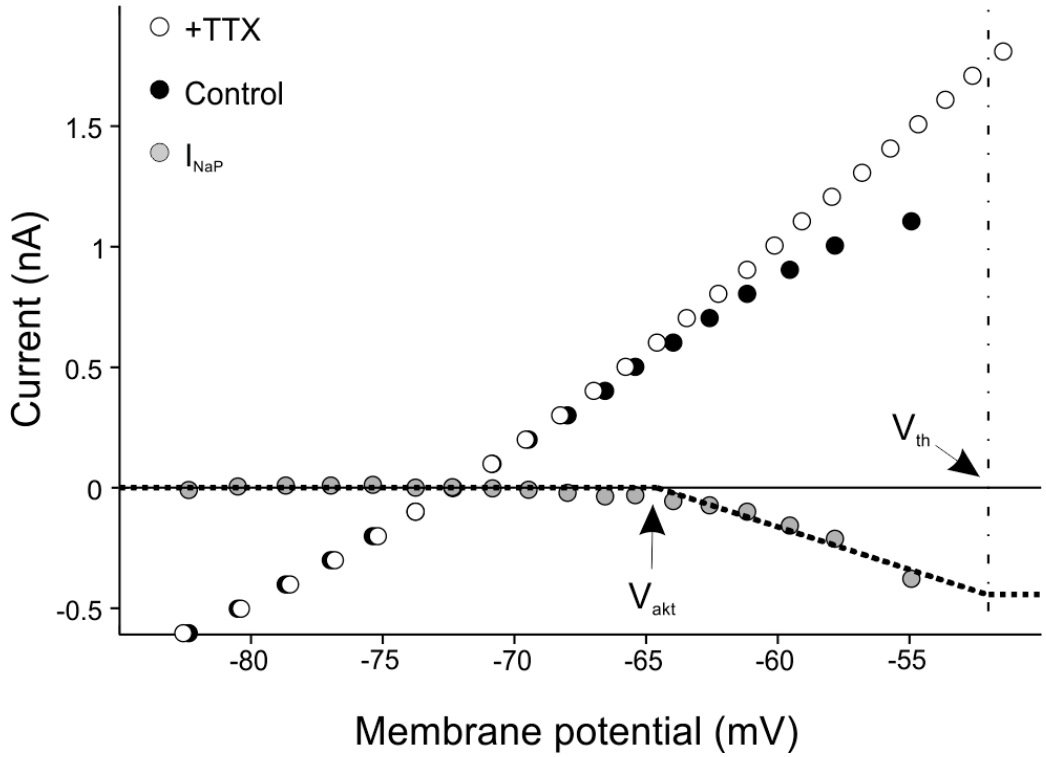

Figure 3.6. TTX application reduced subthreshold excitability due to block of I_{NaP}. Stationary current-voltage relation (I-V) (grey circles) for I_{NaP} was obtained from difference between input I-V values before (black circles) and after application of TTX; the dotted line represents the linear approximation of I_{NaP} I-V in the subthreshold region. Here, V_{akt} corresponds to the membrane potential where the abscissa is crossed by the line in the fitted data and, thus, can be interpreted as the effective voltage at which I_{NaP} activates, V_{th} - threshold for generation of action potential.

3.3.3. Function of the persistent sodium current

Contribution of I_{NaP} to the spiking properties of MNs was investigated by comparing responses to depolarizing current pulses in control and when I_{NaP} was eliminated or reduced by means of dynamic clamp. The injected current in the dynamic clamp was calculated by using equation 1 where the parameter *k* was set to 0.05 μS and V_{akt} was adjusted for each MN to be 10 mV more negative than the threshold for action potential.

As expected, compensation of the persistent sodium current reduced excitability (Fig. 3.7.A.B) in all MNs tested (n=11). A current pulse capable of evoking low frequency firing in control was always subthreshold for spike generation when I_{NaP} was eliminated (Fig. 3.7.A). Current pulses of higher intensity induced more action potentials in control than with I_{NaP} eliminated (Fig. 3.7.B).

The elimination of I_{NaP} also affected the f-I relation. The reduced rheobase was marked by the shift to the right of the f-I relation (Fig. 3.8). Interestingly, the steepness of the f-I relation was altered as well. The compensation of I_{NaP} by dynamic clamp significantly increased the steepness of f-I relation by 64±19 % (n=11) for the first two spikes (Fig. 3.8 left) and by 38±10 % (n=11) for the first four spikes (Fig. 3.8 right). This result implies that I_{NaP} decreases the slope of transient f-I relation, i.e. reduces the transient gain in MNs.

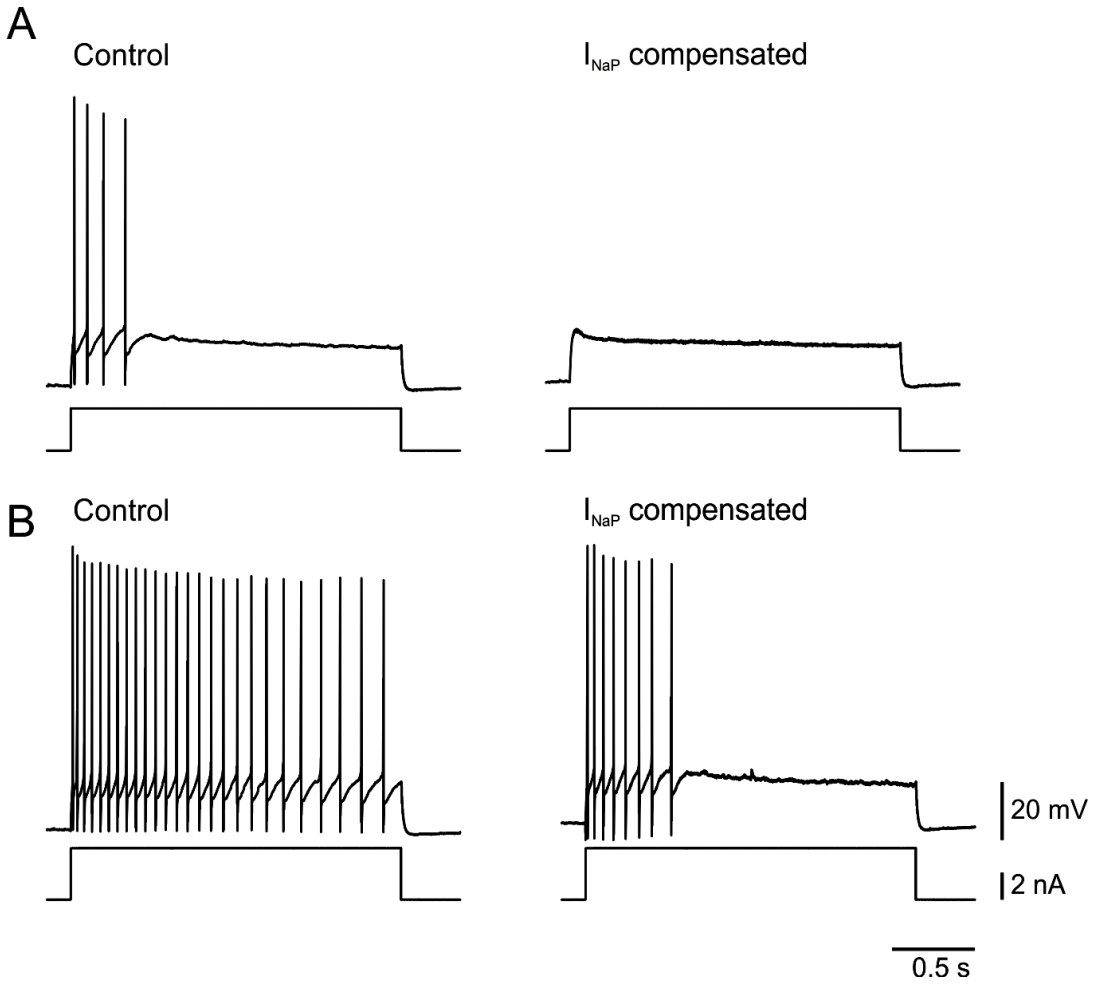

Figure 3.7. Compensation of persistent sodium current reduces excitability of motoneurons. (**A**) response with firing was converted into subthreshold one by dynamic clamp compensation of I_{NaP}; (**B**) the compensation of persistent sodium current also reduced the number of spikes in a response with continuous firing.

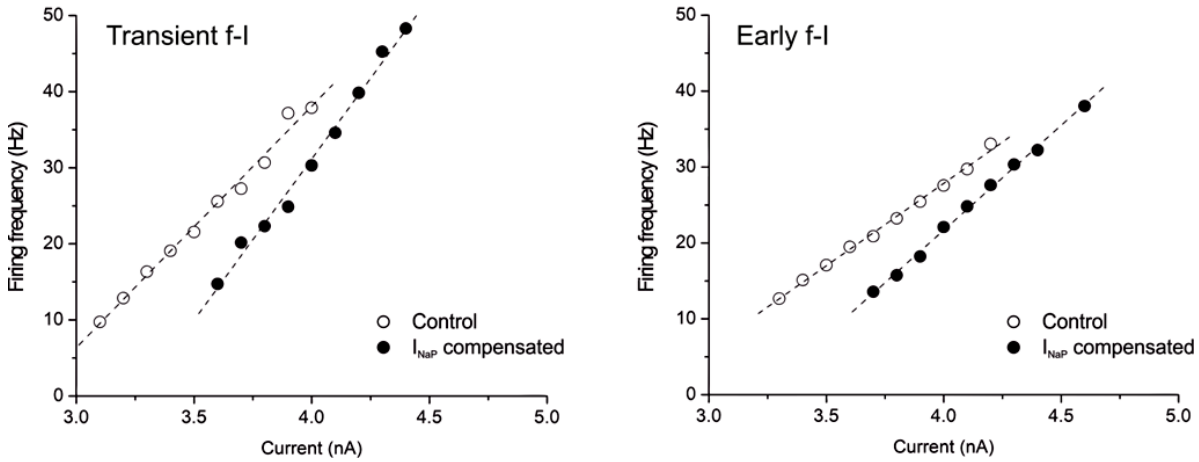

Figure 3.8. Compensation of persistent sodium current reduces the gain of motoneurons. The steepness of transient and early f-I relations was increased by compensation of I_{NaP}.

4. DISCUSSION

4.1. Influence of motoneurons stimulation type on estimation of the gain of motoneurons

The results confirmed that the gain of spinal MNs in the turtle measured from responses to square current pulses decrease significantly with adaptation (Hounsgaard et al., 1988b). We found that the transient gain was more than three times higher than the steady gain. The fact that transient gain is higher than early gain indicates that the decrease in gain begins after the first action potentials generated in response to a square current pulse (Hounsgaard et al., 1988b). Therefore the values of gain estimated strongly depend on protocol used. We show that the gain of spinal MNs, measured with slow current ramps (Bennett et al., 2001a; Button et al., 2006; Harvey et al., 2006b; Hultborn et al., 2004; Lee and Heckman, 1998; Lee and Heckman, 2000) is the same as the steady gain measured from responses to square current pulses. Therefore the gain obtained with ramps is less complete than the gain obtained with square current pulses.

It was reported, that the gain in MNs can be approximated by two linear ranges: primary range below ~20 Hz and a steeper secondary one (Hounsgaard et al., 1988b; Kernell, 1965). However, the weak evidence of primary firing range for the first interspike interval was expressed only in 3 out of 9 MNs tested. Therefore analysis was started at current evoking firing during 2 s stimulus duration. At this stimulus interval the f-I was liner, tending to saturate at significantly higher current values. Based on current stimulus used and response frequency range (20-120 Hz) we conclude that the transient gain in this report corresponds to secondary range of gain reported by others (Hounsgaard et al., 1988b; Kernell, 1965).

4.2. Influence of ramp stimulus speed on the estimation of the gain of motoneurons

Ramp speed could be an important factor influencing the value of the estimated gain. Others have used different ramp speed (0.4-6.0 nA/s) for gain estimation (Bennett et al., 2001a; Button et al., 2006; Harvey et al., 2006b; Hounsgaard et al., 1984; Hounsgaard et al., 1988a; Lee and Heckman, 1998). MNs from different preparations may vary in size, input resistance and threshold. Therefore, the current ramp of the same speed would have different effect on different MNs.

We increased the ramp stimulus speed from 0.5 to 20 nA/s to see if the gain approached the transient gain. The highest gain obtained from ramps was 23.7±8.6 % (n=9) higher than the steady gain obtained from square current pulses. However, the transient gain was still significantly 60.9±4.8 % higher and the early gain was 52.9±3.3 % (n=9) higher than the highest gain obtained from current ramps. The gain of MNs significantly increased when ramp speed increased from 0.5 to 5 nA/s (Fig. 3.2.A). The AHP (Vervaeke et al., 2006; Vogalis et al., 2003) and spike frequency adaptation (Powers et al., 1999; Sawczuk et al., 1995; Sawczuk et al., 1997) are thought to be a major mechanism controlling the f-I relation. During triangle current ramp stimulus the frequency of APs is influenced both by instantaneous current and the level of spike frequency adaptation. Ca^{2+} influx during action potentials influences adaptation and the f-I relation by controlling the slow AHP (Hounsgaard and Mintz, 1988; Powers et al., 1999; Rekling et al., 2000). The results showed that MNs produce more spikes with slower current ramps than with faster ramps (Fig. 3.2.B.C). This may indicate that fewer action potentials during faster ramps produce less adaptation and therefore results in higher gain of MNs. Further increase in ramp speed (from 5 to 20 nA/s) did not significantly affect the gain (Fig. 3.2.A). The number of action potentials decreased with increasing ramp speed (Fig. 3.2.B.C) and the instantaneous current may change significantly between adjacent action potentials. This is probably the reason why the gain obtained with high ramp speed does not approach the levels of the transient and early gain obtained with square current pulses.

4.3. Influence of membrane conductance on the gain of motoneurons

We found that using muscimol (2 μM) MNs input conduction was increased by 38.6±4.9 % (n=10). It is known that during functional spinal network activity peak membrane conductance can increase up to 800 % (Berg et al., 2008). Increased membrane input conductance increased rheobase by 56.4±9.1 % (n=10). Increased MN membrane conductance did not change the gain of MNs. These results confirmed the results from ones reported by other investigators. Previously, using mathematical simulations of cat cerebral cortex neurons it was shown that shunting inhibition does not change the gain of neurons, but only make a shift in rheobase when membrane conductance increases (Holt and Koch, 1997). The dynamic clamp study in anaesthetized

cat lumbar MNs showed that shunting inhibition reduced very substantially the firing frequency in the primary range without changing the slope of f-I curve (Brizzi et al., 2004). Investigating the gain of CA1 (hippocampus zone) pyramidal cells of rat in vitro under inhibitory conductance applied at the soma was found that increasing membrane conductance reduces the steady gain of neuron but has a weaker effect on the transient gain calculated (Fernandez and White, 2010). Otherwise, we do not know how the further increase in membrane conductance would affect the gain of MNs.

4.4. Influence of persistent sodium current on the excitability and gain of motoneurons

We found that the transient and early gain of MNs, as inferred from the steepness of MNs f-I relation, increased after elimination of the persistent sodium current (I_{NaP}) by means of dynamic clamp, while the steepness of the steady f-I relation was unaffected. This indicates that I_{NaP} not only increases excitability, but also decreases the transient and early gain of MNs. During steady firing after adaptation I_{NaP} increases excitability without significant impact on firing gain. This is in agreement with results of dynamic clamp studies in cortical pyramidal neurons (Vervaeke et al., 2006). Most likely the gain regulation is due to interaction of I_{NaP} with conductances shaping the membrane potential between spikes (Chance et al., 2002; Hounsgaard and Mintz, 1988). It was shown that I_{NaP} enhance AHPs by deactivation and it was proposed that this may exert negative feedback regulation of the discharge frequency (Vervaeke et al., 2006).

Previous studies, where I_{NaP} was blocked by persistent depolarization (Lee and Heckman, 2001) or by riluzole (Kuo et al., 2006), concluded that I_{NaP} increases the firing frequency-current gain. As discussed earlier, the steepness of f-I relation decreases during adaptation (Hounsgaard et al., 1988b; Sawczuk et al., 1995). The contrasting conclusion possibly is either due to different methods of elimination of I_{NaP} or due to different stimulation protocols for f-I estimation.

The proposed description of I_{NaP} as a linear function with a threshold and a saturation level works well in the subthreshold region only. In general, our model should be treated as a local effective linear approximation of the full range continuous Boltzmann type conductance based I-V relationship. We used dynamic clamp to compensate the effect of I_{NaP}. Our estimate of I_{NaP} and our use of dynamic clamp both

ignore cable properties and possible inhomogeneous distribution of channels in MNs. However, these simplifications tend to underestimate the magnitude and effect of I_{NaP}. The current was calculated by using stationary current-voltage relation for I_{NaP}.

I_{NaP} could perform an intricate role in integration of synaptic signals in MNs. On the one hand, it increases excitability by reducing the rheobase and amplifying synaptic inputs, while on the other hand, I_{NaP} expands the dynamic range of coding by reducing the relative responsiveness of motoneurons to transient versus steady inputs.

5. CONCLUSIONS

1. The gain of motoneurons estimated using triangular current ramps is the same as steady gain of motoneurons obtained using square current steps.

2. Square, but not triangular current steps are more informative for estimation of transient and early gain of motoneurons.

3. The increased membrane conductance did not change the gain of motoneurons.

4. Persistent inward Na^+ current increases excitability of motoneurons, reduces the transient and early gain, but does not affect the steady gain.

6. SANTRAUKA (Summary in Lithuanian)

Nugaros smegenų neuroninis tinklas valdo gyvūnų galūnių raumenis. Motoneuronai (MN) – tai nugaros smegenų neuronai, kurie tiesiogiai aktyvuoja raumenis. Kaip ir kituose neuronuose, MN įėjimo transformacija į išėjimą charakterizuojama perdavimo funkcija, kuri dažniausiai aprašoma tiese. Didelis perdavimo funkcijos statumas leidžia išvystyti didelę raumens susitraukimo jėgą, o mažas – įgalina tikslų raumenų valdymą.

Eksperimentiškai MN perdavimo funkcijos vertė nustatoma tiriant MN veikimo potencialų dažnio priklausomybę nuo įleidžiamos į ląstelę srovės dydžio. MN elektrinė stimuliacija vykdoma stačiakampiais arba trikampiais srovės stimulais. Nėra žinoma ar skirtingais metodais vertintos perdavimo funkcijos yra vienodos.

Didelė judesių įvairovė sąlygoja, kad specifinės MN fiziologinės savybės ir įvairūs fiziologiniai procesai gali nevienodai įtakoti skirtingus perdavimo funkcijų tipus. Veikiant neuroniniam tinklui MN aktyvuojama labai daug sinapsinių įėjimų, dėl ko padidėja MN membranos laidumas. Nėra žinoma, ar šis veiksnys įtakoja MN perdavimo funkcijos vertę. MN aktyvumui svarbios yra ir nuolatinės įtekančios joninės srovės. Viena tokių srovių yra nuolatinė įtekanti Na^+ srovė. Problema, kad šios srovės poveikis skirtingų gyvūnų MN nėra vienareikšmis.

Neatsakyti tyrėjų klausimai reikalauja detalesnių perdavimo funkcijos tyrimų, todėl atliktų mokslinių tyrimų tikslas ir buvo įvertinti nugaros smegenų MN perdavimo funkcijos ypatybes ir ištirti jos galimus modifikavimo mechanizmus. Buvo tirta MN perdavimo funkcijos verčių priklausomybė nuo neuronų stimuliacijos tipo. Rezultatai parodė, kad MN perdavimo funkcijos vertė stimuliuojant MN trikampiais srovės stimulais sutampa su stacionaria perdavimo funkcija gauta stimuliuojant MN stačiakampiais srovės stimulais. Tiriant trikampių srovės stimulų kilimo greičio galimą įtaką perdavimo funkcijos vertės nustatymui pastebėta, kad stačiakampiais bet ne trikampiais srovės stimulais galima įvertinti pradinę ir ankstyvąją perdavimo funkcijų vertes. Farmakologiškai padidinus MN membranos laidumą tirtas padidėjusio MN membranos laidumo poveikis perdavimo funkcijai. Nustatyta, kad membranos laidumo padidėjimas neįtakoja MN perdavimo funkcijos statumo. Eksperimentiškai parodytas įtekančios nuolatinės Na^+ srovės veikimas vėžlio nugaros smegenų MN, kuriuose ši

srovė didina MN sužadinamumo lygį bei mažina pradinę ir ankstyvą MN perdavimo funkcijos vertę, tačiau neįtakoja stacionarios.

7. PUBLICATIONS

Articles (included in dissertation):

1. **Rokas Buisas**, Robertas Guzulaitis, Osvaldas Ruksenas & Aidas Alaburda. GAIN OF SPINAL MOTONEURONS MEASURED FROM SQUARE AND RAMP CURRENT PULSES. Brain Research. 2012. Vol. 1450. P. 33 – 39.

2. Mantas Gabrielaitis, **Rokas Buisas**, Robertas Guzulaitis, Gytis Svirskis & Aidas Alaburda. PERSISTENT SODIUM CURRENT DECREASES TRANSIENT GAIN IN TURTLE MOTONEURONS. Brain Research. 2011. Vol. 1373. P. 11 – 16.

Articles (not included in dissertation):

1. Aidas Alaburda, **Rokas Buisas**, Robertas Guzulaitis, Osvaldas Kaminskas, Osvaldas Ruksenas. ADAPTATION AND RECOVERY FROM ADAPTATION OF LOCUST WING STRETCH RECEPTOR. Biologija. 2010. Vol. 56. No. 1–4. P. 24–28.

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1. **Rokas Buisas**, Robertas Guzulaitis, Aidas Alaburda. GAIN OF SPINAL MOTONEURONS MESURED FROM RAMPS OF DIFFERENT SPEED. Biomedical Engineering 2011 (Kaunas, Lithuania, 2011). P. 183-186.

2. **Rokas Buisas**, Robertas Guzulaitis, Aidas Alaburda. GAIN OF SPINAL MOTONEURONS MEASURED FROM CURRENT PULSES AND RAMPS. Biomedical Engineering 2010. (Kaunas, Lithuania, 2010). P. 143-145.

3. **Rokas Buisas**, Aidas Alaburda. SPIKE FREQUENCY ADAPTATION INFLUENCES THE GAIN IN SPINAL MOTONEURONS (in Lithuanian). Virtual instruments in biomedicine - 2011 (Klaipeda, Lithuania, 2011). P. 136-139.

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2. **Rokas Buisas**, Aidas Alaburda. MICROELECTRODES: MANUFACTURE AND APPLICATION IN ELECTROPHYSIOLOGY (in Lithuanian). Virtual instruments in biomedicine – 2009 (Klaipeda, Lithuania, 2009). P. 13-19.

3. **Rokas Buisas**, Robertas Guzulaitis, Aidas Alaburda. RECOVERY OF LOCUST WING STRETCH RECEPTOR FROM ADAPTATION (in Lithuanian). 1st Conference of Lithuanian Society of Neurosciences (Vilnius, Lithuania, 2009). P. 25.

Poster presentations:

1. **Rokas Buisas**, Robertas Guzulaitis, Ramunas Grigonis and Aidas Alaburda. THE INFLUENCE OF MEMBRANE CONDUCTANCE ON THE EXCITABILITY OF MOTONEURONS. 8th FENS Forum of Neuroscience (Barcelona, Spain, 2012).

2. Robertas Guzulaitis, Aidas Alaburda, Osvaldas Ruksenas, **Rokas Buisas** and J. Hounsgaard. MOTONEURONS ARE NOT INVOLVED IN TEMPORAL SUMMATION OF SENSORY INFORMATION. 8th IBRO World Congress of Neuroscience (Florence, Italy, 2011).

3. **Rokas Buisas**, Robertas Guzulaitis, Aidas Alaburda. ESTIMATION OF GAIN OF SPINAL MOTONEURONS. Towards research in motoneurons (Paris, France, 2010).

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10. CURRICULUM VITAE

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