Removal of supraspinal input reveals a difference in the flexor and extensor monosynaptic reflex response to quipazine independent of motoneuron excitation

Jeremy W. Chopek, Christopher W. MacDonell, Kevin E. Power, Kalan Gardiner, and Phillip F. Gardiner

Spinal Cord Research Centre, Department of Physiology and Faculty of Kinesiology and Recreation Management, University of Manitoba, Winnipeg, Manitoba, Canada

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The purpose of this study was to determine if the monosynaptic reflex (MSR) of the tibial (extensor) and peroneal (flexor) nerves, by determining the basic and rhythmic properties of extensor and flexor motoneurons, and by recording extracellular Ia field potentials of the tibial and peroneal nerves in the in vivo adult decerebrate rat in both spinal intact and acute spinalized preparations. In the intact spinal preparation, the tibial and peroneal MSR amplitude significantly increased compared with baseline in response to quipazine, with no difference between nerves (P < 0.05). In the spinalized preparation, the MSR was significantly increased in both the tibial and peroneal nerves with the latter increasing more than the former (5.7 vs. 3.6 times; P < 0.05). Intracellular motoneuron experiments demonstrated that the increase in input resistance, afterhyperpolarization amplitude, and the firing rate at a given current injection increased in motoneurons following quipazine administration with no differences between extensor and flexor motoneurons. Both the tibial and peroneal nerve extracellular Ia field potentials increased with the peroneal demonstrating a significantly greater increase (7 vs. 38%; P < 0.05) following quipazine. It is concluded that in the intact spinal preparation quipazine does not have a differential effect on flexor or extensor motor output. However, in the acute spinalized preparation, quipazine preferentially affects the flexor MSR compared with the extensor MSR, likely due to the removal of a descending tonic inhibition on flexor Ia afferents.

The neuromodulating effects of serotonin (5-HT) on motor output are well established (Schmidt and Jordan 2000). Of interest, the 5-HT1 and 5-HT2 receptor (R) families are important for modulating motor output through inhibition and excitation of interneurons, afferent feedback, and motoneuron discharge. Activation of the 5-HT1R results in motoneuron depolarization, increased input resistance and discharge rate (Wang and Dun 1990; Elliott and Wallis 1992; Harvey et al. 2006; White and Fung 1989), whereas activation of the 5-HT2R causes hyperpolarization of the resting membrane potential and decreased input resistance (Wang and Dun 1990). It has been shown that when 5-HT or 5-HT2R agonists are applied on the dendrites of the motoneuron, plateau potentials are facilitated and when 5-HT or 5-HT1AR agonists are applied at the perisomatic region, inhibition of motoneuron output occurs, leading to the notion that 5-HT1ARs are located on the dendrites and 5-HT2Rs are located on the perisomatic region (see Perrier and Cotel 2008). As well, the effects of 5-HT on the monosynaptic reflex (MSR) are well documented. Similar to the motoneuron, activation of the 5-HT2R facilitates the MSR (Miller et al. 1996; Machacek et al. 2001; Shay et al. 2005; Hasegawa and Ono 1996; Gajendiran 2008) and activation of the 5-HT1AR inhibits the MSR (Hasegawa and Ono 1996; Gajendiran 2007; Nagano et al. 1988; Hedo et al. 2002).

5-HT is of interest because indirect evidence suggests that this neuromodulator may have a different effect on flexor and extensor motoneurons, in particular with regards to the ability of 5-HT to induce plateau potentials. Plateau potentials are mediated by both the sodium and calcium persistent inward currents (NaPIC and CaPIC), both of which have been shown to be directly modulated by the 5-HT2R on the motoneuron (Harvey et al. 2006; Perrier and Houngaard 2003; Murray et al. 2011). It was suggested by Houngaard et al. (1988) that in the intact spinal cord, the ability to generate plateau potentials may favor extensor motoneurons compared with flexor motoneurons. Recently, in the in vitro neonate rat preparation, Cotel et al. (2009) showed that extensor motoneurons, but not flexor motoneurons, were able to generate self-sustained firing. The difference between extensor and flexor motoneurons to generate PICs may be due to differences in the 5-HT2R activation or distribution, as immunohistological staining for the 5-HT2R has shown a greater staining in the soleus motoneuron pool compared with that of the extensor digitorium longus (EDL; Vult and Lomo 2005).

If the ability to generate PICs is greater in extensor motoneurons and it is known that the 5-HT2R activates both the CaPICs and NaPICs, then one could suggest that extensor and flexor motoneurons are potentially under different modulations from 5-HT. Following an acute spinalization, the ability to generate PICs is lost and 5-HT has been shown to restore or generate PICs (Houngaard et al. 1988). The purpose of this study was to investigate if the MSR of the tibial and peroneal nerves, as well as the rhythmic and basic properties of identified flexor and extensor motoneurons, responds differently to a supraspinal injection of the 5-HT2R agonist quipazine in the adult decerebrate rat. This was investigated in both spinal intact and acute spinalized preparations.
METHODS

Animal Care

All animal treatment, surgical, and experimental procedures were in accordance with the guidelines of the Canadian Council for Animal Care and approved by the University of Manitoba Animal Ethics Committee.

Adult female Sprague-Dawley rats weighing 275–350 g obtained from the University of Manitoba were used for all experiments described. The rats were housed in groups of three in plastic cages situated in an environmentally controlled room maintained at 23°C with a 12:12-h light-dark cycle. The rats had unlimited access to water and rat chow throughout the experiment period.

General Surgical Preparation

Surgical anesthesia was induced in 5% isofluorane mixed with 100% oxygen and maintained at 2–2.5% isofluorane mixed with 100% oxygen once a tracheotomy was performed. A tracheal tube was inserted to ventilate (Harvard Apparatus, St. Laurent, QC, Canada) the rat on pure oxygen at a tidal volume of 2 ml with a ventilation rate of ~48–60 strokes per min.Expired carbon dioxide levels were measured (Capstar 100 CO₂ analyzer; CWE, Androme, PA) and maintained at 3–4%. Once the tracheal tube was inserted the following procedures were performed. A catheter was inserted into the carotid artery to monitor mean arterial pressure (Pressure Monitor BP-1; World Precision Instruments, Saratosa, FL), which was maintained between 80 and 100 mmHg. As well, a constant infusion of a dextrose/saline solution delivered at 0.9 ml/h was administered through the carotid catheter. The left hindlimb tibial and peroneal nerves were dissected away from the musculature and isolated for mounting of electrodes, and the back musculature was dissected free from the vertebrae. The rat was then moved to a stereotaxic frame where the head, thoracic and lumbar vertebrae, and left foot were immobilized. A T12 to L3 laminectomy was performed to expose the lumbar spinal segments L2-L5. The dura mater was incised, and the large dorsal roots (L3 and L4) were cut and, depending on the experiment protocol, were either mounted for stimulation (MSR) or brushed to the side of the spinal cord (motoneuron intracellular recording). Back and left hindlimb pools were then prepared and were filled with mineral oil. The rectal temperature of the rat was monitored and maintained at 37°C using a Homeothermic Blanket Control Unit and UV lamp (Harvard Apparatus). A craniotomy was performed to limit respiratory related movements. The transection was performed to limit respiratory related movements.

Acute Spinalization

In half of our MSR and intracellular motoneuron recording experiments, an acute complete spinal transection was performed to eliminate descending input to the spinal cord. The transection was performed by dissecting away the back musculature and performing a laminectomy at either C4 (MSR experiments) or T8 (intracellular experiments). The spinal cord was then transected with fine forceps and suction, and the 2-mm incision was then packed with gelfoam. The differences in the location of the spinal transection were due to difficulty in providing a stable preparation for intracellular recording with a C4 spinal transection (i.e., increased movement and large blood pressure increases).

MSR Recordings

Eight female Sprague-Dawley rats were used for the MSR experiments in the acute spinalized preparation, and four rats were used for the MSR experiments with the spinal cord intact. The MSR was elicited by stimulation of the L4/L5 dorsal roots, and the electromyogram (EMG) activity of the tibial (extensor) and peroneal (flexor) nerves was recorded with bipolar Ag-CI hook electrodes. The ENG signal was collected with custom software (capture, SCRC) differentially amplified (10,000 times; band-pass filtered 50–1,000 Hz), digitized at 10 kHz (12-bit A/D), and stored for offline processing. ENG activity was recorded at threshold (T), 1.25T, 1.5T, 2T, and 3T. Threshold for the MSR was defined as the smallest current producing a detectable extracellular compound action potential volley at the cord dorsum recording electrode. The dorsal roots were stimulated at 4 Hz (0.1-ms duration). A minimum of 85 responses were collected and averaged at each time point. A minimum of four baseline averages were taken 5 min apart to ensure a reliable baseline measure. After the baseline measurements were collected, an intraperitoneal injection of quipazine in saline (15 mg/kg) was administered and the MSR was recorded every 5 min for 2 h duration in four experiments. Since it was noted that the largest MSR increase occurred between 5 and 20 min, the MSR response was recorded every 5 min for 60 min in the remaining experiments.

For analysis, the ENG recordings were full-wave rectified and averaged (minimum 85 responses per stimulation). The area under the MSR response was calculated at 1, 1.5, 2, and 2.5 ms following the initiation of the ENG response. The area under the MSR response was then normalized to the average baseline response for each nerve.

Intracellular Recordings

Thin-walled 1.0-mm glass microelectrodes (World Precision Instruments) were pulled to an impedance of 10 MΩ (Kopf Vertical Pipette Puller; David Kopf Instruments, Tujunga, CA) and filled with 2 M potassium citrate. The electrode was advanced through the spinal cord at 6-to 10-μm steps using the Burleigh inchworm microdrive system (Burleigh Instruments). Both the tibial and peroneal nerves were stimulated with bipolar silver electrodes at a frequency of 2 Hz, while the microelectrode was advanced through the spinal cord, and field potentials identified as originating from either extensor motoneurons or flexor motoneurons were continuously monitored. A membrane potential change ≥55 mV in the spinal transected and ≥60 mV in the spinal intact rats, accompanied by an antidromic action potential spike height of ≥55 or 60 mV, respectively, was used to determine successful impalement of a motoneuron. Once the motoneuron was identified and the resting membrane potential stabilized, basic and rhythmic properties were measured using an intracellular amplifier system (Axoclamp 2B; Axon Instruments, Molecular Devices). Only motoneurons, in which both the baseline and postdrug values were recorded in the same motoneuron, were used for analysis.

Measurements of Motoneuron Properties

Basic properties. Rheobase, defined as the minimum current required to elicit an action potential 50% of the time, was recorded in response to a 50-ms intracellular depolarizing pulse in discontinuous current clamp mode (5–10 kHz). Resting membrane potential and voltage threshold, defined as the membrane potential at which depolarization increased at ≥10 V/s (Power et al. 2010), was also determined from these recordings. Input resistance was determined from an averaged membrane response to ~60, 1-nA hyperpolarizing pulses each lasting 150 ms. Afterhyperpolarization amplitude (AHP<sub>amp</sub>) and afterhyperpolarization half decay time (AHP<sub>decay</sub>) were measured from an average of ~40 orthodromic spikes evoked by 0.5-ms supramaximal intracellular current pulses recorded in bridge mode.

Rhythmic properties. Following successful recording of the basic motoneuron properties, the motoneuron was subjected to a slow triangular current ramp (5-s rise, 5-s decay) in discontinuous current clamp mode to determine the active firing properties of the motoneuron. The peak current amplitude of the ramp for each cell was...
determined by trial and error such that the cell would begin firing within 1–2 s of the peak of the ramp, and this amplitude was maintained for the quipazine trial. From the triangular current injection, the following measurements were obtained: 1) slope of frequency-current (F-I) relationship (Hz/nA); 2) instantaneous firing frequency at recruitment and derecruitment; 3) maximum firing frequency achieved on ramp current injection; and 4) the number of spikes achieved on ramp current injection. After completion of a successful intracellular recording, the microelectrode was back out of the motoneuron and the extracellular voltage was recorded to correct membrane voltages recorded intracellularly.

Ia Extracellular Field Potential Recordings

It was noted following the intracellular motoneuron experiments that no difference existed between flexor and extensor motoneurons in response to quipazine (see RESULTS). Therefore, in several experiments (n = 7) extracellular field potentials were recorded pre- and postquipazine injection to determine if presynaptic modulation affected the response of the MSR to quipazine. Recording extracellular field potentials allowed for comparison of pre- and postquipazine values of both the peroneal and tibial nerve in the same experiment. In the Ia extracellular field potential experiments, a low resistance microelectrode (∼1–2 MΩ) was advanced through the spinal cord in 6-µm steps until a location was found with the maximum tibial and peroneal Ia field potentials (minimum depth of 700 µm to ensure microelectrode was in the ventral-lateral horn).

Once the optimal location was determined, stimulation of the tibial and peroneal nerves was set at 1.25T to ensure only the Ia extracellular potentials were recorded via the microelectrode. An average of 60 stimulus responses from each nerve were recorded and analyzed. The negative deflection of both the tibial and peroneal nerve were compared pre- and postquipazine injection for a 20-min period. Recordings at 1.5T, 2T, and 3T were also made to ensure saturation of the field potential had not occurred at 1.25T.

Statistics

The data was subjected to a two-way ANOVA with repeated-measures (RM ANOVA) analysis, with the factors of motoneuron type (flexor vs. extensor) and drug (pre- vs. postquipazine) and time being a repeated measure unless otherwise stated. A Tukey’s post hoc analysis was used when a significant interaction was found. Significance was determined at an alpha level of P < 0.05. Data are presented as means (SD) throughout the text.

RESULTS

In total, eight acute spinalized and four spinal intact rats were used for MSR recordings and seven acute spinalized rats were used for the Ia extracellular field potential experiments. A total of 15 motoneurons were recorded from the spinal intact rat (7 extensors and 8 flexors) and eight motoneurons (4 extensors and 4 flexors) in the acute spinalized rat. For the intracellular experiments, only motoneurons in which both prequipazine and stable postquipazine recordings were made were selected for analysis. Furthermore, to ensure that a stable intracellular penetration occurred during the recording phase, only motoneurons that fired rhythmically and had a stable resting membrane potential (± 5 mV) were used for analysis.

Effect of Quipazine on the MSR

Figure 1 demonstrates the average response obtained from the eight acute spinalized rats (Fig. 1A) and a representative individual response to quipazine (Fig. 1B). Data represent the area under the response collected at 1.5 ms at 2T. The MSR responses at the various stimulus strengths and time epochs described in METHODS produced similar results as described and presented in Fig. 1 (peroneal MSR response was significantly larger than the tibial MSR response). The results are normalized to their own baseline value. The coefficient of variation of the baseline values was 16% (range: 1–22%) for the tibial nerve and 19% (range: 9–27%) for the peroneal nerve. A two-way RM ANOVA indicated a significant drug response and a significant motoneuron type effect (P < 0.05). Post hoc analysis revealed that the area under the peroneal (flexor) MSR response was increased significantly larger compared with the area under the tibial (extensor) MSR following

![Fig. 1. Effect of quipazine on the monosynaptic reflex (MSR).](http://jn.physiology.org/)

* Significant difference between the peroneal and tibial nerve (5.7- vs. 3.6-fold increase, respectively, P < 0.05). Data are means ± SD (n = 8). B: representative rectified electroneurogram (ENG) recordings from the tibial and peroneal nerves from one rat pre- and 5- and 60-min postquipazine. Inset: raw ENG recordings. Responses shown are from 2× threshold.

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quipazine. No interaction of time was found. The average increase in the area under the MSF following quipazine injection in the tibial and peroneal nerve was 3.6- and 5.7-fold, respectively. Although large variability exists in the responsiveness of the MSF to quipazine, it is important to note that in all eight acute spinalized rats from which the MSF was recorded, the increase in the peroneal MSF was always greater than that of the tibial MSF.

To determine the influence of descending inputs, the MSF of spinal intact rats were also evaluated. In these four experiments, the MSF was recorded similarly to the spinalized animals. In the spinal intact animals, the average increase was 1.8- and 2.0-fold for the tibial and peroneal MSF, respectively (data not shown). A significant drug effect was found ($P < 0.05$) with no difference between nerves. Therefore, the difference in the increase in the tibial and peroneal MSF following quipazine requires the removal of a descending tonic influence on the peroneal reflex pathway.

In two MSF experiments after collecting the MSF response to quipazine, ketanserin (0.45 mg/kg) was administered to determine if the results seen were likely due to the activation of the 5-HT$_{2A}$R (data not shown). Following the injection of ketanserin, both the tibial and peroneal MSF amplitudes reached steady state at 50% of their baseline values indicating that the increase in the tibial and peroneal MSF following quipazine is likely due to the activation of the 5-HT$_{2A}$R.

**Effect of Quipazine on Flexor and Extensor Motoneuron Properties**

In the spinal intact preparation, a two-way RM ANOVA revealed a significant main effect of drug (enhanced excitability of motoneurons) for several properties but no significant difference for type (flexors vs. extensors). Motoneuron basic and $I$-$I$ relationship properties are summarized in Table 1. The basic and rhythmic properties of flexor and extensor motoneurons were similar at baseline and demonstrated a similar responsiveness to quipazine.

Overall, motoneuron excitability increased after quipazine injection. For both flexor and extensor motoneurons, rheobase decreased by $\sim 30\%$ following quipazine injection. Average input resistance at baseline for flexor and extensor motoneurons was 1.9 ± 0.9 and 2.1 ± 1.0 MΩ, respectively. Following quipazine injection, input resistance increased 60% (3.1 ± 1.9 MΩ) for flexor motoneurons and 20% (2.5 ± 0.8 MΩ) for extensor motoneurons ($P < 0.05$). The AHP$_{amp}$ increased for both flexor (0.9 ± 0.4 to 1.4 ± 0.7 mV) and extensor (2.0 ± 1.0 to 2.8 ± 2.1 mV) motoneurons ($P < 0.05$). Quipazine did not have an effect on AHP$_{decay}$ resting membrane potential, or voltage threshold.

The increase in the excitability following quipazine injection was evident when examining motoneuron firing properties from a ramp current injection (see properties 7–12 in Table 1; Fig. 2). Similar to the basic properties, no significant differences were seen between flexor and extensor motoneurons pre- or postquipazine injection. Current at spike recruitment for flexor motoneurons significantly decreased 28% from 10.1 to 7.3 nA and 45% from 8.5 to 4.8 nA for extensor motoneurons ($P < 0.05$). Current at spike derecruitment decreased from 7.8 nA to 3.7 nA (33%) and 8.2 to 5.0 nA (40%) for flexor and extensor motoneurons respectively ($P < 0.05$). The maximum ramp firing rate increased by 40% for flexor motoneurons and 30% for extensor motoneurons ($P < 0.05$). The slope of the $I$-$F$ relationship following quipazine was shifted to the left for both flexor and extensor motoneurons with no changes in the slope.

The motoneurons recorded from the acute spinal transected group (Table 2) following quipazine (four flexors, four extensors) showed a similar enhancement of rhythmic firing as measured by current ramp injections to that was seen in the spinal intact group. Current at spike recruitment for flexor motoneurons significantly decreased 42% from 14.2 to 8.2 nA and 23% from 11.3 nA to 8.6 nA for extensor motoneurons ($P < 0.05$). Current at spike derecruitment decreased from 14.7 to 10.25 nA (30%) and 11.4 to 8.8 nA (23%) for flexor and extensor motoneurons respectively ($P < 0.05$). The $I$-$F$ relationship was shifted to the left for both flexor and extensor motoneurons with no change in the slope.

**Effect of Quipazine on Flexor and Extensor Ia Extracellular Field Potentials**

In seven acute spinalized rats, the extracellular Ia field potentials of both the tibial and peroneal nerves were measured pre- and postquipazine (Fig. 3). A two-tailed Student’s $t$-test revealed a significant difference in the increase of the Ia

### Table 1. Passive and active motoneuron properties of spinal intact rats

<table>
<thead>
<tr>
<th>Motoneuron Property</th>
<th>Flexor Motoneurons</th>
<th>Extensor Motoneurons</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prequipazine</td>
<td>Postquipazine</td>
<td>Prequipazine</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>$-66.8 \pm 5.5$</td>
<td>$-68.5 \pm 3.4$</td>
<td>$-65.3 \pm 2.2$</td>
</tr>
<tr>
<td>Vth, mV</td>
<td>$-47.5 \pm 8.5$</td>
<td>$-48.4 \pm 8.4$</td>
<td>$-44.5 \pm 5.5$</td>
</tr>
<tr>
<td>Rheobase current, nA</td>
<td>8.0 ± 4.3</td>
<td>5.6 ± 2.8</td>
<td>7.4 ± 3.4</td>
</tr>
<tr>
<td>IR, MΩ</td>
<td>1.9 ± 0.9</td>
<td>3.1 ± 1.9</td>
<td>2.1 ± 1.0</td>
</tr>
<tr>
<td>AHP amplitude, mV</td>
<td>0.9 ± 0.4</td>
<td>1.4 ± 0.7</td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td>AHP 1/2 decay time, ms</td>
<td>17.9 ± 9.7</td>
<td>16.8 ± 9.8</td>
<td>17.8 ± 3.9</td>
</tr>
<tr>
<td>Current at spike recruitment, nA</td>
<td>10.1 ± 5.8</td>
<td>7.3 ± 4.8</td>
<td>8.0 ± 4.7</td>
</tr>
<tr>
<td>Current at spike derecruitment, nA</td>
<td>10.4 ± 6.0</td>
<td>7.8 ± 5.6</td>
<td>7.5 ± 4.8</td>
</tr>
<tr>
<td>Instantaneous frequency at spike recruitment, Hz</td>
<td>25.4 ± 13.9</td>
<td>21.6 ± 4.1</td>
<td>21.5 ± 14.7</td>
</tr>
<tr>
<td>Instantaneous frequency at spike derecruitment, Hz</td>
<td>21.5 ± 7.4</td>
<td>17.9 ± 8.5</td>
<td>15.0 ± 7.6</td>
</tr>
<tr>
<td>Max firing frequency on ramp current injection, Hz</td>
<td>47.9 ± 16.7</td>
<td>67.9 ± 19.3</td>
<td>37.8 ± 22.7</td>
</tr>
<tr>
<td>Number of spikes on ramp current injection (#)</td>
<td>52.5 ± 39.8</td>
<td>150.8 ± 57.3</td>
<td>45.0 ± 18.1</td>
</tr>
<tr>
<td>Slope, Hz/nA</td>
<td>9.0 ± 4.5</td>
<td>8.4 ± 4.6</td>
<td>7.3 ± 2.1</td>
</tr>
</tbody>
</table>

Data are means ± SD for each group; $n = 7$ flexor motoneurons and 8 extensor motoneurons. Summary of basic and active motoneuron properties in the spinal intact preparation. RMP, resting membrane potential; Vth, voltage threshold; IR, input resistance; AHP, afterhyperpolarization; ePIC, estimated persistent inward current. *Two-way ANOVA revealed a significant drug effect ($P < 0.05$). No significance of motoneuron type was found.

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extracellular field potential amplitudes following quipazine ($P < 0.05$). The average increase for the tibial and peroneal Ia extracellular field potentials was 7 and 38%, respectively.

**DISCUSSION**

The most important finding of this study is that flexor and extensor motor output is differentially modulated by quipazine, a 5-HT$_2$R agonist in the acute spinal transected model. Furthermore, to our knowledge, this was the first time that a serotonergic agonist was used in an in vivo decerebrate adult rat preparation in which both the flexor MSR and the extensor MSR were examined simultaneously. In doing so, it was shown that quipazine enhanced the flexor MSR to a greater extent than the extensor MSR. When the MSR, MN properties and Ia extracellular field potentials data are considered together, it is evident that the enhanced flexor MSR was due to presynaptic modulation and not due to differences in motoneuron modulation. The difference in presynaptic modulation is believed to result from the removal of a descending inhibition on the flexor Ia afferents as the difference was only seen in the spinal transected preparation.

**Fig. 2.** Rhythmic firing of a motoneuron pre- and postquipazine. $A$: example of increased rhythmic firing of a motoneuron following quipazine. Current at recruitment and derecruitment decreased by 30% and maximum firing rate increased by 36%. $B$: shift to the left in the slope of frequency-current ($F$-$I$) relationship was seen for all motoneurons, with no change in $F$-$I$ slope. Black, prequipazine; grey, postquipazine.

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**Table 2.** Rhythmic motoneuron properties of acute spinalized rats

<table>
<thead>
<tr>
<th>Motoneuron Property</th>
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<th>Extensor Motoneurons</th>
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<td>Current at spike recruitment, nA</td>
<td>14.2 ± 4.6</td>
<td>8.2 ± 2.4</td>
</tr>
<tr>
<td>Current at spike derecruitment, nA</td>
<td>14.7 ± 5.0</td>
<td>10.25 ± 4.0</td>
</tr>
<tr>
<td>Instantaneous frequency at spike recruitment, Hz</td>
<td>20.8 ± 3.0</td>
<td>23.0 ± 3.0</td>
</tr>
<tr>
<td>Instantaneous frequency at spike derecruitment, Hz</td>
<td>18.7 ± 4.8</td>
<td>23.5 ± 9.4</td>
</tr>
<tr>
<td>Max firing frequency on ramp current injection, Hz</td>
<td>35.8 ± 7.6</td>
<td>70.4 ± 12.7</td>
</tr>
<tr>
<td>Number of spikes on ramp current injection (#)</td>
<td>36.5 ± 21.3</td>
<td>178.0 ± 81.9</td>
</tr>
<tr>
<td>Slope, Hz/nA</td>
<td>4.5 ± 2.9</td>
<td>4.0 ± 0.9</td>
</tr>
</tbody>
</table>

Data are means ± SD for each group; $n = 4$ flexor motoneurons and 4 extensor motoneurons. Summary of rhythmic motoneuron properties in the acute spinalized preparation. *Two-way ANOVA revealed a significant drug effect ($P < 0.05$). No significance of motoneuron type was found.
the spinal intact animals, with quipazine having a minimal effect on the enhanced MSR was larger in the flexor compared with flexor MSR. However, the opposite was seen in which the extensor MSR would be preferentially modulated compared with the extensor Ia extracellular field potentials after quipazine administration.

The responses of motoneurons to quipazine are similar to what has been noted previously in the literature but also differ slightly. The increased input resistance (Wang and Dun 1990; Elliott and Wallis 1992; Hsiao et al. 1997) and firing rate (White and Neuman 1983; White 1985) shown here are consistent with previous findings that the activation of 5-HT$_{2A}$R enhances the overall excitability of the motoneuron (Harvey et al. 2006; Miller et al. 1996; Perrier and Hounsgaard 2003). However, we found no change in resting membrane potential (VanderMaelen and Aghajanian 1980; White and Fung 1989; Wang and Dun 1990; Elliott and Wallis 1992; Hsiao et al. 1997; Harvey et al. 2006) or voltage threshold (Fedirchuk and Dai 2004), and the AHP$_{amp}$ increased (White and Fung 1989).

There are several explanations that could account for these differences. First, a 5-HT$_{2A}$R agonist was used rather than 5-HT. Harvey et al. (2006) noted that 5-HT administration caused depolarization and changes in membrane potential, whereas the specific 5-HT$_{3A}$R agonist (DOI) did not change resting membrane potential or input resistance in the spinalized rat preparation. Second, it appears that age influences the effect of 5-HT responses following acute spinalization would be the removal of a descending inhibition, most likely the removal of descending inhibition on the flexor Ia terminals as previous literature has shown that flexor reflex afferents (Holmqvist and Lundberg 1961) and Ia afferents are under descending tonic inhibition (Quevedo et al. 1993). Our results support the idea that flexor and extensor Ia afferents are under different modulation from supraspinal sources and also that a difference in modulation exists between flexors and extensors.

Enhanced Excitability of Motoneuron Properties

The responses of motoneurons to quipazine are similar to what has been noted previously in the literature but also differ slightly. The increased input resistance (Wang and Dun 1990; Elliott and Wallis 1992; Hsiao et al. 1997) and firing rate (White and Neuman 1983; White 1985) shown here are consistent with previous findings that the activation of 5-HT$_{2A}$R enhances the overall excitability of the motoneuron (Harvey et al. 2006; Miller et al. 1996; Perrier and Hounsgaard 2003). However, we found no change in resting membrane potential (VanderMaelen and Aghajanian 1980; White and Fung 1989; Wang and Dun 1990; Elliott and Wallis 1992; Hsiao et al. 1997; Harvey et al. 2006) or voltage threshold (Fedirchuk and Dai 2004), and the AHP$_{amp}$ increased (White and Fung 1989).

There are several explanations that could account for these differences. First, a 5-HT$_{2A}$R agonist was used rather than 5-HT. Harvey et al. (2006) noted that 5-HT administration caused depolarization and changes in membrane potential, whereas the specific 5-HT$_{3A}$R agonist (DOI) did not change resting membrane potential or input resistance in the spinalized rat preparation. Second, it appears that age influences the effect of 5-HT on the motoneuron. For example, embryonic and neonatal rat motoneurons depolarize in response to 5-HT$_{1A}$ and 5-HT$_{2A}$R activation (Ziskind-Conhaim et al. 1993; Takahashi and Berger 1989), whereas in the juvenile rat, the 5-HT$_{1A}$R and the 5-HT$_{3A}$R activation results in hyperpolarization and depolarization of the motoneuron respectively (Talley et al. 1997).
This age-dependent effect is also highlighted by the differential effect 5-HT has on the AHP\textsubscript{amp}. In the neonatal rat, 5-HT\textsubscript{1A}R activation results in the suppression of the AHP\textsubscript{amp}, whereas in the juvenile rat, the AHP\textsubscript{amp} is unaffected (Talley et al. 1997). As well, in the adult in vivo cat preparation, 5-HT and the 5-HT\textsubscript{2}R agonist DOI, increased the AHP\textsubscript{amp} (Zhang 1991), which is consistent with our finding that quipazine increased the AHP\textsubscript{amp} in the adult in vivo rat preparation.

**Differences in Flexor and Extensor Motoneurons**

Contrary to what was hypothesized, no differences in modulation of flexor and extensor motoneurone properties or discharge rates occurred following quipazine injection in both the spinal intact and acute spinalized preparations. Based on previous studies in which differences in the ability of extensor and flexor motoneurones to activate PICs in the adult in vivo cat (Hougsgaard et al. 1988) or self-sustained firing in the in vitro neonatal rat preparation (Cotel et al. 2009), it was unexpected to see no difference in modulation of the motoneurone type with quipazine, as 5-HT activates both the NaPIC (Harvey et al. 2006) and CaPIC (Perrier and Hounsgaard 2003). However, it has been recently shown that the 5-HT\textsubscript{2B/C} receptors are responsible for mediating the CaPIC (Murray et al. 2011). As well, immunohistological staining for the 5-HT\textsubscript{2AR} has shown that identified slow extensor motoneurones (soleus motoneurones) express a larger number of the 5-HT\textsubscript{2AR} compared with fast flexor motoneurones (Vult and Lomo 2005). However, as the authors suggest, this may be due to differences between fast and slow motoneurones. As well, a previous study that examined spontaneous alpha- and gamma-motoneurone discharge rates in response to 5-hydroxytryptophan found no difference between extensor filament (gastrocnemius) and flexor filaments (semitendinosus) (Mysslnski and Anderson 1978). Our results seem to support this initial finding, which suggests that no differences between flexor and extensor motoneuron discharge rate and excitability exist in both the spinal intact and acute spinalized preparation.

**Conclusions**

This study concludes that, in the spinal intact state, quipazine does not have a differential effect on the MSR, due to a descending tonic inhibition on the flexor reflex pathway. In the acute spinal preparation, with the descending tonic inhibition removed, quipazine is found to have a preferential effect on the flexor MSR compared with the extensor MSR. The difference in excitation is believed to be the result of presynaptic modulation as evident by a consistently larger increase in the peroneal extracellular I\textsubscript{a} field potential compared with the tibial I\textsubscript{a} field potential and by the lack of a differential effect of quipazine on the biophysical or rhythmic firing properties of the flexor and extensor motoneurones.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


