The influence of a 5-wk whole body vibration on electrophysiological properties of rat hindlimb spinal motoneurons

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Whole body vibration (WBV) is now widely applied as a form of physical training or as a rehabilitation method preventing muscle force decrease. It is generally accepted that WBV induces acute responses of the neuromuscular system, yet the actual effects on skeletal muscle contractile activity as well as mechanisms of influence on motoneurons and motor units (MUs) are vague. The most noticeable is an enhancement in muscle electromyographic (EMG) activity (Abercromby et al. 2007; Roelants et al. 2006), most likely elicited on a basis of the tonic vibration reflex (Roelants et al. 2006), yet the actual effects on motoneurons and motor units (MUs) are vague. The experimental group subjected to the WBV consisted of seven rats, and the control group of nine rats. The WBV treatment induced no significant changes in the passive membrane properties of motoneurons. However, the WBV-evoked adaptations in excitability and firing properties were observed, and they were limited to fast-type motoneurons. A significant decrease in rheobase current and a decrease in the minimum and the maximum currents required to evoke steady-state firing in motoneurons were revealed. These changes resulted in a leftward shift of the frequency-current relationship, combined with an increase in slope of this curve. The functional relevance of the described adaptive changes is the ability of fast motoneurons of rats subjected to the WBV to produce series of action potentials at higher frequencies in a response to the same intensity of activation. Previous studies proved that WBV induces changes in the contractile parameters predominantly of fast motor units (MUs). The data obtained in our experiment shed a new light to possible explanation of these results, suggesting that neuronal factors also play a substantial role in MU adaptation.

Motoneuron; membrane properties; training; whole body vibration; rat

MATERIALS AND METHODS

Animal housing. The study was performed on 16 adult male Wistar rats (body mass 440–470 g), which were assigned to the experimental

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group of rats subjected to the WBV (n = 7), and the control group (C) (n = 9). Animals were housed in standard cages (2/each), in a room with humidity and temperature maintained constant, with the 12/12-h dark/light daily cycle, and had unrestricted access to food and water. All experimental procedures were approved by the Local Ethics Committee. The rats’ maintenance and care followed the European Union guidelines and the Polish Law on the Protection of Animals.

WBV. The WBV training was performed on a vibration platform (Power Plate), producing sinusoidal vertical vibrations, 5 days a week for 5 wk. Rats were placed in a plastic container, attached to the platform by rigid belts, and were allowed to move freely inside the container during the procedures. WBV was superimposed onto the animal standard cage activity, provoking corrective postural reactions and body-supporting activities during which fast MUs were activated (Gorassini et al. 2000; Grimby 1984). The maximal vertical displacement of a platform was 2.5 mm, and the maximal acceleration of vibrations (measured by accelerometer ACL300, Biometrics) reached 4.79 g of the gravitational acceleration on earth. Four bouts of 30-s vibrations separated by 60-s rest intervals were applied in each daily session. Thirty-second exposures were used to minimize the possibility of high-threshold MU derecruitment, observed during prolonged muscle vibration (Bongiovanni et al. 1990). Four repetitions of the WBV were used to fit in principles of strength-training protocols, which recommend a small number of repetitions during a short period to provoke strength rather than endurance adaptations in a muscle (Ratamess et al. 2009). Vibration frequency was set to 50 Hz, since oscillations of hindlimb muscles during natural activity are estimated to be within a range between 30 and 50 Hz (Ettema and Huijing 1994).

Surgery. All procedures were performed under pentobarbital anesthesia (initial dose 60 mg/kg ip). The depth of anesthesia was controlled by lack of withdrawal reflexes during preparation and the continuous ECG monitoring of the heart rate (300–360 bpm) during the recording session. Additional doses of anesthetics (10 mg/kg) were given when the heart rate rose, and experiments were not continued when the heart rate continuously decreased. Rats were artificially ventilated (Columbus Instruments; volume 4–5 ml, stroke rate 100–120/min), and expired CO₂ level was measured (CAPSTAR-100, CWE) and maintained between 2 and 4% by adjusting tidal volume or ventilation rate. Pancuronium bromide (Pancuronium, Jelfa, Poland) was administered at regular intervals (the first dose of 0.4 mg/kg iv, supplementary doses of 0.2 mg/kg, applied every 30 min) to induce paralysis when recording from the motoneurons. At the end of each experiment, rats were euthanized by an overdose of pentobarbital (180 mg/kg). The femoral vein was catheterized for drug administration. Tracheotomy was made and a tracheal tube was inserted for artificial ventilation. The sciatic nerve (Sci) and its branches to the medial gastrocnemius (MG) and lateral gastrocnemius and soleus muscles (LGS) were dissected and placed on bipolar silver wire electrodes for stimulation. A laminectomy over L4–L5 spinal cord segments, using a remotely controlled stepping motor, was performed. Animals were placed in a thermostatically controlled heating system. Body temperature was kept within physiological limits (37°C ± 1°C) and humidity and temperature maintained constant, with the 12/12-h light/dark cycle, and had unrestricted access to food and water.

All recordings from motoneurons with the resting membrane potentials of at least ~50 mV and the amplitudes of action potentials over 50 mV were taken into consideration (Gardiner 1993; Gardiner and Kernell 1990). After successful implantation of a motoneuron, antidromic action potentials were recorded in a bridge mode, and 10 spikes were averaged. From the obtained antidromic recordings, basic membrane properties were calculated: the resting membrane potential; the action potential amplitude; the action potential duration; the action potential rise time; the afterhyperpolarization amplitude; the AHP time-to-peak; and the AHP half-decay time (AHP-HDT). The latter parameter was used to separate fast from slow motoneurons: AHP-HDT was ≤20 ms for fast and >20 ms for slow motoneurons (Beaumont and Gardiner 2002, 2003; Gardiner 1993).

Measurement of cell input resistance was performed from an average of 40 short pulses (100 ms) of 1-nA hyperpolarizing current recorded in a DCC mode (4–8 kHz). Rheobase was determined as a minimum amplitude of a 50-ms square-wave current resulting in spikes >50% of the time. After measuring the basic properties, the steady-state firing (SSF) was evoked in a series of 500-ms square pulses of depolarizing current injections at amplitudes increasing or decreasing in steps of 0.1–5 mV in a DCC mode. Stimulations were separated by 5-s intervals. The minimum and maximum currents for the SSF were measured, and minimum and maximum SSF frequencies were calculated from the last three interpulse intervals during the 500-ms square pulse (Fig. 1). For each motoneuron, the frequency-current (f-I) relationship was plotted, and the f-I slope was determined. Moreover, from the recordings with 500-ms current injections, the doublet threshold (DT) was determined as a minimum current to evoke the initial doublet of action potentials (with the first interspike interval <10 ms), and the relative ratio of DT to rheobase (DT/Rh) was calculated.

Statistical analysis. For statistical comparison between WBV and control groups, Student’s t-test was used for data with equal variance and normal distribution, whereas U-Mann Whitney test was used for data with unequal variance and unequal distribution. Significance levels were set at P < 0.01 or P < 0.05.

RESULTS

Sampling. A total of 174 motoneurons with stable recordings were analyzed: 93 in the WBV group, and 81 in the control group. 53 cells were antidromically identified as MG, 37 as LGS, and 84 as Sci motoneurons. 69 motoneurons in the WBV group (23 MG, 11 LGS, 35 Sci) and 65 in the control group (20 MG, 16 LGS, 29 Sci) were classified as fast, whereas 24 motoneurons in the WBV group (7 MG, 4 LGS, 13 Sci) and 16 in the control group (3 MG, 6 LGS, 7 Sci) were classified as slow.

Five-hundred-millisecond current steps were applied in a subset of motoneurons (n = 106), so the SSF properties, doublet threshold, and f-I relationship could be determined in a proportion of the whole sample: from 48 and 33 fast motoneurons, as well as 16 and 9 slow motoneurons of the WBV and control groups, respectively.

Passive and threshold properties. All the passive membrane properties of motoneurons remained unchanged after the 5-wk WBV treatment (Table 1). However, significantly lower mean values of the rheobase (P < 0.05) and the doublet threshold (P < 0.01) were observed in fast-type motoneurons of the WBV group compared with the untrained control (Table 1), indicating an increase in excitability of these cells. The relative
threshold for doublet generation (measured as a ratio of current necessary to evoke doublet to the rheobase) exceeded 2 times rheobase, and there were no differences between motoneurons of the C and WBV groups. It should be mentioned here that doublets were observed frequently when intensity of current injection into motoneurons increased, but not in all the cases. In the WBV group, doublets could be determined in all 48 fast, and in 13 out of 16 slow-type motoneurons, while in the control group in 30 out of 33 fast, and 8 out of 9 slow-type cells.

**Rhythmic firing properties.** Rhythmic firing responses were measured in a subset of motoneurons (Table 2). Examples of the steady-state firing for two motoneurons (fast and slow) from the WBV group are given in Fig. 1. The minimum and maximum frequencies during SSF were not different between control and WBV groups, neither for fast nor slow-type motoneurons (Fig. 2A). However, the 5-wk WBV treatment resulted in a significant decrease in the minimum SSF current ($P < 0.05$) and in the maximum SSF current ($P < 0.01$) in fast motoneurons (Table 2, Fig. 2B). No significant changes with respect to these parameters were found in slow motoneurons.

The above changes in rhythmic firing properties of fast motoneurons influenced $f$-$I$ relationships, reflecting minimum and maximum SSF frequencies, minimum and maximum current thresholds for SSF, and $f$-$I$ slopes. Figure 3 presents examples of such $f$-$I$ relationships, illustrating differences between fast- and slow-type motoneurons as well as a tendency of adaptive changes observed in fast-type motoneurons following the WBV training. The $f$-$I$ relationships for the whole sample of motoneurons is summarized in Fig. 4. The $f$-$I$ relationship for fast-type motoneurons of the WBV group was shifted leftwards compared with the control group, which was evidenced by significantly lower currents necessary for both minimum and maximum SSF (Table 2). Moreover, an increased slope (from 2.85 Hz/nA to 4.51 Hz/nA, $P < 0.01$) of the $f$-$I$ relationship was determined in the group of fast-type motoneurons following the WBV (Table 2). Again, no differences in $f$-$I$ relationship were found between C and WBV groups of slow motoneurons, with only a minor tendency to a leftward shift of the $f$-$I$ relationship.

**DISCUSSION**

**Diverse effects of the WBV on fast and slow motoneurons.** The study indicates that a short-term, 5-wk WBV is capable to induce type-specific adaptations in electrophysiological properties of rat spinal motoneurons. Changes in excitability and firing characteristics concerned only fast motoneurons, whereas membrane properties of slow motoneurons remained unaltered. The diversity of WBV-evoked responses between two types of motoneurons might partly be justified by the training characteristics that favor fast motoneurons with respect to their relative daily time of activity. As mentioned earlier, MUs (and their corresponding motoneurons) are usually recruited in accordance to Henneman’s size principle (Henneman et al. 1965); however, significantly lower recruitment thresholds of entire populations of MUs have been observed during the WBV (Romaiguère et al. 1993). There-
Table 1. Passive and threshold properties of motoneurons

<table>
<thead>
<tr>
<th></th>
<th>Fast (n = 65)</th>
<th>WBV (n = 69)</th>
<th>P</th>
<th>Slow (n = 16)</th>
<th>WBV (n = 24)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP, mV</td>
<td>60.53 ± 7.87</td>
<td>59.09 ± 7.88</td>
<td>ns</td>
<td>56.63 ± 6.04</td>
<td>58.38 ± 8.02</td>
<td>ns</td>
</tr>
<tr>
<td>AP amp, mV</td>
<td>65.51 ± 10.66</td>
<td>65.66 ± 11.29</td>
<td>ns</td>
<td>57.68 ± 9.52</td>
<td>62.92 ± 11.47</td>
<td>ns</td>
</tr>
<tr>
<td>AP dur, ms</td>
<td>1.72 ± 0.27</td>
<td>1.72 ± 0.27</td>
<td>ns</td>
<td>1.86 ± 0.27</td>
<td>1.88 ± 0.37</td>
<td>ns</td>
</tr>
<tr>
<td>AP rise, ms</td>
<td>0.94 ± 0.15</td>
<td>0.92 ± 0.15</td>
<td>ns</td>
<td>0.99 ± 0.18</td>
<td>1.02 ± 0.20</td>
<td>ns</td>
</tr>
<tr>
<td>AHP amp, mV</td>
<td>3.36 ± 1.45</td>
<td>3.59 ± 1.77</td>
<td>ns</td>
<td>6.13 ± 2.72</td>
<td>4.67 ± 2.36</td>
<td>ns</td>
</tr>
<tr>
<td>AHP time-to-peak, ms</td>
<td>9.93 ± 2.66</td>
<td>9.90 ± 3.16</td>
<td>ns</td>
<td>10.32 ± 4.98</td>
<td>10.38 ± 3.12</td>
<td>ns</td>
</tr>
<tr>
<td>AHP-HDT, ms</td>
<td>11.68 ± 2.91</td>
<td>12.34 ± 2.82</td>
<td>ns</td>
<td>25.80 ± 6.18</td>
<td>25.11 ± 4.89</td>
<td>ns</td>
</tr>
<tr>
<td>IR, mV</td>
<td>1.97 ± 1.16</td>
<td>1.98 ± 1.28</td>
<td>ns</td>
<td>3.97 ± 2.03</td>
<td>3.36 ± 2.25</td>
<td>ns</td>
</tr>
<tr>
<td>Rh amp, mV</td>
<td>57.73 ± 8.94</td>
<td>58.49 ± 8.57</td>
<td>ns</td>
<td>50.97 ± 50.97</td>
<td>57.25 ± 8.48</td>
<td>ns</td>
</tr>
<tr>
<td>DT, nA</td>
<td>17.90 ± 8.35</td>
<td>13.48 ± 6.96</td>
<td>†</td>
<td>7.44 ± 3.31</td>
<td>10.31 ± 5.62</td>
<td>ns</td>
</tr>
<tr>
<td>DT/Rh</td>
<td>2.41 ± 1.18</td>
<td>2.14 ± 0.73</td>
<td>ns</td>
<td>3.24 ± 1.20</td>
<td>3.62 ± 1.42</td>
<td>ns</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. Comparisons between control and whole body vibration (WBV) groups were made with Student’s t-test for data with equal variance and normal distribution or the Mann-Whitney Rank Sum Test for data with unequal variance and unequal distribution; ns, P ≥ 0.05; *0.01 ≤ P < 0.05; †P < 0.01.

Table 2. Rhythmic firing properties of motoneurons

<table>
<thead>
<tr>
<th></th>
<th>Fast (n = 33)</th>
<th>WBV (n = 48)</th>
<th>P</th>
<th>Slow (n = 9)</th>
<th>WBV (n = 16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>maxSSF current, nA</td>
<td>28.50 ± 9.19</td>
<td>22.18 ± 7.71</td>
<td>†</td>
<td>13.24 ± 5.86</td>
<td>14.00 ± 7.41</td>
<td>ns</td>
</tr>
<tr>
<td>minSSF, Hz</td>
<td>33.01 ± 9.68</td>
<td>34.24 ± 10.56</td>
<td>ns</td>
<td>22.65 ± 5.40</td>
<td>29.91 ± 11.79</td>
<td>ns</td>
</tr>
<tr>
<td>maxSSF, Hz</td>
<td>67.70 ± 27.02</td>
<td>79.10 ± 35.54</td>
<td>ns</td>
<td>66.41 ± 28.61</td>
<td>81.82 ± 27.47</td>
<td>ns</td>
</tr>
<tr>
<td>f/I slope, Hz/nA</td>
<td>2.85 ± 1.32</td>
<td>4.51 ± 2.54</td>
<td>†</td>
<td>4.93 ± 1.84</td>
<td>5.62 ± 2.43</td>
<td>ns</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. Comparisons between control and WBV groups were made with Student’s t-test for data with equal variance and normal distribution or the Mann-Whitney Rank Sum Test for data with unequal variance and unequal distribution; ns, P ≥ 0.05; *0.01 ≤ P < 0.05; †P < 0.01.

Adaptations in threshold and rhythmic properties of motoneurons. In this study, several threshold and rhythmic properties of fast-type motoneurons were altered by the WBV, without corresponding significant alterations of the passive membrane properties (Tables 1 and 2). This is somehow surprising, as the previous studies have indicated that alterations of the basic properties induce alterations in the threshold and rhythmic properties (Gardiner et al. 2006). An increase in the cell excitability (expressed principally by a reduction in the rheobase, the minimum current necessary to evoke SSF, and an increase in the slope of f-I relationship) is generally linked to an increase in a cell’s input resistance and the AHP amplitude; however, this was not the case in our study (Tables 1 and 2). Therefore, other factors should be considered as responsible for the observed changes after 5 wk of the WBV.

One may speculate about a possible increase in activation of specific persistent inward currents (PICs), which are known to decrease rheobase of motoneurons (Lee and Heckman 2000). A decrease in rheobase current and an increase in the f-I slope reported after the spinal cord transection (Button et al. 2008) have also been connected with the increase in PIC currents. However, in our study we did not measure PIC activation, as the use of pentobarbital anesthesia abolishes PICs in motoneurons (Button et al. 2006; Hultborn 1999; Hultborn and Kiehn 1992). Therefore, we can only hypothesize about a role of PIC activation in the observed WBV-evoked changes in motoneuronal properties. For another explanation, one might consider mechanisms of presynaptic inhibition. It has been suggested that presynaptic inhibition of Ia afferents is highly dependent on the type of muscle activation, as motor tasks involving high...
motor control result in the increase of presynaptic inhibition, contrary to its inhibition by more automated tasks (Tahayori and Koceja 2012). In our study, muscle activation during the WBV was more reflexive than controlled, and it is supposed that levels of Ia presynaptic inhibition became considerably downregulated. However, it is difficult to assess how alterations in presynaptic inhibition could have affected discharge properties of motoneurons using current clamp technique. One may conclude that the factors responsible for the change in excitability of fast motoneurons in this study must await explanation.

**Functional considerations.** Lower values of rheobase current and a leftward shift of the f-I relationship observed for fast motoneurons after the 5-wk WBV program (Fig. 4) indicate their ability to become recruited earlier (and possibly more frequently) and to achieve the same or even higher firing rates at lower stimulus intensities compared with the control group. Thus, one might draw a conclusion that in the trained group, a weaker synaptic input was needed for a motoneuron activation. However, this leftward shift of the relationship was not followed by a decrease in the minimum or maximum SSF frequencies. Therefore, the motoneurons of WBV-treated animals maintained their ability to generate high frequency of discharges, necessary to evoke high levels of force, while the “cellular effort” needed to induce such activations was significantly lower.

Fig. 2. A: distribution of the minimum and maximum frequencies of SSF for slow and fast motoneurons of the control and WBV groups. B: distribution of the minimum and maximum SSF current values for slow and fast motoneurons of the control and WBV groups. Note that frequently similar or identical values were measured in different motoneurons that resulted in overlapping of symbols in the charts. Horizontal lines indicate mean values, and asterisks point to significant differences between the control and WBV groups (*P < 0.05, **P < 0.01, the Mann-Whitney Rank Sum Test).

Fig. 3. Examples of frequency-current relationships for fast (A, C) and slow (B, D) motoneurons of control and WBV groups. Considerable differences are visible between fast and slow motoneurons with respect to the minimum and maximum SSF frequencies (A and C vs. B and D). Moreover, a substantial difference in the slope between fast motoneurons of the control and WBV groups is presented (A vs. C; note different scale on abscissa), the latter closer to values observed for slow-type MNs. The regression lines were plotted according to $f = y_0 + ax$ equations with individual values of $y_0 = 6.5, a = 4.6$ for A; $y_0 = -1.2, a = 7.7$ for B; $y_0 = -4.1, a = 6.1$ for C; $y_0 = -1.3, a = 9.1$ for D.

Fig. 4. Summary of the frequency-current relationships for the whole sample of fast and slow motoneurons of the control and WBV groups. Note the significant effect of the WBV treatment on fast motoneurons, reflected by a change in the slope of the f-I relationship combined with a significant leftward shift towards values observed for slow motoneurons (*P < 0.05, **P < 0.01).
The increased slope of the f-I relationship (Fig. 4) indicates higher susceptibility of fast motoneurons after the WBV to an increased or decreased intensity of a stimulus (i.e., more or less effective synaptic drive under natural conditions). Therefore, our study suggests that the WBV treatment can influence control of the force output of fast MUs. The instantaneous force developed by a MU during a tetanic contraction depends both on the interpulse interval in a motoneuronal discharge pattern and on the initial force level (Krutki et al. 2008). Modifications of a motoneuronal firing rate by small changes in intensity of depolarization current (i.e., strength of synaptic input) appear to be more effective in fast motoneurons after the WBV compared with control.

Whole body vibration has already become an attractive alternative treatment for the strength training regime. Various studies have shown that WBV may be beneficial for inducing strength gains (Delecluse et al. 2003), osteoporosis prevention (Belavý et al. 2009), poststroke conditioning (Van Nes et al. 2004), or prevention of muscle atrophy (Verschueren et al. 2004). However, there is still much debate about mechanisms involved in the observed adaptive changes in the neuromuscular system (Cochrane 2010). This is the first study to demonstrate directly that merely four bouts of 30-s WBV daily, performed for 5 wk, induce changes in electrophysiological properties of the spinal motoneurons, and therefore, is the first study to provide direct evidence on motoneuronal plasticity following the WBV. Our observations performed in rats cannot be easily verified in human subjects. They may, however, contribute to our understanding of neuronal adaptations to the WBV and serve as a source of likely explanations of the WBV-evoked adaptations in the neuromuscular system.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.B. and P.K. conception and design of research; M.B., A.H., W.M., and P.K. performed experiments; M.B., A.H., and P.K. analyzed data; M.B., W.M., J.C., and P.K. interpreted results of experiments; M.B. prepared figures; M.B., A.H., W.M., and P.K. conceived and designed the research; M.B., A.H., W.M., and P.K. interpreted results of experiments; M.B. and P.K. conception and design of research; M.B., A.H., W.M., and P.K. performed experiments; M.B., A.H., W.M., and P.K. prepared figures; M.B., and P.K. conception and design of research; M.B., A.H., W.M., and P.K. performed experiments; M.B., A.H., W.M., and P.K. analyzed data; M.B., W.M., J.C., and P.K. interpreted results of experiments; M.B., A.H., W.M., and P.K. conceived and designed the research.

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