

Axonal tuning by GABA_A receptor unveils novel tricks from an old dog

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In the last years, axonal conductance of action potential trains became a novel subject of study, changing the view of axons, from a static cable-like compartment to a more complex and dynamic system (Debanne et al., 2011). Axonal computation, indeed, is canonically constituted by the action of voltage-gated ion channels, such as the classic Na⁺ and K⁺ channels, but recent studies demonstrated that it can be modulated by the action of other ion channel pumps, and metabolic factors (Byczkowicz et al., 2019; Zang and Marder, 2021). These non-canonical mechanisms have been studied mainly in the central nervous system (Byczkowicz et al., 2019; Kamiya, 2019), and little is known about axonal conductance modulation in peripheral nerve fibers. Interestingly, the peripheral projecting neurons possess a pseudounipolar conformation and exceptionally long axons, an anatomical characteristic that make propagation and tuning of the axonal action potential more easily adjustable. Notably, unmyelinated axons (i.e. C-fiber nociceptors) prevail in all peripheral fibers as the most affected by changes in conduction velocity, since relatively small alterations cause substantial delays in action potential incoming time (Zang and Marder, 2021). Peripheral C-fiber nociceptors, indeed, are defined by specific activity-dependent slowing properties, whereby repetitive firing of nerve fibers results in the progressive slowing of their conduction velocity and concomitant increase in response latency (Gee et al., 1996). Activity-dependent slowing is so preserved to be designated as a signature mark able to discriminate among different functional C-fiber subtypes (Werland et al., 2021).

Gamma-aminobutyric acid A (GABA_A) receptor and Na-K-Cl cotransporter type 1 (NKCC1) are important regulators of axonal conduction: Very recently, Bonalume et al. (2021) characterized axonal GABA_A receptors in peripheral nerves. These receptors mediate depolarizing Cl⁻ currents, which in turn modulate peripheral C-fiber nociceptors computation. Thereby, the endogenous GABA_A receptor activation represents a novel physiological mechanism able to increase C-fibers' fidelity, stabilizing their excitability and enabling prolonged firing. Such a non-canonical location and function of tonic GABA_A receptor currents may represent an intriguing target of study in several physio-pathological conditions, such as the neuropathies occurring after nerve injury, chronic pain as well as in pain insensitivity. Furthermore, Bonalume et al. (2021) demonstrated that any dysregulation of the Cl⁻ gradient upstream GABA_A receptor fluxes leads

to a significant alteration in C-fiber nociceptors' excitability and conduction velocity. Deepening the comprehension of this innovative pathway may unveil alternative pharmacological targets for pain treatment, besides the GABA_A receptor itself, avoiding most of central side effects resulting from its activation. The main Cl⁻ transporter engaged in this process is NKCC1. Peripheral neurons possess a high intracellular Cl⁻ concentration even in adults, compared to central neurons that gain a low intracellular Cl⁻ concentration upon maturation. This reverse gradient results from the physiological predominance of inward Cl⁻ transport, mediated by NKCC1, versus the outward transport, mediated by KCC2, being the last faintly expressed in peripheral neurons. Accordingly to the literature, GABA_A receptor opening in peripheral axons mediates depolarizing Cl⁻ currents. Bonalume et al. (2021) further demonstrated that NKCC1 activity is dynamically coupled with C-fiber nociceptors firing. The high-frequency stimulation of C-fibers induces a feedforward activation of NKCC1, leading to an increase in Cl⁻ gradient upon axonal membrane, within a consequent potentiation of GABA_A receptor currents. In this context, GABA_A receptor serves as a regulator of the "Cl⁻ conductance", acting downstream the NKCC1 control. Overall, the mutual activity of these two players elicits tonic depolarizing currents along the peripheral C-fiber nociceptors, able to sustain a prolonged firing, and preventing fast desensitization mediated by the activity-dependent slowing mechanism. The blockade of NKCC1 or the absence of functional GABA_A receptor in C-fibers, indeed, causes a strong uprise in the activity-dependent slowing. The increased conduction velocity of axons upon mild depolarization is consistent with previous observations correlating the shift of membrane potential in the subthreshold range to the raise in firing frequency (Carp et al., 2003). Intuitively, such a GABA_A receptor-mediated hypothesis of the "acceleration of conduction" in C-fibers can be figured out as follows: during the action potential sustained activity, the NaV channels-mediated current in one axonal site depolarizes the neighboring locations faster whether the axon is already depolarized through the GABA_A/NKCC1/Cl⁻ control, rather than in an axon still in a resting state.

Where does the GABA come from? This paradigm rises an intriguing question: how would the axonal GABA_A receptor be activated without a classical synaptic-like release of GABA? In the mouse sural nerve, Bonalume et al. (2021) demonstrated that the GABA_A receptor is endogenously activated

under physiological conditions, exhibiting a tonic profile. In fact, different pharmacological approaches (that is a competitive GABA_A receptor antagonist, the allosteric agonist allopregnanolone, as well as an NKCC1 blocker) confirmed the effect mediated by the endogenous GABA. Undoubtedly, the release of GABA from *ex vivo* desheathed nerves has been detected in the concentration range of tens of nanomolar (Bonalume et al., 2021). Altogether, these observations prove that GABA is physiologically released within peripheral nerves, allowing a tonic modulation of axonal excitability and supporting the action potential propagation and tuning, from the periphery to the central nervous system. However, in line of principle, it should not be excluded that other extrasynaptic ligands of GABA_A receptors such as glycine and taurine (Le-Corronc et al., 2011) could participate in the modulation of axonal excitability.

Surprisingly the novel interpretation of GABA_A receptor-mediated actions in peripheral nerves corroborates the illuminated observation by Jessen et al. (1979), that formerly showed the presence of GABAergic neurotransmission in the peripheral nervous system. The scientific challenge, however, to fully understand the role of Cl⁻-mediated currents modulation of axonal conductance, would be to define precisely the local origin of GABA, studying the pathways involved in its homeostasis and the putative triggers for its release. In principle, both neuronal and glial compartments of peripheral fibers could be considered as local sources and reuptake of GABA (Colciago et al., 2020). Both GABA_A and the metabotropic GABA_B receptors are expressed and functional in Schwann cells, whereas their endogenous activation participates in axonal sorting and myelination, likely during the development (Castelnuovo et al., 2017). Furthermore, the Schwann cells have been proved to synthesize and release the neuroactive steroid allopregnanolone (Bonalume et al., 2020), which can modulate their fate as well as the peripheral neuronal activity (Meyer et al., 2019) via a fast GABA_A receptor activation (Cociago et al., 2020). In this regard, the presence and importance of endogenous tonic GABA in peripheral nerves have been already demonstrated, for peculiar physiological aspects, in those cellular compartments. Interestingly, the concomitant modulation of axonal conduction velocity adds more complexity to the GABAergic puzzle in the peripheral nervous system, emphasizing a more dynamic role for GABA, GABA_A receptor, and Cl⁻ conductance in the context of sustained firing and strong axonal fidelity.

Concluding remarks: In the context of this novel interpretation of GABA_A-mediated depolarizing Cl⁻ currents, it is noteworthy that the peripheral nociceptors are not the unique mature neurons characterized by high intracellular Cl⁻ concentration. Somatosensory trigeminal neurons, post-ganglionic sympathetic neurons, and olfactory sensory neurons are equally characterized by high intracellular Cl⁻ and subsequent outward

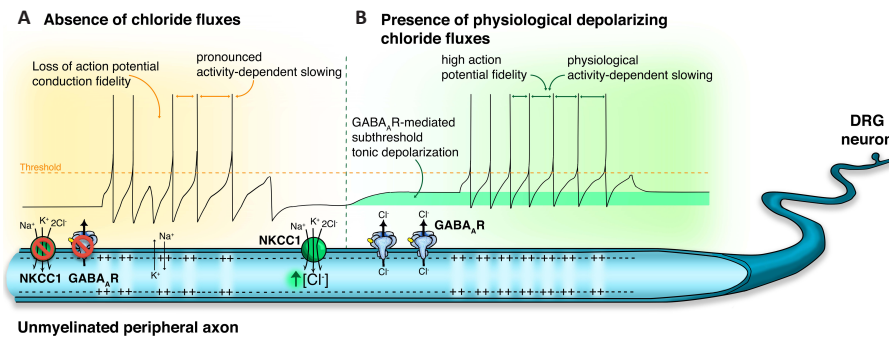


Figure 1 | Modulation of action potential conductance by GABA_A receptor-mediated currents.

(A) In kinds of Cl⁻ dysregulation, such as NKCC1 blockade, GABA_A receptor absence or inhibition, the peripheral nociceptor firing is curtailed. Unmyelinated axons (i.e. C-fiber nociceptors) are characterized by excessive slowing and decreases in action potential fidelity. (B) In presence of a high intracellular Cl⁻ concentration, mediated by NKCC1 inward currents, GABA_A receptor tonic activation induces a subthreshold depolarization that stabilizes excitability, triggering high action potential fidelity and enabling prolonged firing in time. DRG: Dorsal root ganglion; GABA_A: gamma-aminobutyric acid A receptor; NKCC1: Na-K-Cl cotransporter type 1.

depolarizing Cl⁻ currents. All these neurons show strong and characteristic desensitizing/adaptive mechanisms, which go together with the inability to hold high-frequency firing for a prolonged period. Interestingly, such a conservative mechanism is physiologically controlled by the presence of opposite currents, which stabilizes fibers, avoiding axonal failure. For instance, Ca²⁺-activated Cl⁻ currents (CaCCs) have been proved to modulate repetitive firing in mouse sympathetic ganglion cells (Martinez-Pinna et al., 2018). In this subset of neurons, the Ca²⁺-activated Cl⁻ currents serve as a regulator of Cl⁻ conductance instead of GABA_A receptor, acting in a comparable dynamic manner by means of depolarizing currents.

Therefore, the “GABA_A/Cl⁻” regulation adds further workpieces to the “non-cable-like” theory of axonal function, posing an alternative overview of the axonal computation and focusing on C-fiber unmyelinated axons, which are affected by pain physiopathology. The pharmacologic possibility to modulate the GABA_A/NKCC1/Cl⁻ paves the way to alternative approaches for the treatment of neuropathic and/or chronic pain.

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