Neuro Forum Title:
Neuronal integration and the depolarizing effects of axonal GABAₐ receptors

Abbreviated Title:
GABA depolarizes axons

Article Reviewed:

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Keywords:
GABAₐ receptor, axon, excitability, release probability

Acknowledgements:
We thank Pablo E. Castillo and Sung-Min Park for comments on the manuscript.
Abstract

Despite their presence throughout the central nervous system, the impact of axonally expressed gamma-amino-butyric-acid type-A receptors (GABA_ARs) on neuronal signaling is just beginning to be understood. A recently published article by Jason Pugh and Craig Jahr (2011) tackled this important issue by investigating GABA_AR-mediated function in axons of cerebellar granule cells. Their results indicate parallel fiber GABA_ARs enhance neurotransmitter release probability and boost axonal and somatic excitability.

Introduction to the work of Jason Pugh and Craig Jahr

Bernard Katz once said, “The more one finds out about properties of different synapses, the less grows one’s inclination to make general statements about their mode of action.” This maxim can be extended beyond synaptic transmission to neuronal signaling in general. For example, it is widely appreciated that dendritic integration leads to an all-or-none action potential (AP) initiated near the soma which then propagates to the axon terminal. The terminal bouton is depolarized, leading to Ca^{2+} influx and subsequent neurotransmitter release. Although this model is highly instructive, research over the past few decades revealed additional computational features of neurons. It is now known APs can travel backwards in an antidromic fashion away from the soma and into dendrites (Stuart et al. 1997). Moreover, mounting evidence indicates APs can also be generated in the axon where they travel antidromically towards the soma (Paradiso and Wu 2009).

Action potentials are not alone in their ability to influence neural computation. While sub-threshold membrane potential fluctuations can lead to the production or negation of APs, they themselves can provide meaningful information to the system (Alle and Geiger 2008). Sub-threshold events generally originate from ligand-gated ion channels expressed in the plasma membrane. While the role that somato-dendritic ion channels play in supporting graded potentials and AP generation has been relatively well characterized, the contribution of channels
expressed in axonal compartments to these signals is less clear. Interestingly, it is now known that GABA\(_A\)Rs are expressed in certain axons throughout the brain and spinal cord (Trigo et al. 2008). In mature animals, GABA\(_A\)R activation typically hyperpolarizes the membrane potential due to low intracellular Cl\(^-\) concentrations. However, compared with the soma and dendrites, axonal compartments can maintain more Cl\(^-\), resulting in a higher Cl\(^-\) reversal potential, and GABA\(_A\)R activation *depolarizes* the membrane potential (Price and Trussell 2006). Given the widespread expression of GABA\(_A\)Rs in the central nervous system, determining when and where these channels exert their depolarizing action is essential to advancing our knowledge of brain function.

A recent report by Pugh and Jahr (2011) adds to our growing understanding of how axonal GABA\(_A\)Rs impact neural excitability and synaptic output. Working in the cerebellum of mature animals, the authors combined *in-vitro* slice electrophysiology, high resolution calcium imaging, and various pharmacological tools to probe GABA\(_A\)R-mediated affects in parallel fibers. They provide compelling evidence that axonal GABA\(_A\)R activation modulates at least three aspects of granule cell physiology: neurotransmitter release probability (P\(_r\)), axonal excitability, and somatic excitability.

**Stimulating parallel fiber GABA\(_A\) receptors leads to more than one outcome**

Pugh and Jahr (2011) build their story off a previous counterintuitive observation that application of the GABA\(_A\)R agonist muscimol to parallel fibers increased excitatory postsynaptic currents (EPSCs) recorded from Purkinje or stellate cells (Stell et al. 2007). Several initial observations suggested this plasticity was due to enhanced P\(_r\). Consistent with the idea that transmitter release is stochastic, sometimes leading to a postsynaptic response and other times failing to release transmitter, the failure rate of synaptic responses decreased in response to muscimol. Second, the paired-pulse ratio, which is inversely related to changes in P\(_r\), decreased. Thirdly, and strikingly, locally applied GABA onto parallel fibers caused an axonal
Ca\textsuperscript{2+} transient, suggesting that GABA directly depolarizes the axon, thereby activating voltage-gated Ca\textsuperscript{2+} channels. Since changes in P_r can arise from multiple sources like increased transmitter release or parallel fiber excitability, Pugh and Jahr (2011) wanted to distinguish between these possibilities. They performed the same experiment mentioned above, but this time in the presence of EGTA-AM, a membrane permeable Ca\textsuperscript{2+} chelator. EGTA-AM limits Ca\textsuperscript{2+}-dependent processes like presynaptic transmitter release and could expose additional contributions to muscimol-induced potentiation of the EPSC. As expected, GABA\textsubscript{A}R-induced changes in paired-pulse ratio were no longer observed. Surprisingly, however, GABA\textsubscript{A}R activation still enhanced EPSCs, albeit to a lesser extent than control. The authors reasoned that another mechanism must be at hand.

Parallel fiber GABA\textsubscript{A} receptors boost axonal and somatic excitability

Several experiments were performed to determine whether GABA\textsubscript{A}R activation influenced granule cell excitability. Using a technique known as excitability testing, Pugh and Jahr (2011) stimulated parallel fibers such that antidromic spikes elicited from the axon were detected at the soma approximately 50% of the time. Focal GABA application on the axon was then shown to increase spiking probability, indicating that axonal excitability was enhanced. Notably, GABA alone could sometimes trigger a spike in the soma. These depolarizing GABA-spikes were not full blown APs, probably because they failed to fully invade the soma. Nevertheless, their waveform was indistinguishable from electrically evoked antidromic spikes, suggesting a full blown AP might be achieved in the axon. Indeed, Ca\textsuperscript{2+} transients measured from axonal varicosities in response to ortho- and antidromic stimulation elicited identical Ca\textsuperscript{2+} signals.

Although exogenous GABA application was shown to increase axonal excitability, it was still unknown whether this was a naturally-occurring phenomenon. Pugh and Jahr (2011) capitalized on the fact that stimulating local molecular layer interneurons can trigger
endogenous GABA release and subsequent spillover onto neighboring synapses. If GABAAR activation leads to enhanced axonal excitability, then one would expect to see a concomitant increase in the probability of observing AP-dependent Ca$^{2+}$ transients in parallel fiber boutons. By imaging Ca$^{2+}$ transients in single parallel fiber boutons, the authors found that endogenous and exogenous GABAAR activation led to the predicted result. When the GABAAR antagonist picrotoxin was present, GABA no longer increased AP-dependent Ca$^{2+}$ transients. Collectively, these experiments demonstrate endogenous and exogenous GABA augments parallel fiber axonal excitability in a GABAAR-dependent manner.

Interestingly, Pugh and Jahr (2011) found that GABA applied to parallel fibers would on occasion trigger sub-threshold depolarizations, in addition to truncated spikes, recorded hundreds of microns away at the soma. With this in mind, the authors realized sub-threshold depolarizations could electrotonically spread back toward the cell body and affect somatic excitability. Excitability testing was again applied but this time APs were elicited by somatic current injection, and their success rate was compared in the absence and presence of GABA locally applied to the axon. The AP success rate increased in response to GABA, indicating that somatic excitability can also be enhanced by axonal GABAAR activation.

Up to this point, Pugh and Jahr (2011) provide solid evidence that GABAAR activation enhanced granule cell axonal and somatic excitability. However, it should be noted that for most experiments the intracellular recording pipette contained large amounts of Cl$^-$. Under these conditions, GABA will always depolarize the cell. It was therefore crucial to test whether GABAAR activation was depolarizing in the axon when Cl$^-$ was unperturbed. The authors show that even when endogenous Cl$^-$ gradients were left intact, the probability of observing AP-dependent Ca$^{2+}$ influx at single boutons still increased as a result of GABA locally applied to the axon. Although this finding supports the notion that GABA is depolarizing in an endogenous intracellular medium, it is not clear whether those effects can influence somatic excitability when Cl$^-$ gradients are left intact.
Neurotransmitter release probability is amplified by GABA<sub>A</sub> receptor activity

Pugh and Jahr (2011) came full circle and returned to the finding that muscimol increased parallel fiber-evoked EPSCs in a manner consistent with enhanced Pr. Although changes in failure rate and paired-pulse ratio often indicate presynaptic effects, the authors decided to test this possibility more directly using a powerful imaging technique known as optical quantal analysis (Oertner <i>et al.</i> 2002). In the context of Pugh and Jahr’s (2011) work, the method requires glutamate release from parallel fibers and subsequent binding to and opening of postsynaptic Ca<sup>2+</sup>-permeable AMPA receptors expressed on stellate cells. Probabilistic postsynaptic Ca<sup>2+</sup> transients were then detected, directly reflecting probabilistic presynaptic transmitter release. Using this technique, GABA applied locally at the axon was shown to increase Pr. Importantly, when this experiment was performed in EGTA-AM, a change in Pr was no longer observed, indicating they were able to dissociate release from excitability. The exact mechanism underlying the increased Pr is not entirely clear because it could result from more vesicles being released or a decreased number of failures. If multi-vesicular release was occurring, one would expect a larger postsynaptic Ca<sup>2+</sup> transient in the presence of GABA, which was not the case. A decreased failure rate is therefore the more likely explanation.

Putting the brakes on GABA<sub>A</sub> receptor-mediated synaptic activity and excitability

According to the model put forth by Pugh and Jahr (2011), molecular layer interneurons activated by granule cells release GABA, which spills over to parallel fibers in a paracrine manner, leading to enhanced Pr and granule cell excitability. If depolarizing GABA<sub>A</sub>R-dependent signals are not checked, one can imagine a positive feedback loop leading to runaway excitation. In the simplest case, GABA<sub>A</sub>R-mediated depolarizations could inactivate voltage-gated sodium conductances to terminate the loop. In addition, and as the authors point out, the system may be kept in check by GABA<sub>B</sub>Rs which are also expressed on parallel fibers.
Although blocked in the present study, it has been shown that GABA<sub>B</sub>R activation quickly depresses parallel fiber to Purkinje cell EPSCs by limiting presynaptic Ca<sup>2+</sup> influx (Dittman and Regehr 1997). The coincident activation of Purkinje cells by granule cells also needs to be considered. Activation of metabotropic glutamate receptors on Purkinje cells triggers short and long-term depression of neurotransmitter release from parallel fibers and is mediated by endogenous cannabinoid signaling (Carey <i>et al.</i> 2011). Thus, it is conceivable that endocannabinoids are potent negative regulators of parallel fiber GABA<sub>A</sub>R activity. Another possibility includes the traditional inhibitory role played by GABA. GABA<sub>A</sub>Rs are expressed across the somato-dendritic axis where they can differentially affect neural excitability (Rojas <i>et al.</i> 2011). If somato-dendritic GABAergic inputs reduce the effects of axonal GABA<sub>A</sub>Rs on granule cell excitability, then local inhibition may be a way to short-circuit the positive feedback loop.

**GABA<sub>A</sub> receptors in action potential generation and sub-threshold signaling**

Recent findings have demonstrated that APs do not operate in a vacuum, but rather, can integrate with sub-threshold events to enhance computational capacity (Alle and Geiger 2008). This is remarkable because when graded synaptic potentials merge with all-or-none events, they can cause the AP waveform to be modified, and this will be directly reflected in the postsynaptic cell due to more or less transmitter having been released. The study by Pugh and Jahr (2011) adds to this theme by revealing a surprising mechanism by which GABA<sub>A</sub>Rs influence AP generation and sub-threshold signaling. GABA<sub>A</sub>R-mediated depolarizations spread back up the axon to modulate somatic AP initiation. From the soma’s point of view, the axon and dendrites are quite similar: both serve as discrete points of input which would then be integrated by the soma, driving the neuron closer to, or farther from, AP threshold. Collectively, these reports elucidate how the final output of a neuron can be modulated by a multitude of inputs. These studies also imply that the number and rate of APs inherent in all-or-none
signaling are not the only relevant factors for information processing. Location, direction, velocity, amplitude, and kinetics of sub-threshold events all contribute to the dynamic landscape over which information processing occurs.

Several questions about axonal GABA<sub>A</sub>R signaling remain. Are the phenomena described by Pugh and Jahr (2011) generalizable to other synapses and cell types? How do other ligand-gated ion channels expressed in the axon mediate changes in transmitter release or excitability (Sasaki <i>et al.</i> 2011)? What is the relative contribution of phasic versus tonic axonal GABA<sub>A</sub>R activation? It will be very interesting to determine how sub-threshold events integrate with APs and contribute to network function in vivo.

The common view that most neurons act in a strictly binary manner is not altogether accurate. Rather, the language that neurons use to communicate with each other is more nuanced than previously thought, with APs being modified by many factors which influence how they are generated, if at all.
References:


