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Assessment of $GABA_A$ mediated changes in excitability in mouse somatosensory neurons

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GABA and glycine are the two main inhibitory neurotransmitters in the central nervous system mediating fast inhibitory transmission. In general, inhibitory neurotransmitters are expected to be analgesic owing to their hyperpolarising and shunting actions that reduce neuronal excitability. The expression of the NKCC1 chloride transporter in primary afferent neurons maintains a high intracellular chloride concentration, in the absence of the KCC2 transporter to extrude chloride. Thus, upon $GABA_AR$ activation the ensuing efflux of chloride will depolarise these cells. Indeed, $GABA_AR$ activation in dorsal root ganglion neurons of the mouse stimulate calcium influx. Interestingly, primary afferent depolarisation will still reduce excitability of presynaptic endings of nociceptors via presynaptic inhibition. Importantly, GABA-mediated chloride currents can have opposite effects on nociceptor excitability along the axon, in particular for activity-dependent changes. In general, nociceptor excitability decreases upon repetitive discharge that initiates a negative feedback loop via hyperpolarisation, increase in intracellular sodium and inactivation of sodium channels. On the other hand, GABA-mediated chloride currents are facilitated by hyperpolarisation and thus, they are optimally positioned to limit activity-dependent reduction of excitability. Thus, $GABA_AR$ activation and modulation of the chloride gradient can maintain neuronal excitability despite ongoing activity.
Therefore, it was of particular interest to study the role of GABA-mediated chloride currents in limiting activity-dependent inhibition in peripheral primary afferents. Traditionally, mechanisms inducing hyperexcitability are in the forefront of concepts for chronic pain. This work focused on axonal and somal GABA effects, which were hypothesised to provide evidence for a complementary concept of chronic pain, namely reduced activity-dependent inhibition via regulation of the chloride gradient.
In this study, the threshold tracking technique was used to assess the functional role of $GABA_AR$-mediated chloride currents on excitability of axons within the sural nerve, which are primarily skin afferents. The excitability index was used to indirectly determine a change in the membrane potential. We find that GABA-induced excitability changes can be dynamically regulated by activity after bouts of high frequency firing, however these effects may be obscured by parallel $GABA_A$ receptor desensitization.
Additionally, this study employed the calcium imaging technique to determine excitability changes by GABA application in the DRG of mice. We find calcium transients in response to GABA in approx. 50% of DRG neurons (664/1317). These transients were mediated by calcium influx through L-type voltage-gated calcium channels, indicating a depolarising action of GABA in the DRG in line with previous studies. This calcium signal is further dependent on the existence of a chloride gradient, which we manipulated by reduction of the extracellular chloride concentration. The use of high frequency stimulation, in contrast to axonal threshold tracking experiments, did not yield evidence of an activity-dependent increase in NKCC1 inward chloride transport in the cell somata, but rather reduced the subsequent GABA-mediated calcium signal. The expected activity-dependent increase in NKCC1 activity was not even evident after depletion of intracellular chloride via a reduced extracellular chloride concentration and a repetitive $GABA_AR$ activation. Based on our results in axonal threshold tracking, we cannot rule out that receptor desensitization might have contributed to the prolonged reduction of calcium responses to $GABA_AR$ activation. Additional explanations include excessive intracellular calcium increases due to the electrical stimulation protocols and low expression density of NKCC1 in DRG neurons, that would prolong effective re-establishment of an original chloride gradient.
Our study provides insights into the complexity of chloride regulation via the $GABA_AR$ and resulting excitability changes in primary somatosensory neurons and their peripheral axons. We provide evidence

that $GABA_AR$ activation and NKCC1-mediated inward chloride transport can counteract activity-dependent reduction of excitability in nociceptors.
New methods such as voltage-sensitive dyes that allow direct quantification of membrane potential changes and action potentials may further clarify the role of $GABA_AR$ activation on nociceptor excitability and potential clinical implications.
Despite not clarifying the exact determinants for $GABA_AR$-mediated effects, our results have major implications for our main hypothesis that GABA counteracts an activity-dependent reduction of excitability in nociceptors. We verify a transient effect in primary afferent neurons that may be of clinical relevance for brief bursts in nociceptors underlying the experience of short-lasting intermittent pain.