Compensatory changes in rapid A-type potassium channel function in the superficial dorsal horn of the spastic mouse; studied *in vitro* and *in vivo*

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The spastic mouse has a naturally occurring mutation in the inhibitory glycine receptor. Previously we have shown that the spastic mutation disrupts synaptic transmission in both motor (Graham *et al.*, 2006) and sensory (Graham *et al.*, 2003) pathways by reducing glycinergic inhibition. In addition to disrupted glycinergic inhibition, we have also identified a concomitant increase in $GABA_A$ergic inhibition in the superficial dorsal horn (SDH) of the spastic mouse (Graham *et al.*, 2003). The impact of this altered inhibitory drive on signal processing and neuronal excitability in the SDH of the spastic mouse has not been investigated. In this study, we used a combination of *in vitro* and *in vivo* patch-clamp recording techniques to examine how altered inhibitory drive affects neuron excitability and signal processing in the SDH of the spastic mouse. For *in vitro* experiments, mice (C57Bl/6 or spastic; ~ P23) were anaesthetised with Ketamine (100 mg/kg, i.p.) and decapitated. Transverse slices (300 µm thick) were prepared from the lumbar spinal cord (L3-L5 segments). Intrinsic membrane properties and excitability of spastic and wildtype SDH neurons (n = 91 and 97 neurons, respectively) were compared using whole-cell current and voltage-clamp recording techniques (KCH_3SO_4 internal solution; 23ºC). Apart from a modest reduction (-69.2 ± 0.8 *vs.* -66.6 ± 0.8 mV; $p < 0.05$) in resting membrane potential, neurons in the spastic mouse had membrane and action potential (AP) properties identical to those of wildtype mice. There was, however, a substantial reorganization of AP discharge patterns in response to step current injection (800 ms, 20 pA increments). Recordings in spastic, showed a significant increase (14%) in the proportion of delayed firing neurons, compared to wildtype controls. We also observed a depolarising shift in the steady-state inactivation of rapid A-currents (I_{Ar}), an important potassium conductance in SDH neurons. We propose that this shift in voltage sensitivity of I_{Ar} enhances its impact on delaying AP discharge and increases the proportion of delayed firing neurons in the SDH. To assess the functional consequences of the reorganization of AP discharge patterns and enhanced I_{Ar} current on signal processing in the SDH we next made *in vivo* patch-clamp recordings from spastic and wildtype SDH neurons (n = 32 and 37, respectively). Mice (~ P37) were deeply anaesthetised (Urethane 2.2 g/kg, i.p.) and SDH neuron responses to innocuous (brush) and noxious (pinch) hindpaw stimulation were quantified. Overall, responses recorded in wildtype and spastic mice were similar (p = 0.21); however, in spastic mice a small population of spontaneously active neurons (~ 10 %) exhibited elevated spontaneous discharge frequency and post-pinch discharge rates. Together, these results are consistent with the altered intrinsic membrane properties of SDH neurons, observed *in vitro*, having functional consequences for pain processing mechanisms *in vivo*. We propose that alterations in I_{Ar} current properties in the spastic mouse compensate, in part, for disrupted inhibition and maintain normal signal processing in the SDH.

Graham BA, Schofield PR, Sah P, & Callister RJ. (2003) *Journal of Physiology*, **551:** 905-16.
Graham BA, Schofield PR, Sah P, Margrie TW, & Callister RJ. (2006) *Journal of Neuroscience,* **26:** 4880-90.