

Neuronal integration and the depolarizing effects of axonal GABA_A receptors

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Submitted 11 April 2011; accepted in final form 2 August 2011

Younts TJ, Makani S. Neuronal integration and the depolarizing effects of axonal GABA_A receptors. *J Neurophysiol* 106: 2105–2107, 2011. First published August 3, 2011; doi:10.1152/jn.00328.2011.—Despite their presence throughout the central nervous system, the impact of axonally expressed gamma-aminobutyric acid type-A receptors (GABA_ARs) on neuronal signaling is just beginning to be understood. A recently published article (Pugh JR and Jahr CE, *J Neurosci* 31: 565–574, 2011) tackled this important issue by investigating GABA_AR-mediated function in axons of cerebellar granule cells. The results of Pugh and Jahr indicate parallel fiber GABA_ARs enhance neurotransmitter release probability and boost axonal and somatic excitability.

GABA_A receptor; axon; excitability; release probability

Introduction to the work of Jason Pugh and Craig Jahr. Bernard Katz, winner of the 1970 Nobel Prize in Physiology or Medicine for his work on the quantal mechanisms of neurotransmitter release, 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 Ca²⁺ 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).

APs are not alone in their ability to influence neural computation. While subthreshold 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). Subthreshold 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 gamma-aminobutyric acid type-A receptors (GABA_ARs) are expressed in certain axons throughout the brain and spinal cord (Trigo et al. 2008). In mature animals, GABA_AR 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_AR activation depolarizes the membrane potential (Price and Trussell 2006). Given the widespread expression of GABA_ARs 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_ARs 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_AR-mediated effects in parallel fibers. They provide compelling evidence that axonal GABA_AR 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_AR 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. Third, and strikingly, locally applied GABA onto parallel fibers caused an axonal Ca²⁺ transient, suggesting that GABA directly depolarizes the axon, thereby activating voltage-gated Ca²⁺ 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 Ca²⁺ chelator. EGTA-AM limits Ca²⁺-dependent processes like presynaptic transmitter release and could expose additional contributions to muscimol-induced potentiation of the EPSC. As expected, GABA_AR-induced changes in paired-pulse ratio were no longer observed. Surprisingly, however, GABA_AR activation still enhanced EPSCs, albeit to a lesser extent than control. The authors reasoned that another mechanism must be at hand.

Parallel fiber GABA_A receptors boost axonal and somatic excitability. Several experiments were performed to determine whether GABA_AR activation influenced granule cell excitability. Using a technique known as excitability testing, Pugh and

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Jahr (2011) stimulated parallel fibers such that antidromic spikes elicited from the axon were detected at the soma ~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^{2+} transients measured from axonal varicosities in response to ortho- and antidromic stimulation elicited identical Ca^{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 GABA_{A} 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 GABA_{A} activation led to the predicted result. When the GABA_{A} 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 GABA_{A} -dependent manner.

Interestingly, Pugh and Jahr (2011) found that GABA applied to parallel fibers would on occasion trigger subthreshold depolarizations, in addition to truncated spikes, recorded hundreds of micrometers away at the soma. With this in mind, the authors realized subthreshold 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 GABA_{A} activation.

Up to this point, Pugh and Jahr (2011) provide solid evidence that GABA_{A} 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 GABA_{A} 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_{A} 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 P_{r} .

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 et al. 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^{2+} -permeable AMPA receptors expressed on stellate cells. Probabilistic postsynaptic Ca^{2+} transients were then detected, directly reflecting probabilistic presynaptic transmitter release. Using this technique, GABA applied locally at the axon was shown to increase P_{r} . Importantly, when this experiment was performed in EGTA-AM, a change in P_{r} was no longer observed, indicating they were able to dissociate release from excitability. The exact mechanism underlying the increased P_{r} 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^{2+} 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_{A} 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 P_{r} and granule cell excitability. If depolarizing GABA_{A} -dependent signals are not checked, one can imagine a positive feedback loop leading to runaway excitation. In the simplest case, GABA_{A} -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_{B} Rs that are also expressed on parallel fibers. Although blocked in the present study, it has been shown that GABA_{B} R activation quickly depresses parallel fiber to Purkinje cell EPSCs by limiting presynaptic Ca^{2+} 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 et al. 2011). Thus, it is conceivable that endocannabinoids are potent negative regulators of parallel fiber GABA_{A} R activity. Another possibility includes the traditional inhibitory role played by GABA. GABA_{A} Rs are expressed across the somato-dendritic axis where they can differentially affect neural excitability (Rojas et al. 2011). If somato-dendritic GABAergic inputs reduce the effects of axonal GABA_{A} Rs on granule cell excitability, then local inhibition may be a way to short-circuit the positive feedback loop.

GABA_{A} receptors in action potential generation and subthreshold signaling. Recent findings have demonstrated that APs do not operate in a vacuum, but rather, can integrate with subthreshold 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_{A} Rs influence AP generation and subthreshold signaling. GABA_{A} -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 that 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 subthreshold events all contribute to the dynamic landscape over which information processing occurs.

Several questions about axonal GABA_AR 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 et al. 2011)? What is the relative contribution of phasic vs. tonic axonal GABA_AR activation? It will be very interesting to determine how subthreshold 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 that influence how they are generated, if at all.

ACKNOWLEDGMENTS

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

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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