Please cite this article in press as: McClelland TJ, Parker D. Inverse modulation of motor neuron cellular and synaptic properties can maintain the same motor output. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.07.047

Neuroscience xxx (2017) xxx–xxx

INVERSE MODULATION OF MOTOR NEURON CELLULAR AND SYNAPTIC PROPERTIES CAN MAINTAIN THE SAME MOTOR OUTPUT

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Abstract—Although often examined in isolation, a single neuromodulator typically has multiple cellular and synaptic effects. Here, we have examined the interaction of the cellular and synaptic effects of 5-HT in the lamprey spinal cord. 5-HT reduces the amplitude of glutamatergic synaptic inputs and the slow post-spike afterhyperpolarization (sAHP) in motor neurons. We examined the interaction between these effects using ventral root activity evoked by stimulation of the spinal cord. While 5-HT reduced excitatory glutamatergic synaptic inputs in motor neurons to approximately 60% of control, ventral root activity was not significantly affected. The reduction of the sAHP by 5-HT increased motor neuron excitability by reducing spike frequency adaptation, an effect that could in principle have opposed the reduction of the excitatory synaptic input. Support for this was sought by reducing the amplitude of the sAHP by applying the toxin apamin before 5-HT application. In these experiments, 5-HT reduced the ventral root response, presumably because the reduction of the synaptic input now dominated. This was supported by computer simulations that showed that the motor output could be maintained over a wide range of synaptic input values if they were matched by changes in postsynaptic excitability. The effects of 5-HT on ventral root responses were altered by spinal cord lesions: 5-HT significantly increased ventral root responses in animals that recovered good locomotor function, consistent with a lesion-induced reduction in the synaptic effects of 5-HT, which thus biases its effects to the increase in motor neuron excitability. © **2017 Published by Elsevier Ltd on behalf of IBRO.**

Key words: spinal cord, neuromodulation, 5-HT, lamprey, spinal cord injury.

INTRODUCTION

Neuromodulators confer behavioral flexibility by modifying the functional properties of hard-wired circuits. A single neuromodulator typically affects several cellular and synaptic properties (Buonomano and Merzenich, 1998; Harris-Warrick and Johnson, 2010). These effects can be synergistic (e.g. an increase in excitatory and decrease in inhibitory inputs) or antagonistic (e.g. increased presynaptic transmitter release combined with reduced postsynaptic sensitivity; see Harris-Warrick and Johnson, 2010; Lillvis and Katz, 2013). The interactive effects of different modulators (Brezina, 2010) and multiple effects of a single modulator, together with the potential for concentration, time, and state-dependent influences (Levitan and Levitan, 1988; Power and Sah, 2008; Parker, 2015), provide the potential for considerable flexibility of modulatory effects.

5-HT is among the best studied neuromodulators. Its effects have been studied in detail in the spinal cord of several animals where both sensory inputs and motor outputs are affected (Schmidt and Jordan, 2000; Jordan et al., 2008). In the lamprey, 5-HT slows the frequency of fictive locomotor activity (Harris-Warrick and Cohen, 1985) as it does in most other systems (Jordan et al., 2008; but see Sillar et al., 1998), although its effects differ somewhat in intact animals (Kemnitz et al., 1995; Becker and Parker, 2015). This network effect has been linked to a 5-HT-mediated reduction of a calcium-dependent potassium conductance underlying the slow afterhyperpolarization (sAHP) following action potentials, which can influence spike frequency adaptation and increase neuronal excitability (Wallen et al., 1989). While this effect has been claimed to account for the changes in fictive and simulated network activity (e.g. Grillner et al., 1995, 2005; Hellgren et al., 1992), this conclusion is complicated by the wide range of cellular and synaptic effects of 5-HT. These include a hyperpolarization of the resting membrane potential, a reduction of glutamatergic synaptic transmission, and an increase or decrease in inhibitory inputs (Biró et al., 2006; Buchanan and Grillner, 1991; Harris-Warrick and Cohen, 1985; Parker and Grillner, 1999, 2000; Parker, 2006; Svensson et al., 2001).

We have shown that the cellular and synaptic effects of 5-HT differ after spinal cord lesions (Becker and Parker, 2015). We aimed to determine the mechanisms underlying this change in 5-HT modulation by examining 5-HT effects on ventral root activity evoked by spinal cord stimulation in lesioned and unlesioned animals. This approach was chosen as it offers a simpler assay for 5-HT effects than fictive locomotion, where the marked variability of the fictive output complicates analyses (Wallen and Williams, 1984; McClellan, 1990; Parker and Srivastava, 2013). However, we found that 5-HT did not reduce the ventral root response in unlesioned animals as expected, despite a significant reduction of glutamatergic synaptic inputs. Here, we provide evidence that inverse effects of 5-HT on neuronal excitability and synap-

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http://dx.doi.org/10.1016/j.neuroscience.2017.07.047
0306-4522/

tic inputs can maintain the same motor neuron output, providing an example of interactive cellular and synaptic effects by a single modulator (Harris-Warrick and Johnson, 2010). We also show that this interaction is altered after spinal cord lesions, consistent with a change in neuromodulatory effects after spinal injury.

EXPERIMENTAL PROCEDURES

Juvenile adult lampreys (Pertomyzon marinus) were obtained from commercial suppliers (Acme Lamprey Company, Maine, USA). All experiments were conducted under license of the UK Home Office (Animals Scientific Procedures Act 1986) and the approval of the local ethics committee. All attempts were made to minimize the number of animals used and any suffering.

Animals were anesthetized with MS-222 (300 mg/mL, pH adjusted to 7.4) and the spinal cord and notochord were removed from the trunk region (i.e., between the last gill and the start of the dorsal fin) in oxygenated lamprey Ringer at 4 °C (Ringer contents: 138 mM NaCl, 2.1 mM KCl; 1.8 mM CaCl2; 2.6 mM MgCl2; 4.0 mM D-(+)-glucose; 2.0 mM HEPES; 0.5 mM L-glutamine, bubbled with O2 and adjusted to pH 7.4 with 1 M NaOH). The spinal cord and notochord were pinned to a Sylgard lined chamber at 10 °C and superfused with lamprey Ringer at 10 °C.

The spinal cord was left attached to the notochord to prevent potential damage to the ventral roots upon isolation of the cord. Ventral root activity was evoked using an extracellular stimulating electrode placed on the dorsal surface of the spinal cord to cover the lateral tract on one side. 5-HT consistently reduces the amplitude of reticulospinal inputs from descending axons in this tract (Buchanan and Grillner, 1991). Extracellular activity was recorded from an electrode placed on a ventral root 3–5 segments caudally to the stimulation electrode. A single stimulation of the dorsolateral tract to evoke a single or unpatterned burst of ventral root activity was given at 1.5–2 times the threshold needed to evoke a single ventral root spike: this stimulation was delivered ten times at a frequency of 0.1 Hz. The stimulation strength and frequency of delivery was not altered once the experiment started. The cord-evoked ventral root activity was quantified from the peak of the averaged rectified and integrated activity over a period of either 50 ms or 150 ms after the stimulation (Ullström et al., 1999; Cooke and Parker, 2009).

Single or paired intracellular recordings were made from motor neurons and spinal cord interneurons using thin walled micropipettes filled with 3 M potassium acetate and 0.1 M potassium chloride. Motor neurons were identified by recording orthodromic spikes in a ventral root following current injection into their somata. Excitability was examined by injecting 100-ms depolarizing current pulses (0.5–2.5 nA) into the cells using discontinuous current clamp (DCC; sampling frequency between 2 and 3 kHz). The sAHP was assessed from single action potentials evoked in motor neurons by 1-ms depolarizing current pulses using DCC. The sAHP amplitude was measured as the peak hyperpolarization that occurred >10 ms following the action potential. Synaptic inputs were evoked in motor neurons by stimulation of the dorsolateral column in the same way as used to evoke ventral root activity above. Unless stated otherwise, all cellular and synaptic properties in control and after 5-HT application were examined from a membrane potential of −65 mV maintained using current injection in DCC (5-HT could hyperpolarize cells by 1–2 mV). In some cases when the response of individual motor neurons was examined to cord stimulation, depolarizing current injection was needed so that the synaptic input caused the cell to spike. In these cases the effects of 5-HT were examined at the same membrane potential before and after 5-HT application.

To examine the effects of 5-HT after spinal cord lesions, animals received a complete transection of the spinal cord approximately 1cm behind the last gill (see Cooke and Parker, 2009). Animals were then left to recover for 8–10 weeks. By the end of this period the incision site had healed and most animals had recovered full locomotor function (McClellan, 1994). Animals were scored behaviorally on a six point scale that ranged from stages 1–2 (no recovery of locomotor function) to stages 5 or 6 (almost complete or complete recovery; see Cooke and Parker, 2009 for details). Once the swimming behavior had been scored the animal was anesthetized and the spinal cord removed for experiments as above.

Drugs were purchased from Sigma–Aldrich and applied to the isolated spinal cord by superfusion using a peristaltic pump. 5-HT (1–10 μM) was superfused for ten minutes after it had replaced the Ringer superfusing the cord (the time needed for the 5-HT solution to replace the normal Ringer solution was determined from the time needed for a dye to fill the bath). Because the initial response to stimulation could vary markedly in different experiments, all values were normalized to the mean of the control values. Statistical analyses were performed using t-tests or a one-way ANOVA with a Tukey post hoc test.

Model

A simple model was built in MATLAB using Simulink (Version 6.3 R14SP3). The simulation used a generic neuron to represent the motor neuron and a presynaptic neuron to represent the inputs stimulated extracellularly in this study.

The neuron block had a Na^+, delayed rectified K^+, leak K^+ current, and a calcium-dependent potassium channel (KCa). The resting potential for model neurons was -70 mV. The peak value of the action potential was +50 mV. Voltage-dependent Na^+ and K^+ channels were modeled using Hodgkin–Huxley equations:

$$I_{Na} = \overline{G_{Na}} m^3 h (V - E_{Na})$$

$$I_K = \overline{G_K} n^4 (V - E_K)$$

$$I_{leak} = G_{leak} (V - V_{rest})$$

in which m, h and n were defined as:

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