

Generally Physiological

Of changing glial responses, modular interactions, and why a worm may turn



This month's installment of *Generally Physiological* considers developmental changes in signaling between neurons and astrocytes, construction of a functional chimeric K^+ channel, and a surprising molecular link between salt aversion in nematodes and human deafness.

Developmental changes in neuroglial signaling

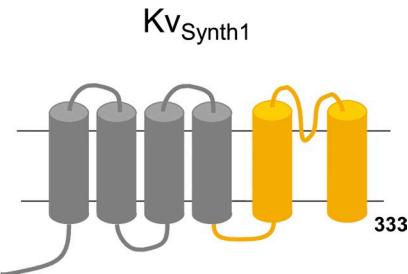
The discoveries that the neurotransmitter glutamate elicits astrocytic Ca^{2+} signals—and that increased intracellular Ca^{2+} is associated with the release of gliotransmitters—led to the notion that synaptic transmission involves not only presynaptic and postsynaptic neurons but also neighboring glia, which can both respond to and modulate synaptic activity. Glutamate signals through $G_{q/11}$ -coupled type 5 metabotropic glutamate receptors (mGluR5) to elicit astrocytic Ca^{2+} waves; noting that most such studies had analyzed cultured astrocytes or slices from young rodents, Sun et al. (2013) investigated developmental changes

in astrocytic mGluR expression and signaling. Quantitative real-time fluorescence polymerase chain reaction (qPCR) indicated that mGluR5 expression in mouse hippocampal astrocytes plummeted after the first postnatal week. Indeed, a combination of microarray analysis and qPCR revealed little mGluR5 expression in adult human or mouse cortical astrocytes or adult mouse hippocampal astrocytes, whereas mGluR3 (a $G_{i/o}$ -coupled receptor that acts to decrease adenylate cyclase activity) was expressed in adult astrocytes as well as during development. Consistent with these data, the strong astrocytic Ca^{2+} signals elicited by mGluR5 agonists in pups were not apparent in adult mice. Thus, the authors propose that neuroglial signaling at glutamatergic synapses may be fundamentally distinct in the adult and developing brain. Grosche and Reichenbach (2013) provide thoughtful commentary, hypothesizing that, with maturation, mGluR5-dependent increases in astrocytic Ca^{2+} (and gliotransmitter release) may shift from a

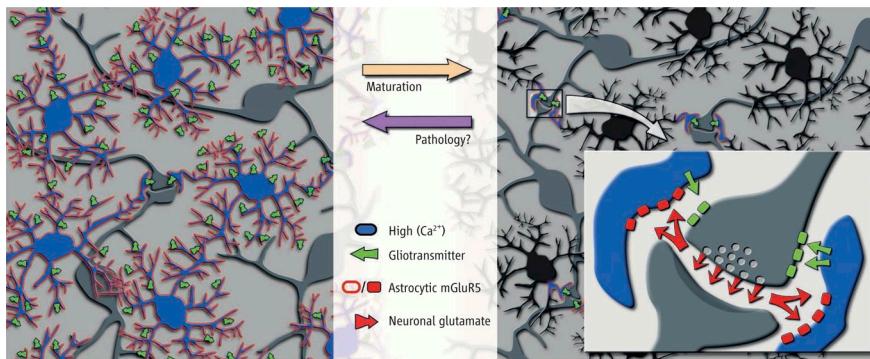
networked multicellular response to one localized to glial microdomains adjacent to the active synapse.

Constructing a functional K^+ channel chimera

Voltage-gated potassium (K_v) channels are characterized by a modular architecture, with the voltage-sensing domain (VSD) that enables activation by membrane depolarization distinct from the pore domain that enables ions to cross the membrane. Indeed, a VSD homologous to those



Kv_{Synth} consists of the C-VSP VSD coupled to Kcv. (From Arrigoni et al., 2013.)



A hypothetical shift from global to local mGluR5-mediated calcium signals and gliotransmitter release in the maturing brain (neurons in gray). (From A. Grosche and A. Reichenbach. 2013. *Science*. 339:152–153. Reprinted with permission from AAAS.)

of K_v channels is coupled to a phosphatase, in a protein initially identified in the sea squirt *Ciona intestinalis* (*C. intestinalis* voltage sensor-containing phosphatase [Ci-VSP]), and the isolated pore domain of a bacterial K_v channel forms a functional K^+ channel, underlining the modular nature of K_v channel function. In this issue, Arrigoni et al. connected the Ci-VSP VSD to the (voltage-independent) viral Kcv channel (essentially an isolated pore domain) and determined that the resulting fusion protein, Kv_{Synth}, was activated by depolarization, behaving as a delayed-rectifier K^+ channel with voltage

dependence like that of Ci-VSP and permeability like that of Kcv. The VSD of Kv channels connects to the pore domain through a cytoplasmic

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linker region and, remarkably, experiments in which the length and composition of the Kv_{Synth} linker was varied indicated that linker ability to enable voltage-dependent gating by the VSD depended on length (optimal at 6–12 amino acids) rather than specific amino acid sequence. Their data (Arrigoni et al., 2013) thus argue that simple mechanical coupling of voltage sensing and pore modules from distinct proteins can enable voltage-dependent gating of a channel and provide insight into the criteria governing the mechanism of sensor coupling to the gate.

Hearing about too much salt?

Chatzigeorgiou et al. (2013) uncovered an intriguing molecular link between human deafness and the aversive response to salt in the nematode *Caenorhabditis elegans*. The

membrane protein transmembrane channel-like 1 (TMC-1) has been implicated in hair cell mechanotransduction, and its mutation is associated with deafness in humans and mice. However, its precise role—even whether it functions as a channel per se—has been unclear (see commentary by Coste and Patapoutian, 2013). Chatzigeorgiou et al. (2013) found that, in transgenic worms, expression of a fluorescent reporter under the control of the *tmc-1* promoter was restricted to a small number of neurons; these included ASH sensory neurons, which are involved with avoidance of noxious stimuli (including hyperosmolarity, heavy metals, nose touch, and high concentrations of salt). Although avoidance of high concentrations of NaCl was impaired in *tmc-1* mutant worms, as was the *in vivo* calcium transient elicited by high NaCl concentrations in the ASH neurons of wild-type worms, their responses to nose touch, CuCl₂, and glycerol were unaffected. *tmc-1* mutation had little or no effect on behavioral responses or ASH calcium transients elicited by high concentrations of CaCl₂, MgCl₂, or KCl₂, but attenuated those elicited by sodium gluconate and sodium acetate, suggesting that TMC-1 is specifically involved in the ASH response to sodium ions. Sodium gluconate produced calcium signals in normally salt-insensitive worm neurons ectopically expressing TMC-1.

Moreover, electrophysiological analyses of cultured mammalian cell lines expressing worm TMC-1 revealed Na⁺-activated cationic currents (with an activation threshold of ~140 mM NaCl). The authors thus propose that TMC-1 acts as a salt sensor in *C. elegans* and postulate that it may function as a salt-activated ion channel.

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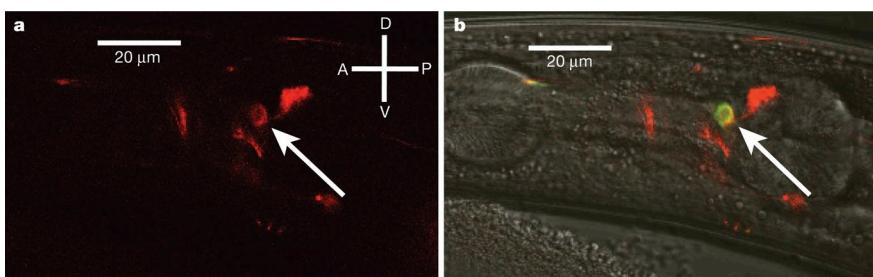
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Expression of a fluorescent fusion protein under the control of the *tmc-1* promoter in transgenic worms. The arrow indicates ASH. (Reprinted by permission from Macmillan Publishers, Ltd. *Nature*. Chatzigeorgiou et al., 494:95–99, copyright 2013.)