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Dopamine Pumping Up Spinal Locomotor Network Function

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Review of Picton et al.

Central pattern generator networks in the lumbar spinal cord of mammals control a variety of rhythmic movements of the hind limbs. Although these networks can function in isolation, modulatory inputs configure the network to create diversity and stability in network operation. Modulators have traditionally been thought to exert their influence on networks by altering cellular intrinsic properties and synaptic weight by changing ion channel permeability (Harris-Warrick and Marder, 1991; Marder and Calabrese, 1996; Harris-Warrick, 2011). More recent work is beginning to reveal that modulators also alter neuronal activity by acting on ion pumps that regulate neuronal excitability homeostatically (Bos et al., 2013; Ding et al., 2014). Picton et al. (2017) demonstrate that the electrogenic sodium-potassium ATPase pump not only plays a role in regulating locomotor network function in the developing mammalian spinal cord but is also under the modulatory control of dopamine.

The sodium pump acts homeostatically to maintain resting ionic gradients and uses ATP to pump three sodium ions out of the cell and two potassium ions into the cell.

The net effect of the pump on neuronal excitability is a hyperpolarizing outward current due to the asymmetric movement of sodium and potassium ions. Pump function is also enhanced in response to activity-dependent increases in intracellular sodium. Thus, the sodium pump not only maintains ion gradients homeostatically but also regulates neuronal excitability dynamically in response to activity. Dopamine has been well documented to alter sodium pump activity in the lungs, kidney, intestine (Zhang et al., 2013), and brain (Wu et al., 2007), but the direction and mechanisms vary depending on the organ system. For example, in alveolar epithelial cells of the lung, dopamine increases pump activity via second-messenger signaling to dephosphorylate and increase pump insertion into the cell membrane (Lecuona et al., 2000, 2006). This is different from bovine striatal neurons where dopamine receptors have been reported to form protein complex with the pump and can either increase or decrease pump activity (Hazelwood et al., 2008). In rhythmic motor networks of invertebrate (Tobin and Calabrese, 2005; Kueh et al., 2016) and aquatic vertebrate (Zhang and Sillar, 2012; Zhang et al., 2015) species, the pump keeps network excitability in check but its contribution to mammalian locomotor network function remains poorly understood despite reports of the pump being expressed ubiquitously within the spinal cord (Watts et al., 1991; Edwards et al., 2013). Therefore, Picton et al. (2017) explored the contribution of the sodium

pump to locomotor network function in neonatal mice.

The authors first evoked fictive locomotion in isolated spinal cords either neurochemically (by applying 5-HT, NMDA, and dopamine) or electrically (by stimulating dorsal-root nerves). Blocking the pump with ouabain dose-dependently increased the frequency of the neurochemically or electrically evoked locomotor rhythm. In contrast, increasing pump function with monensin slowed the rhythm. Importantly, the effect of ouabain on the locomotor rhythm was not elicited in the absence of dopamine, suggesting that the sodium pump only influences the rhythm when dopamine is present.

Two possibilities could account for this observation. First, the sodium pump may play a greater role when rhythms are slow, as they are in the presence of dopamine. Indeed, the contribution of the sodium pump to rhythmicity in the heart central pattern generator of the leech depends on the presence of an h-current (Kueh et al., 2016), which slows the rhythm. In line with this, the sodium pump also contributes to the generation of the slow disinhibited rhythm evoked by picrotoxin and strychnine in the isolated spinal cord (Ballerini et al., 1997; Darbon et al., 2003). This rhythm is not dopamine-dependent but is modulated by dopamine (Humphreys and Whelan, 2012). An alternative possibility is that dopamine affects the network by regulating sodium pump activity. If this is the case, dopamine should have an attenuated effect on the rhythm in presence of ouabain.

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Similar to previous work (Sharples et al., 2015), Picton et al. (2017) show that dopamine reduces the frequency and increases the stability of the locomotor rhythm. The stabilization of rhythmic activity was previously attributed in part to a depolarization of motor neurons by reducing an A-type (I_A) and calcium-dependent (SK_{Ca}) potassium conductance (Han et al., 2007). The resulting increase in membrane input resistance amplifies synaptic currents (Han et al., 2007), and indeed dopamine also directly increases glutamatergic transmission via D_1 -receptor-PKA-dependent increase in motor neuron AMPA conductances (Han and Whelan, 2009).

Picton et al. (2017) provide an additional cellular mechanism to account for dopamine's modulatory effect on locomotor network. To test pump function more directly, they investigated the ultra-slow hyperpolarization (usAHP) (Zhang and Sillar, 2012; Zhang et al., 2015) that persists for 20–80 s following the removal of a 10 s step or train of depolarizing current. The usAHP depends on spiking activity, and it is reduced by the sodium pump inhibitor ouabain, suggesting that it is produced by the sodium pump. The usAHP was expressed in a large proportion of motor neurons, and the duration of the usAHP was increased by dopamine. Increasing the usAHP via sodium pump might therefore counter the depolarizing effects of dopamine and delay the next burst contributing in part to a slower rhythm.

Another cellular mechanism that could account for changes in rhythm frequency is the modulation of rhythm generating interneurons, and the possibility is supported by the results of altering pump function with ouabain or monensin. Picton et al. (2017) report the activity-dependent usAHP in a large proportion of Pitx2 and unidentified lamina X interneurons of the lumbar spinal cord, which is an area where rhythm-generating cells can be found. Pitx2-expressing interneurons are a subpopulation of cells located around the central canal of the spinal cord and are composed of nonoverlapping populations of cholinergic and glutamatergic cells. Although these cells are not rhythm generators, they do modulate locomotor activity (Zagoraïou et al., 2009). Although not tested, the presence of the usAHP in these cells may serve as a target for dopamine to alter locomotor activity.

Dopamine might also regulate locomotor rhythms by promoting oscillations in Hb9 interneurons (Han et al., 2007), which are located in lamina VII-X and have been suggested to be important for rhythm gen-

eration (Wilson et al., 2005; Caldeira et al., 2017). Picton et al. (2017) found that ~40% of unidentified interneurons located in this area had a usAHP. Although they did not directly test whether dopamine modulated the usAHP in these cells, this remains a possibility. If so, dopamine may promote these oscillations by modulating sodium pump function.

Together with previous work, these results suggest that dopamine slows and stabilizes the locomotor rhythm by influencing multiple molecular targets in both motor neurons and interneurons. If dopamine drives rhythmicity in premotor rhythm-generating cells via a pump-dependent mechanism, the effects would be amplified postsynaptically by dopamine increasing motor neuron input resistance and AMPA currents. Amplified rhythmic inputs would be further augmented by a parallel reduction in I_A , which would produce more rapid initiation of spiking activity, whereas a reduction in SK_{Ca} would sustain high spike rate during a burst. An increase in the activity of the sodium pump by dopamine would then contribute to more rapid and prolonged hyperpolarization between bursts, which would slow the rhythm. Prolonged hyperpolarization would also result in greater recruitment of hyperpolarization-activated cation channels, including the h-current (I_h) and T-type calcium current (I_T), which would augment postinhibitory rebound and the transition to next burst. Together, the combined effects could contribute to a slower and more robust rhythm.

The receptors mediating dopamine's effect on the sodium pump remain to be elucidated; however, in cultured bovine striatal neurons, D_1 and D_2 receptors have been documented to exist as a complex with the sodium pump providing bidirectional modulation (Hazelwood et al., 2008). In the spinal locomotor network, excitatory D_1 -like receptors appear to be key in augmenting the rhythmic network output (Sharples et al., 2015). Interestingly, increasing inhibitory D_2 -receptor signaling with quinpirole not only reduces the frequency but also stabilizes the rhythm. It was previously suggested that the D_2 -mediated augmentation of rhythm stability was a network property of a slower rhythm and not a D_2 -specific effect (Sharples et al., 2015). But D_2 -receptor mediated dephosphorylation has also been reported to increase pump activity in striatal neurons (Bertorello and Aperia, 1990; Wu et al., 2007). It is therefore possible that D_2 receptors interacting with the sodium pump could contribute to rhythm stabilization.

This poses a potential explanation for dysfunction in D_2 -like receptor-sodium

pump interactions as a contributor to sensory-motor dysfunction in restless leg syndrome (RLS). RLS is characterized by involuntary leg movements usually during sleep, and D_3 receptor signaling dysfunction has been hypothesized to contribute to spinal hyperexcitability in this condition (Clemens et al., 2006). Given that the symptoms of RLS are treated with D_2/D_3 agonists (Earley et al., 2016), it is possible that dysfunction in this system contributes to symptoms of sensory-motor hyperexcitability in RLS.

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