Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Nucleus of Tractus Solitarius Astrocytes as Homeostatic Integrators

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Review of McDougal et al.

Astrocytes, the main glial population in the CNS, have long been considered a type of support cells that ensure an adequate environment for the proper operation of neurons. In recent years, novel experimental approaches revealed that these cells play an active role in the regulation of neurophysiological functions (Hamilton and Attwell, 2010). For example, astrocytes help to fine-tune blood and energy supplies to the brain as neuronal activity fluctuates and engage in bidirectional interactions with neurons (Pellerin and Magistretti, 2011).

Communication between astrocytes and other cell types is mediated by activation of membrane receptors by a wide array of signals. The subsequent induction of Ca^{2+} transients induces release of intercellular mediators. Some of those, such as ATP, glutamate, and D-serine, act on neurons to modulate excitability and support some forms of synaptic plasticity (Hamilton and Attwell, 2010).

Astrocytes contact plasma via astrocytic endfeet that surround brain capillaries, as well as in a set of specialized brain structures devoid of blood–brain barrier, the sensory circumventricular organs (CVOs). This suggests that astrocytes could relay information about the internal milieu to neuronal networks. Indeed, recent data have confirmed that astrocytes monitor blood-born signals, such as electrolytes, metabolites, hormones, and cytokines, that reflect the homeostatic status of the body, and that they communicate changes in these parameters to neuronal centers that regulate vital body functions (Marty et al., 2005; Shimizu et al., 2007; Gourine et al., 2010).

The nucleus of tractus solitarius (NTS) is an important viscerosensory center in the dorsal brainstem. It collects information about the internal environment via vagal afferents and from the area postrema (AP), a CVO juxtaposed to the fourth ventricle. The NTS regulates body homeostasis by producing autonomic reflexes and modulating behavior. Interestingly, NTS has a distinctive astrocytic organization. It has a high density of classical GFAP-positive astrocytes as well as a more specialized population of glial cells that acts as a diffusion barrier at the AP/NTS border (Dallaporta et al., 2010). The reasons for this high astrocytic density in NTS are unknown. However, NTS astrocytes respond to peripheral chemical signals such as TNFα and thrombine (Hermann and Rogers, 2009; Hermann et al., 2009), and they participate in homeostatic responses such as thrombin-induced gastric stasis and α2-adrenergic receptor-dependent cardiovascular responses (Bhuiyan et al., 2009; Hermann et al., 2009). A recent paper published by McDougal et al. (2011) in *The Journal of Neuroscience* showed that NTS astrocytes can be activated directly by vagal afferents through an atypical mechanism, suggesting that these cells could participate in the modulation of NTS integrative function in physiological conditions.

McDougal et al. (2011) used Ca^{2+} imaging to probe astrocyte activation by afferent stimulation of the tractus solitarius (TS) in horizontal slices of rat NTS. Astrocytes were identified as cells retaining red fluorescent marker sulforhodamine 101 (SR101). Astrocytes responded to TS stimulation with large Ca^{2+} transients that were blocked by tetrodotoxin, indicating that they resulted from fiber activation. This does not rule out the participation of second-order neurons, however.

Astrocytic response to TS stimulation was mediated by glutamate, as expected from the activation of vagal afferents to NTS, and depended on AMPA receptors (AMPARs), but not NMDA or group I metabotropic glutamate receptors. The presence of functional AMPARs on NTS astrocytes was confirmed by detection of AMPAR subunit GluR1 expression on GFAP-labeled astrocytic processes. Interestingly, the diffuse pattern of GluR1 staining suggests that a substantial part of these receptors are located on distal processes where astrocytes contact synapses rather than on proximal GFAP+ branches. Demonstration of a colocalization with markers present on distal processes such as glial glutamate transporters GLT-1 or GLAST would clarify this point.

AMPA-mediated Ca^{2+} transients depended both on extracellular Ca^{2+} and on

Received Dec. 5, 2011; revised Jan. 10, 2012; accepted Jan. 11, 2012.

This work was supported by a grant from the Swiss National Science Foundation to Jean-Yves Chatton.

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DOI:10.1523/JNEUROSCI.6049-11.2012

 0270-6474/12/322579-03$15.00/0

internal stores, as shown by the effects of Ca^{2+}-free medium and thapsigargin, respectively. A decrease in Ca^{2+} response produced by philanthotoxin (PhTx) indicated that Ca^{2+} influx was partly mediated by Ca^{2+}-permeable AMPARs. The partial effect of PhTx suggests that other modes of Ca^{2+} entry coexist. This might include activation of the Na^+/Ca^{2+} exchanger in the reverse mode following Na^+ influx through AMPARs (Verkhratsky et al., 2011). Activation of voltage-gated Ca^{2+} channels by cell depolarization or of purinergic P2X autoreceptors by ATP released from stimulated astrocytes could also contribute to the Ca^{2+} rise. The release of Ca^{2+} from internal stores by Ca^{2+}-gated ryanodine receptors shown in this paper would serve as an amplification step to the AMPA-dependent Ca^{2+} influx.

Astrocytes are a heterogeneous population (Matyash and Kettenmann, 2010). Those recorded by McDougal et al. (2011) displayed staining by GFAP, S100B, and SR101, which are typical markers of classical astrocytes. In cortical regions, classical astrocytes have also been defined by a linear current–voltage relationship, gap-junctional coupling, the presence of glutamate transporter currents, and the lack of AMPARs, whereas complex astrocytes have opposite characteristics (Matyash and Kettenmann, 2010). Brainstem astrocytes seem to follow the same classification scheme except that AMPARs are expressed by all subpopulations (Grass et al., 2004). It would be of great interest to have a more detailed characterization of TS-responsive astrocytes at electrophysiological and molecular levels and to compare them to other NTS astrocytes. Subspecialization of brainstem astrocytes has been shown to support different responses to homeostatic signals (Erlichman et al., 2004). Similarly, distinct subgroups of NTS astrocytes responding to different peripheral signals could participate in specific homeostatic pathways.

McDougal et al. (2011) focused on receptor-mediated astrocytic responses to glutamate that could potentially lead to modulation of neural circuits through the release of gliotransmitters (Hermann et al., 2009). However, glutamate from vagal afferents could also affect neuronal networks via activation of astrocytic metabolism by a mechanism similar to that of the astrocyte-to-neuron lactate shuttle (ANLS) (Pellerin and Magistretti, 2011). According to the ANLS theory, glutamate is taken up by astrocytes together with Na^+, leading to an increase in intracellular Na^+ and to the subsequent activation of Na/K ATPase. The pump in turn stimulates astrocytic glycolysis, resulting in the production and release of lactate that is then used as an energy substrate by neurons. Recently, it appeared that lactate not only fuels neurons but could also increase their excitability by closing K_{ATP} channels (Song and Routh, 2005; Parsons and Hirasawa, 2010). In agreement with this observation, ANLS-like mechanisms were shown to regulate homeostatic circuits such as those in the Na^+-sensitive subfornical organ. In this CVO, astrocytes monitor systemic Na^+ levels and proportionally stimulate neighboring GABAergic interneurons by the release of lactate (Shimizu et al., 2007).

Lactate has also been shown to affect neuronal responses in regions regulating energy metabolism. In NTS, lactate blunted the response of glucose-sensing neurons to glucose and the counter-regulatory response to hypoglycemia (Himmi et al., 2001; Patil and Briski, 2005a,b). Similar effects were discovered in hypothalamic metabolic centers, such as ventromedial and lateral areas, in which lactate appeared to differentially regulate glucose-inhibited and glucose-exited cells (Song and Routh, 2005; Parsons and Hirasawa, 2010). This suggests a general role for lactate as an indicator of energy balance. Activation of lactate release by vagal afferents would thus be especially significant for the regulation of energy metabolism and feeding behavior by NTS. Because astrocytes potentially also respond to metabolism-related hormones, they could act as integrators of homeostatic signals driving NTS responses.

To summarize, the findings of McDougal et al. (2011) confirm previous reports of astrocyte activation by vagal primary afferents (Ballanyi et al., 1993) and describe an uncommon mechanism leading to direct stimulation of Ca^{2+} responses by AMPARs. These experiments show that astrocytes in NTS are positioned to control the first relay in vago-vagal reflexes. Given that these astrocytes are also receptive to blood-born peripheral signals and can modulate neuronal networks through neuroactive mediators and neurometabolic coupling, they appear to be integrators of organism homeostatic state set to tune autonomic and behavioral adaptive responses to varying internal conditions. These astrocytes could also regulate the plasticity of NTS microcircuits, via mechanisms similar to those present in hypothalamus, by the release of gliotransmitters (Gordon et al., 2009), and through modifications of astrocytic synaptic coverage during homeostatic challenges (Oliet et al., 2006). It is also predictable that alterations of NTS astrocytic physiology will be involved in pathological conditions such as metabolic disorders (Yi et al., 2011). Further work is needed to better characterize NTS astrocyte subpopulations, to investigate the modulation of astrocytic function by chemical peripheral signals, and to clarify the interaction of these astrocytes with specific neuronal subcategories driving homeostatic responses.

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