Changes in the estimated time course of the motoneuron afterhyperpolarization induced by tendon vibration

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ABSTRACT

Group Ia afferents are activated vigorously with high frequency tendon vibration and provide excitatory input to the agonist muscle and inhibitory input to the antagonist muscle group via inhibitory interneurons. The purpose of this experiment was to determine whether the afterhyperpolarization (AHP) time course in humans is altered in response to tendon vibration. The AHP time course is estimated using the Interval Death Rate (IDR) analysis, a transform of the motor unit action potential train. Single motor units from tibialis anterior (TA) were recorded as subjects held low force dorsiflexor contractions for 600 s with and without vibration. The vibratory stimulus was superimposed upon the low force contraction, either to the tendon of the TA or the antagonist Achilles tendon. During TA tendon vibration, the time course of the AHP, as expressed by its time constant ($\tau$), decreased from 35.5 ms in the pre-vibration control condition to 31.3 ms during the vibration ($p = 0.003$) and returned to 36.3 ms after the vibration was removed ($p=0.002$). The AHP $\tau$ during vibration of the antagonist Achilles tendon (38.6 ms) was greater than the pre-vibration control condition (33.6 ms; $p = 0.001$). It is speculated that the reduction in AHP time constant with TA vibration may have resulted alone or in combination with a modulation of motoneuron gain, an alteration of persistent inward currents and/or the restructuring of synaptic noise. A decrease in firing probability, possibly reflecting Ia reciprocal inhibition, may have been responsible for the larger AHP time constant.
INTRODUCTION

Motoneuron output is a result of its intrinsic properties and the summation of the input it receives, both inhibitory and excitatory (Binder et al. 1993). Group Ia afferent input has been shown to increase motoneuron firing probability in animal models (Heckman and Binder 1988). These large diameter afferents are mainly sensitive to the velocity of stretch and are activated vigorously with high frequency tendon vibration (Eklund and Hagbarth 1966; Brown et al. 1967; Hagbarth and Vallbo 1968). High frequency vibration in the cat results in stable synaptic inward currents (Lee and Heckman 1998; Heckman and Binder 1988) that are distributed extensively throughout the motoneuron pool (Burke et al. 1979; Mendell and Henneman 1971). Group Ia afferents also project to inhibitory interneurons; this reciprocal Ia inhibitory reflex pathway acts to inhibit the motoneuron pool of the antagonist muscle group in both animals (Eccles et al. 1956) and humans (Mizuno et al. 1971; Katz et al. 1991). This disynaptic inhibitory pathway (Jankowska 1992) has been shown to project evenly to the heteronymous motoneuron pool in the cat (Heckman and Binder 1991) and has reduced motor unit firing probability in elbow flexors and extensors during antagonist activation (Katz et al. 1991).

The action of vibration on the motoneuron is complex and includes monosynaptic and oligosynaptic excitation (Jankowska et al. 1983). The response of the motoneuron has been shown to be locked to the frequency of the vibratory stimulus in relaxed human muscles (Burke et al. 1976a; Godaux 1975) but this phase lock decreases in the presence of a tonic vibration reflex (Burke et al. 1976b) and voluntary isometric contraction (Burke et al. 1976c). A number of human studies have shown the motor unit firing rate to be
altered with vibration, but the effects are dependent upon the protocol and the instructions
to the participants. For example, Griffin et al. (2001) showed that intermittent vibration
applied to the triceps brachii tendon during a fatiguing isometric contraction reversed the
previously decreasing motor unit discharge rate. Grande and Cafarelli (2001), however,
demonstrated that intermittent vibration led to brief decreases in motor unit firing rate
during a non-fatiguing contraction of the knee extensors. In the latter study, subjects
were instructed to keep the force level constant and 'disregard' the vibration, which likely
accounted for the decrease in firing rate. Lastly, Gorassini et al. (1998) showed that brief
vibration can lead to motor unit recruitment that is self-sustaining and increase the rate of
the original motor unit (c.f. Figure 1). Gorassini et al. (1998) also showed that the
original motor unit firing rate can remain stable if a second motor unit is recruited during
vibration (c.f. Figure 2). On balance, vibration tends to increase motoneuron excitation
and the firing rate of motor units in an unconstrained system.

Motoneuron excitability, as reflected by the gain of the motoneuron, is determined
by its frequency-current relationship. The post-spike afterhyperpolarization (AHP)
influences the slope of the motoneuronal frequency-current relationship, and modification
of the AHP should alter motoneuron excitability (Rekling et al. 2000). Several studies
have shown that the AHP is modifiable. Miles et al. (2007) found that endogenous
activation of m2 type muscarinic receptors increased the gain of the motoneuron by
altering the amplitude of the AHP. Similarly, 5-hydroxytryptamine (5-HT), an
endogenous neurotransmitter, induces a decrease in amplitude, half-decay time, and
duration of the AHP in the turtle (Hounsgaard and Kiehn 1989; Hornby et al. 2002) and
has been shown to decrease the amplitude of the AHP (White and Fung 1989) and its
duration in the cat (Berger et al. 1992). Using a chronic model, Beaumont and Gardiner
(2002) showed that 12 weeks of habitual spontaneous physical activity in rats increased
the amplitude of the AHP in slow motoneurons, while Cormery et al. (2005) showed a
decreased AHP amplitude following two weeks of hindlimb suspension in the rat. These
studies suggest that modulation of the motoneuron AHP may be associated with altered
motoneuron function.

Over the years, more attention has focused on anaesthetized animals whose basic
motoneuron properties are different from those properties measured when the animal is
engaged in a motor activity. There are a number of examples that highlight this
difference. For instance, spike-frequency adaptation, a phenomenon whereby the
discharge rate of the motoneuron decays, is a well-established motoneuron property in
response to a constant amplitude current injection (Granit et al. 1963; Kernell 1965;
Kernell and Monster, 1982; Spielmann et al. 1993). However, spike frequency
adaptation is absent during fictive locomotion in which a motor output is elicited through
mesencephalic locomotor region (MLR) stimulation (Brownstone et al. 1992). Also, an
alteration of the afterhyperpolarization period occurs during fictive movement compared
to that seen when the motoneuron is activated with current injected directly into the soma
(Kernell 1999; Binder et al. 1993). With current injection, there is a relatively large,
measurable AHP that is modifiable in the presence of neuromodulators (see below). In
contrast, the AHP during fictive locomotion in the cat (Brownstone et al. 1992), rat
(MacDonell et al. 2010), and during fictive scratch in the cat (Power et al. 2010) is
largely abated. In addition to the spike frequency adaptation and AHP examples, a
noticeably reduced input resistance occurs during the weight-bearing phase of fictive
scratch (Perreault 2002). The evidence, therefore, suggests that motoneuron properties
are different during motor output compared to properties measured during a quiescent
state where the drive to the motoneuron occurs via intracellular current injection. The
latter technique does not take into account the different synaptic inputs (spinal and
supraspinal) that modify motoneuron output and therefore the potential effects of specific
synaptic inputs on motoneuron AHP parameters have not been studied extensively in
animal preparations. The AHP may be altered differently depending upon the
convergence of inputs onto the motoneuron because different inputs may evoke different
currents that modify the intrinsic properties of the motoneuron (Stauffer et al. 2007).

The extent to which intrinsic motoneuron properties are modified in response to
sensory inputs is not known in humans. In humans, the window into motoneuron
function is the motor unit action potential. Transformation of the motor unit interspike
intervals into an estimate of the motoneuron AHP time course occurs with the use of
Matthews’ Interval Death Rate (IDR) analysis (Matthews 1996). The ability of the IDR
analysis to estimate the AHP time course has been tested with noisy current injection in
the anaesthetized cat, and was found to be a reasonable estimate of the AHP rate of decay
(Powers and Binder 2000). In humans, Gossen et al. (2003) found the temporal
properties of the motor unit twitch to be positively related to the estimated AHP time
course in the first dorsal interosseus muscle. This finding supports animal research in
which a "speed match" between the motoneuron and its muscle fibers exists in both cats
and rats (Bakels and Kernell 1993; Gardiner 1993; Gardiner and Kernell 1990; Zengel et
al. 1985; Dum and Kennedy 1980; Eccles et al. 1958). The agreement between the
results in animal and human studies, as well as its established reliability (MacDonell et al. 2007), suggest that the IDR analysis is a valid indicator of the AHP time course. The purpose of this investigation was to determine whether the AHP time course is modified by tendon vibration. It was hypothesized that the AHP time course, as expressed by the time constant calculated from the IDR transform, would be reduced upon agonist tendon vibration. In addition to this, an increase in the time constant was hypothesized during antagonist tendon vibration.

METHODS

Subjects

Fourteen subjects (7 males and 7 females; age 32 ± 8.6 yrs) with no known neuromuscular disorders participated in this study after providing informed written consent. This study conformed to the standards established by the Declaration of Helsinki and was approved by the Review Board for Health Sciences Research Involving Human Subjects at the University of Western Ontario.

Force and electromyographic (EMG) recording

Ankle dorsiflexion torque was measured with a transducer (Model: FR5-300-B000; Tovey Engineering, Inc., AZ, USA) mounted to an aluminum plate. Subjects were seated in a chair with their hips and knees at 90° and the right foot secured to the aluminum plate with two 2.5 cm wide non-compliant straps. The right heel was prevented from moving backward on the aluminum plate by a metal heel cup. Movement of the knee was prevented by a padded U-shaped aluminum brace positioned 3 cm proximal to the patella.
that exerted a constant downward pressure onto the leg. The transducer signal was sampled at 2500 Hz.

The skin overlying the tibialis anterior (TA) and soleus (SOL) muscles was cleansed prior to affixing bipolar surface electrodes (silver-silver chloride electrodes 10 mm in diameter; 2.5 cm center-to-center distance). The surface EMG signals were band-pass filtered between 5 Hz and 1000 Hz, and digitized at 2500 Hz. Tibialis anterior motor unit potentials were recorded with insulated Teflon-coated fine wire intramuscular electrodes with a cut tip as the recording surface. Examples of motor unit and surface EMG recordings with and without vibration are depicted in Figure 1. Motor unit recordings were band-passed filtered (10-10 000 Hz), and differentially amplified (CMMR > 90 dB at 60 Hz; input impedance 10 Mega Ohms; Colbourn Electronics, PA, USA) prior to digitization at 25000 Hz. Each electrode was comprised of three bonded stainless steel wires (50 μm diameter; California Fine Wire Company, CA, USA) passed through a 25 gauge hypodermic needle for intramuscular insertion. Two of the 3 wires formed a bipolar electrode. The third wire allowed the freedom to configure the bipolar electrode differently, should the first configuration yield an undesirable signal. A small hook at the terminal end of the fine wire electrode held the electrode in place after the needle was removed. All EMG and force signals were monitored online and stored for processing (16 bit, Power 1401, Cambridge Electronic Design, UK). Offline motor unit identification was performed using the Spike2 software package (Cambridge Electronic Design, UK) and care was taken to ensure the motor unit was identified correctly.

In separate experiments, tendon vibration was applied either to the tendons of the tibialis anterior or Achilles tendon with a vibrator (110 Hz; 2 mm displacement) suspended from
a custom support that allowed adjustment of the application point of the vibration according to the anatomical requirements of each participant. In an attempt to reduce the noise from the motor, the vibrator housing was wrapped in copper foil and grounded. The applicator head was extended by 20 cm to keep the motor further away from the muscle and was held in place with a non-compliant strap wrapped around the lower leg. Pilot testing demonstrated that the extension did not change the frequency or amplitude of the vibration. Despite this, noise created from the vibrator increased on all collected channels (Figure 1), so surface EMG (sEMG) and force were not compared across conditions. Autocorrelation histograms of the motor unit spike trains were constructed to examine whether the tendon vibration induced fluctuations in motor unit discharge; no evidence of systematic modulation of motor unit firing rate occurred at the frequency of the vibration or its subharmonics.

Protocol

Two dorsiflexion and plantarflexion maximal voluntary contractions (MVCs), 3 s in duration and separated by 30 s, were collected at the beginning and upon completion of the protocol. Once a single motor unit was identified, subjects performed control and experimental conditions; each of which consisted of holding a low force contraction and varying the discharge rate of the single motor unit. During the experimental condition, a vibratory stimulus was superimposed upon the low force contraction. In each condition, single motor unit discharge rates were varied in order to satisfy the requirements of the Interval Death Rate (IDR) analysis (Matthews 1996). The duration of the complete experiment was at least 1200 s. After the first 600 s (pre-vibration control), the vibrator was turned on and the participant repeated the 600 s protocol. Following completion of
the 600 s vibration protocol, the vibrator was turned off and participants repeated the
control condition. No rest was given between the control and experimental conditions.
Participants were required to vary the firing rate of a single motor unit between its
lowest firing rate and approximately 10 Hz, according to a predetermined protocol that
allowed achievement of the necessary range of firing rates along with smooth transitions
between firing rates (MacDonell et al. 2007). Motor unit action potentials were captured
with a dual window discriminator (BAK Electronics, Inc. MD, USA) to provide
participants with motor unit discharge rate feedback. Visual feedback of motor unit
firing rate was given to participants in the form of a solid line indicating the one-second
running average of motor unit firing rate on a computer monitor. Audio feedback of
motor unit discharge was also given through a speaker each time the motor unit fired.
To determine how well the subjects were able to follow the firing rate protocol
across conditions, the inter spike interval (ISI) distribution was evaluated with the
following parameters: mean ISI and standard deviation (SD), coefficient of variation
(CV) of ISI, skewness and kurtosis of the ISI distribution histogram. Skewness and
kurtosis describe the degree of symmetry and the broadness of the distributions,
respectively (Glass and Hopkins 1984). The normal distribution has a kurtosis of 3. A
sample distribution with a kurtosis > 3 is narrower than the normal distribution and a
kurtosis < 3 describes a broader distribution than normal

Motoneuron AHP properties

The IDR utilizes the ISIs of a train of motor unit action potentials. Each ISI
between 50 ms and 300 ms was sorted into one of 3 or more discharge frequency sub-
populations. A one-second running mean of the intervals 500 ms prior to and following
the ISI being sorted was calculated. The running mean was then used to classify each
instantaneous ISI into a distinct firing frequency subpopulation. Therefore, each ISI was
categorized as occurring at different mean level of central drive.

Histograms with 5 ms bin widths were created for each subpopulation that
represented the distribution of ISIs for a given mean firing rate. Almost all histograms
contained at least 1000 intervals and the total number of intervals used for analysis was
never less than 3000. After the death rate of the ISIs for each histogram was calculated, a
transform was applied (cf Figure 13, Matthews 1996).

The Matthews (1996) transform was obtained via computer modeling. The data
points resulting from the transform were based on how a motoneurone model responded
to structured time smoothed noise (Matthew, 1996). The single compartment threshold
crossing model of a motoneurone with a 4 ms membrane time constant, fixed membrane
potential and spike threshold, was overlaid with exponentially time-smoothed Gaussian
distributed noise. When the applied noise caused the model to reach spike threshold, a
spike was produced, and the interval between successive spikes recorded. In this way,
model-generated histograms were created, and their death rate calculated. A series of
iterations with different levels of synaptic drive led to a 'death rate versus distance from
threshold plot' and by inverting their axes, a calibration curve was produced (cf. Figure
13B, Matthews, 1996). The curve fitted with the sum of two exponentials, yielded the
transform described Figure 13, Matthews (1996); see also the equation, Matthews (2002),
which was re-published in MacDonell et al. (2007).

Applying the transform to the death rate of the ISI yields a voltage trajectory plot,
where the voltage is expressed as 'noise units' (equivalent to the standard deviation of the
noise), and the trajectory over time is represented as a distance from threshold, rather than an absolute voltage. Each histogram separated from the original spike train was transformed into a single trajectory that represented a different level of synaptic drive, as well as a different rate of decay in the AHP. As stated by Matthews (1996), in the final step a "compound trajectory" is produced by overlaying each trajectory onto a fixed point or anchor. The slowest moving trajectory, or the trajectory constructed from the longest ISIs provided the anchor to which all other trajectories were aligned. After data point alignment, a 1st order exponential equation fitted the multi-trajectory curve. The rate constant of the exponential equation represented the time constant of the AHP decay.

With this model, at membrane potentials below threshold (low levels of synaptic drive) the underlying noise structure at the motoneuron varies the AHP. The level of synaptic drive also affects the distribution of the intervals. As drive increases, the structure of the histograms tend towards a Gaussian distribution; as drive decreases, and ISI duration increases, a skewed histogram that deviates from a Gaussian distribution results. Matthews transform argues that the AHP, at a low level of drive, decays to the resting membrane potential and two things can trigger an action potential: increased synaptic drive or noise. The transform itself describes the probability of a spike in relation to the characteristics of the synaptic noise and synaptic drive.

**Statistical analysis**

Statistical analysis was performed using Statistica v6.1 (StatSoft Inc, Tulsa, OK). One-way repeated measures ANOVAs with three levels (pre-vibration control; vibration; post-vibration control) or paired Student’s t-tests (pre-vibration control, vibration) were used to determine if any difference existed in the AHP time course between conditions.
Dependent samples Student's t-tests were used to compare the ISI distribution parameters between control and vibration conditions. Means ± standard deviation (SD) are presented in the text. The difference between means was considered significant at p<0.05.

RESULTS

Tibialis anterior tendon vibration

Maximal dorsiflexion torques at the start of the experiment (35.1 ± 16.6 Nm) and following the experiment (34.2 ± 15.4 Nm) were similar. In addition, no statistically significant difference was found between the maximal plantarflexion torques collected before (115.8 ± 40.8 Nm) and after (106.0 ± 53.7 Nm) the experiment.

Twenty TA motor units were recorded from 11 subjects, age 33.0 yrs ± 9.3 yrs. The mean number of ISIs in the pre-vibration control condition was 4815 ± 797 ISIs and 4418 ± 726 ISIs for the vibration condition. The mean AHP time constants for the pre-vibration control and vibration conditions were 35.5 ± 5.3 ms and 31.3 ± 5.4 ms, respectively (p = 0.002; Figure 2A). In ten experiments, subjects were able to keep the motor unit discharging for the full 1800 s. The other 10 experiments did not have complete post-vibration data either because of deterioration of the motor unit signal or subject’s discomfort with the apparatus. The mean number of ISIs in the histograms for the 10 motor units with all three conditions was 4646 ± 855 ISIs, 4218 ± 747 ISIs, and 4466.3 ± 876.0 ISIs, for the pre-vibration control, vibration, and post-vibration control conditions, respectively. In these 10 motor units, the AHP time constant during vibration was significantly less (29.5 ± 3.6 ms; p = 0.001) than the pre-vibration control (35.6 ± 6.1 ms) and post-vibration control (36.3 ± 5.5 ms) conditions and the pre-vibration and post-
vibration control conditions did not differ from each other (Figure 2B). The goodness of
fit for the trajectories (n=20) between control (0.98 ± 0.008) and agonist vibration (0.97 ±
0.01) were found to be statistically different (p = 0.01), although the fit for the curves was
high and likely did not contribute to the difference found in the time constant. For the
sample of motor units in which post-vibration data was collected (10 experiments), the
goodness of fit for post-vibration control (0.98 ± 0.005) was found to be similar to pre-
vibration control (0.978 ± 0.006) and agonist vibration (0.97 ± 0.01) trajectories.

Subjects were instructed to control the motor unit firing rate (and hence
motoneuron output). To determine the ability of subjects to control the firing rate, the ISI
distribution histograms were compared between pre-vibration control and vibration
conditions (Table 1). The mean ISIs differed by 2.8 ms between pre-vibration control
(136.4 ± 7.6 ms) and vibration conditions (133.6 ± 7.4 ms; p < 0.005), with no significant
difference in the CV of ISI. The ISI SD was not significantly different between the pre-
vibration (38.3 ± 5.4) and vibration conditions (39.0 ± 4.6). In case the modest 2%
difference in motor unit firing rate between the pre-vibration control and vibration
conditions accounted for the difference in the AHP $\tau$, a secondary sample of 11 motor
units was examined in which there was no statistically significant difference in the ISIs or
the SD of the ISIs. In these experiments, the mean difference in ISI between pre-
vibration control and vibration was 1.3 ms. The AHP time course during vibration in this
secondary sample remained less (30.5 ± 6.4 ms) than the pre-vibration control (34.6 ± 4.8
ms; p = 0.045). The ISI distributions for each condition were positively skewed (1.3 ±
0.1, pre-vibration control; 1.5 ± 0.2, agonist vibration; p = 0.001), as a large number of
low firing rates were required. An example of ISI distributions for a single motor unit
during pre-vibration control and TA vibration conditions together with corresponding AHP trajectories are presented in Figure 3. A difference in the extent of kurtosis between the pre-vibration control and vibration conditions was found for both the original analysis (p = 0.006) and the secondary analysis of 11 experiments with similar ISIs (p = 0.046); whereby the vibration condition had greater values of kurtosis (6.3 ± 1.3, original; 6.7 ± 1.5, secondary) compared to pre-vibration control (5.7 ± 0.9, original; 5.9 ± 0.9, secondary). The greater kurtosis of the vibration ISI distribution indicates that the excitatory stimulus resulted in a narrower distribution of ISIs than in the pre-vibration control condition.

The time constant derived from the IDR analysis is strongly influenced by the trajectory created from the sub-histogram made with the longest ISIs. For this reason, the number of intervals assigned to the histogram constructed from the long duration ISI was compared for pre-vibration control (1143 ± 199), vibration (1088 ± 116), and post-vibration (1184 ± 278) conditions. The numbers of intervals did not differ among conditions.

Achilles tendon vibration

Maximal dorsiflexion torque and maximal plantarflexion torque collected at the start of the experimental protocol (dorsiflexion, 33.3 ± 15.6 Nm; plantarflexion, 116.1 ± 49.5 Nm) were not significantly different from the peak torque collected after the protocol ceased (dorsiflexion, 30.9 ± 14.7 Nm; plantarflexion, 106.4 ± 48.2 Nm). The mean number of ISIs used to calculate the AHP $\tau$ for 10 TA motor units (7 subjects, age 34.0 yrs ± 11.0 yrs) was 4363 ± 453 ISIs for the pre-vibration control condition and 3748
± 465 ISIs for the antagonist vibration condition. Only 3 of the 10 motor units were followed for the full 1800 s which comprised an insufficient sample for statistical analysis. Therefore, only pre-vibration control and vibration data are reported. The mean AHP time constant for the pre-vibration control and antagonist vibration conditions were 33.6 ± 4.3 ms and 38.6 ± 5.0 ms, respectively (Figure 4A,B) and were found to be significantly different (p < 0.001). The mean goodness of fit of the trajectories was compared and found to be similar between pre-vibration control (0.97 ± 0.01) and antagonist tendon vibration (0.96 ± 0.02).

An assessment of the ISI distribution histograms between pre-vibration control and antagonist vibration conditions revealed no difference in the mean ISIs (pre-vibration control, 136.8 ± 7.4 ms; Achilles tendon vibration, 135.4 ± 7.3 ms; n.s.) but the CV of ISIs in the pre-vibration control condition (28.7 ± 2.8%) was smaller than the antagonist vibration condition (33.1 ± 6.2%; p < 0.02; Table 1). The ISI SD was significantly less in the pre-vibration condition (39.5 ± 3.5) than in the antagonist vibration condition (44.7 ± 7.6; p < 0.02). An example of ISI distributions for a single motor unit from TA during pre-vibration control and antagonist vibration conditions with corresponding AHP trajectories are presented in Figure 5.

In addition to the positively skewed ISI distribution (pre-vibration control, 1.4 ± 0.2; antagonist vibration, 1.2 ± 0.1; p = 0.002), a significant difference in kurtosis between pre-vibration control and antagonist vibration conditions was found (p < 0.006). The kurtosis of the ISI distribution for the pre-vibration control condition was greater (5.7 ± 0.6) than the antagonist vibration condition (5.0 ± 0.8; Table 1). It should be noted that the degree of kurtosis changed in opposite directions for agonist vs. antagonist vibration.
As with agonist tendon vibration, the intervals that made up the subhistogram with the longest mean ISIs were examined between pre-vibration control and the antagonist tendon vibration conditions. This analysis also demonstrated that the number of intervals used for the long duration trajectories was similar between pre-vibration control (1098 ± 64) and antagonist tendon vibration (1125 ± 91).

[Figures 4 and 5 about here]

DISCUSSION

This investigation showed that tendon vibration results in modification of the motoneuron AHP time course. Vibration of the agonist tendon induces a significantly reduced motoneuron AHP time constant, whereas vibration of the antagonist tendon led to an increased AHP time constant, compared to the pre-vibration control conditions in which no vibration was applied. This effect did not simply result from a change in motor unit firing rate, as subjects maintained careful control over the firing rate. TA vibration was associated with a narrower ISI distribution histogram (greater kurtosis) with no change in overall CV of ISI, whereas Achilles tendon vibration increased the variability of ISIs as seen by an increase in CV of ISI and a broader distribution of ISIs. These results suggest that a change in the balance of inputs that produce a given motor unit firing rate (motoneuron output) may have resulted in a modification of the AHP time course.

The shorter AHP time course with agonist vibration was reversible. The return of the AHP time constant to pre-vibration control values in the post vibration period is important in two ways: 1) in the absence of vibration, the model renders consistent results
and 2) the reduced AHP time course is not long lasting after cessation of the sensory
input.

The mechanisms underlying the shortened AHP remain elusive. Three possible
mechanisms include 1) an increased motoneuron gain, 2) induction of plateau potentials,
and 3) restructuring of synaptic noise through vibration-induced EPSPs.

Neuromodulation of the motoneuron has been shown to affect the AHP amplitude and
duration, acting to alter the apamine sensitive outward K+ current largely responsible for
medium duration hyperpolarized post-spike membrane potential (Sah 1996). The
duration and/or amplitude of the AHP have been shown to be reduced in response to NE
(White et al. 1991), ACh (Chevallier et al. 2006) and muscarinic receptor activation
(Miles et al. 2007). In a similar manner, serotonin (5-HT) reduces the amplitude of the
slow/late AHP in cat spinal motoneurons (White and Fung 1989; Wallen et al. 1989; Van
Dongen et al. 1986) and turtle motoneurons (Hounsgaard and Kiehn 1989). Modulation
of the AHP is believed to occur via a decrease in the Ca++ mediated K+ conductance,
which is thought to increase spinal motoneuron gain (Miles et al. 2007; Chevallier et al.
2006; Wallen et al. 1989). The decrease in AHP time course resulting from TA tendon
vibration in the current experiment suggests an increase in the motoneuron gain.

A second possibility for the shortened AHP is that vibration has been shown to
change the input/output relations of the motoneuron by activating plateau potentials in the
presence of neuromodulators (Wand 1976; Lee and Heckman 1996). For example,
Gorassini et al. (1998) found prolonged motor unit activity, or self-sustained firing, in
humans that lasted several minutes after removal of high frequency vibration applied
during a steady low force contraction (up to 10% MVC). Gorassini et al. (1998)
proposed that the increase in robustness of the self-sustained firing, as the number of
times the stimulation was applied increased, is similar to the warm-up phenomenon
(Bennett et al. 1998) seen with plateau potentials in rats. It is possible that the
neuromodulator acting to induce plateau potentials, i.e. serotonin, is reducing the AHP.
In addition, the initiation of the plateau potential may increase the discharge probability,
and the membrane trajectory rises to threshold sooner. The increased incidence of
discharge may lead to increased central tendency in the ISI distribution (see below).
Further investigation would be needed to examine the involvement of persistent inward
currents in vibration-induced shortened AHPs.
A third possibility is that the underlying noise bombarding the motoneuron from
the afferent input results in increased EPSPs, which brings the membrane potential to
threshold sooner, and this would be associated with a shortened AHP. In the present
experiment, participants were asked to discharge a single motor unit for 600 s according
to a predetermined firing rate protocol. The mean interspike interval between control and
vibration conditions only differed by 2%, albeit this difference was statistically
significant. The kurtosis, a measure that describes the peak of a distribution, was greater
during TA tendon vibration, reflecting a distribution that has a more pronounced peak
and less frequent extreme deviations from its central tendency. The finding that the
kurtosis of the ISI distributions was greater during TA tendon vibration suggests that the
motor control scheme for successfully achieving the firing rate protocol differed between
control and vibration conditions. In order for subjects to maintain a similar mean firing
rate during agonist vibration, (excitatory input) it may have been necessary to reduce
volitional drive to the motoneuron pool. Although speculative, the increased likelihood
of a motor unit action potential may be reflected in the narrower ISI distribution
histogram. The narrow histogram, i.e. a histogram with a distinct central tendency, is
reflective of an output that is more structured or highly probable.

Figure 6 presents a schematic model of motoneuron output during control
conditions and with excitatory and inhibitory synaptic inputs. During TA vibration,
excitatory input to the TA may facilitate a plateau potential that may increase the ability
of the motoneuron to achieve self-sustained firing. A reduction in supraspinal drive to
the motoneuron would be needed to balance the increase in excitation from peripheral
sources. It is speculated that the change in motoneuron gain (as suggested by the smaller
AHP time constant) resulted from TA tendon vibration-mediated facilitation of already
activated persistent inward currents, that are shown to be enhanced by Ia afferent
stimulation in the cat (Lee and Heckman, 1996).

AHP time course with SOL tendon vibration

Vibration of the Achilles tendon likely induced Ia reciprocal inhibition.
Reciprocal Ia inhibition occurs by activating Ia afferents from the antagonistic muscle via
a disynaptic pathway (Jankowska 1992) and is diffusely distributed across the
motoneuron pool of the opposing muscle group (Heckman and Binder 1991). Inhibition
of flexor and extensor ankle motoneurons has been found during conditioning stimuli of
the antagonist tendon (Mizuno et al. 1971; Iles 1986; Crone et al. 1987). As such, the
reflex response produced by Achilles tendon vibration would tend to inhibit the tibialis
anterior motoneuron pool and would likely succeed if not for the voluntary drive used to
achieve the motor unit discharge frequencies required by a predetermined protocol (see
Methods and MacDonell et al. 2007). The task is therefore composed of the motor command and inhibitory command, which compete (Figure 6). This competition between conflicting inputs to the motoneuron pool is, perhaps, responsible for the increased ISI CV during antagonist tendon vibration. The fact that the motor unit discharge rates in the current experiment were quite low may have promoted a decrease in firing probability because inhibition of soleus motor units by tibialis posterior nerve stimulation was most effective when motor unit discharge rate was at or near the minimal firing rate (Kudina 1980, 1999).

In Figure 6C, the disynaptic Ia inhibitory input from the plantarflexors is associated with an increase in the AHP time course in TA. The inhibition to the TA motoneuron pool may be offset by an increased supraspinal descending command to the TA motoneuron pool. In this way, the same motoneuron output is achieved with antagonist vibration as in the control condition. The competing inputs (inhibition and descending excitation) probably induce significant variance in the resting membrane potential, which is then reflected in motor unit firing (Figure 6C). The increased variability in motor unit firing found with antagonist tendon vibration reflects a decrease in the likelihood of a motor unit action potential and will lead to an increased time constant.

**Dependence upon the discharge rate protocol**

A number of investigations show that motoneuron output is structured to the phase of the vibration cycle (Burke 1976a,b,c), although this tendency is less with prolonged vibration or muscle contraction. Auto-correlation of the motor unit spike train was used to determine whether the vibration impacted motor unit discharge frequency in
a similar manner. We found no systematic fluctuations of motor unit discharge at the 110 Hz frequency of the vibrator or its subharmonics. This implies that the input to the motoneuron was not dominated solely by the frequency of vibration. The difference in this investigation, compared to those where phase lock of the vibration to motoneuron discharge occurred, is that the participants had to execute a protocol that adjusted the firing rate of the motoneuron volitionally. The participants did not follow a protocol where the force level was matched and maintained, nor were participants instructed to attend to the vibration; the only requirement was to follow a prescribed firing rate that allowed the muscle to vary in a small range of intensity. The focus upon the motor unit discharge rate may have contributed to the lack of phase dependency.

Matthews (c.f. Figure 12) argues that the shorter the AHP time constant, the larger the CV of the ISI. In this experiment, a shift to a shorter AHP time constant occurred without change in the CV for ISIs during agonist vibration, while a shift to a longer AHP was associated with a larger CV during antagonist vibration when compared to pre-vibration. The data presented in Matthews (1996) were from manipulations that allowed both the ISIs and CV of ISIs to vary substantially. In the current experiment, constraining the ISIs in the face of added synaptic input resulted in the CV being the primary variable to vary. As such, the distributions change their shape (i.e. increased kurtosis) with limited to no change in the mean firing rate. Forcing this separation of mean ISI and variability of ISI was critical for the detection of AHP changes in response to the synaptic input.

The SD of the ISIs was similar for both agonist vibration and pre-vibration control conditions. The finding that the ISI SD, as well as the ISI CV, remained similar suggests
that the motoneuron is receiving similar amounts of synaptic noise but is more excitable. However for antagonist vibration, the mean ISI was similar, but the ISI CV and ISI SD were greater than the pre-vibration control. The increase in SD could reflect an increase in synaptic noise or a decrease in discharge probability as a result of competing excitatory and inhibitory drive at the motoneuron synapse through the action of the Ia reciprocal inhibition pathway.

Summary

This investigation has shown that the AHP duration is modified with the application of vibration. Tendon vibration of the agonist (TA) induced a decrease of the AHP time course in TA motoneurons, whereas vibration of the antagonist Achilles tendon was associated with an increase of the AHP time course. Despite changing the peripheral inputs to the motoneuron in the control and vibratory input conditions, similar motoneuron output (in terms of average firing rate) was achieved. It is speculated that the reduction in AHP time constant with TA vibration may have resulted from modulation of motoneuron gain, alteration of persistent inward currents and/or the restructuring of synaptic noise, while a decrease in firing probability, possibly reflecting Ia reciprocal inhibition, may have been responsible for the larger AHP time constant.
REFERENCE LIST


ACKNOWLEDGEMENTS

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Figure 1. EMG recordings with and without vibration. A single motor unit recording from tibialis anterior (TA MU, second row) with instantaneous frequency (top row) and insets of overlaid motor unit potentials above the control (left) and vibration (right) conditions is presented in the first row. The third and fourth rows are the surface EMG signals from the tibialis anterior (TA sEMG) and soleus (SOL sEMG). Application of vibration increased the baseline noise but did not affect the integrity of the motor unit recordings.

Figure 2. Tibialis anterior tendon vibration. Estimated afterhyperpolarization (AHP) time constants (τ), with line of unity for 20 motor units collected during pre-vibration control and vibration conditions. Two groups of motor units are shown: (●) with a small difference in the mean interspike interval (ISI) during both conditions (n=9) and (Θ) with similar mean ISI (n=11) during both conditions (A). Below are presented the means and standard deviations of the estimated AHP time constant (τ) for the 10 motor units that were collected during pre-vibration control (white), tibialis anterior tendon vibration (grey), and post-vibration control (black) conditions (B). * Indicates a significant difference from the pre-vibration control and post-vibration control conditions, p < 0.01.

Figure 3: Single motor unit estimates for tibialis anterior tendon vibration. Afterhyperpolarization trajectories for a single motor unit calculated during pre-vibration control (dashed line) condition and during vibration of tibialis anterior tendon (solid line) are shown (A). Four slices were used to create the estimate. The histograms are for each
slice containing the interspike intervals used in the calculation of a separate trajectory (inset; trajectories prior to alignment) for pre-vibration control (B) and tibialis anterior tendon vibration (C). The left-most trajectory in the inset corresponds with the left-most histogram (fastest ISIs) and the right-most trajectory in the inset corresponds with the right-most histogram (longest ISIs). The threshold represents the point at which a spike would be produced.

Figure 4. Achilles tendon vibration. Estimated afterhyperpolarization (AHP) time constants ($\tau$) with line of unity for 10 TA motor units collected during pre-vibration control and antagonist vibration conditions (A). Below are presented the means and standard deviations of the estimated AHP time constant ($\tau$) for all experiments during pre-vibration control (white) and Achilles tendon vibration (grey) conditions (B). * Indicates a significant difference from the pre-vibration control, $p < 0.001$.

Figure 5: Tibialis anterior AHP estimate for pre-vibration control and achilles tendon vibration: Afterhyperpolarization trajectories for a single motor unit calculated during pre-vibration control condition (dashed line) and during vibration of the Achilles tendon (solid line) conditions are shown (A). Three slices were used to create the estimate. The histograms from each slice contain the interspike intervals used in the calculation of a separate trajectory (inset; trajectories prior to alignment) for pre-vibration control (B) and Achilles tendon vibration (C). The left-most trajectory in the inset corresponds with the left-most histogram (fastest ISIs) and the right-most trajectory in the
inset corresponds with the right-most histogram (longest ISIs). The threshold represents
the point at which a spike would be produced.

Figure 6. Schematic representation of motoneuron output during excitatory and
inhibitory input. Schematic model of motoneuron output during control conditions (A)
and during conditions of excitatory (B) and inhibitory (C) inputs is presented. The
thickness of the lines corresponds to more (thick) or less (thin) input. A schematic action
potential with threshold (dotted lines) and relative length of the afterhyperpolarization is
shown at the inferior right corner of the motoneuron in each panel. Panel A represents
the control condition whereby a given descending supraspinal drive is associated with a
given motoneuron output (motor unit firing rate) depicted by the thick arrow leaving the
axonal hillock of the motoneuron. Panel B illustrates how increasing the excitatory input
with agonist vibration, represented by the monosynaptic pathway synapse on the left of
the motoneuron, contributes to a decrease in the time course of the AHP. To keep the
motoneuron output constant, there is a concomitant reduction in supraspinal drive.
Conversely, in panel C, the time course of the AHP is increased due to inhibition
represented in the disynaptic pathway to the right of the motoneuron. In this case, the
supraspinal drive increases to keep the motoneuron output constant, which may result in a
variable resting membrane potential and decreased firing probability. In all three
scenarios, a similar motoneuron output, as indicated by the motor unit firing rate, is
proposed to be achieved through alterations in both intrinsic and supraspinal control
mechanisms.
**Table 1. Interspike interval (ISI) statistics.** Mean and coefficient of variation (CV) of ISI, and skewness and kurtosis for ISI distribution histograms collected during agonist (TA tendon) and antagonist (Achilles tendon) vibration.

<table>
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<tr>
<th></th>
<th>Control†</th>
<th>Agonist Vibration†</th>
<th>Post‡</th>
<th>Agonist Vibration†</th>
<th>Control†</th>
<th>Vibration†</th>
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<td>ISI</td>
<td>136.4 ± 7.6</td>
<td>133.6 ± 7.4*</td>
<td>135.9 ± 6.7</td>
<td>136.8 ± 7.4</td>
<td>135.4 ± 7.3</td>
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<td>ISI SD</td>
<td>38.3 ± 5.4</td>
<td>39.0 ± 4.6</td>
<td>39.7 ± 7.3</td>
<td>39.5 ± 3.5</td>
<td>44.7 ± 7.6*</td>
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<td>CV</td>
<td>29.2 ± 2.3</td>
<td>28.1 ± 4.0</td>
<td>29.2 ± 5.3</td>
<td>28.7 ± 2.8</td>
<td>33.1 ± 6.2*</td>
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<td>Skewness</td>
<td>1.3 ± 0.1</td>
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<tr>
<td>Kurtosis</td>
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<td>5.1 ± 0.9</td>
<td>5.7 ± 0.6</td>
<td>5.0 ± 0.8*</td>
<td></td>
</tr>
</tbody>
</table>

* indicates significant difference from control or post vibration. † Indicates data for 20 motor units and ‡ indicates data for 10 motor units.