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Defining the Role of Astrocytes in Neuromodulation

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Astrocytes undergo elevations in intracellular calcium following activation of metabotropic receptors, which may trigger glutamate secretion and excitation of surrounding neurons. In this issue of *Neuron*, Fiacco et al. use transgenic mice that express a foreign G_q-coupled receptor in astrocytes to show that selective stimulation of astrocytes is not sufficient to induce the release of glutamate.

Calcium transients are as prevalent in astrocytes as action potentials in neurons. They are induced by activation of metabotropic receptors following transmitter release from nerve terminals and can propagate as waves through processes and among neighboring astrocytes via ATP release. Recent studies indicate that elevation of cytosolic Ca^{2+} within astrocytes can also trigger the release of glutamate through fusion of secretory vesicles, resulting in the activation of glutamate receptors on surrounding neurons. These results suggest that astrocytes not only listen and react to ongoing neural activity but also have the ability to rapidly modulate this activity. If correct, the implications of these findings would be profound, with astrocytes representing a potent feedforward excitatory influence able to synchronize the firing of pyramidal neurons in spatially restricted domains. However, a recent study by Fiacco et al. (2007) reported in this issue of *Neuron* suggests that the primary role of astrocyte Ca^{2+} signaling may not be to release glutamate and excite neurons. They show that selective elevation of Ca^{2+} within hippocampal astrocytes through activation of metabotropic receptors is not sufficient to trigger glutamate release and activation of NMDA receptors in surrounding pyramidal neurons.

Astrocytes extend highly ramified processes between cells and around excitatory synapses throughout the CNS. The close proximity of astrocytes to synapses allows them to place glutamate transporters at sites of release and restrict interactions between neighboring synapses. Although previously thought to be passive elements because of the conspicuous absence of electrical potentials, astrocytes have been shown to express metabotropic receptors for many neurotransmitters, including glutamate, GABA, norepinephrine, and acetylcholine. Activation of these receptors, either by exogenous agonists or through endogenous activity-dependent release from terminals, results in an elevation of cytosolic Ca^{2+} through activation of G_q, formation of IP_3, and subsequent Ca^{2+} release from internal stores. In response, astrocytes often exhibit oscillations or repetitive spikes in Ca^{2+} that outlast agonist exposure, events that can be restricted to discrete microdomains within a portion of an astrocyte process. Although initially shown for astrocytes in culture, astrocytes in vivo also exhibit spontaneous Ca^{2+} transients (Hirase et al., 2004), and astrocytes in the barrel cortex undergo a robust elevation in $[Ca^{2+}]_i$ in response to repetitive whisker deflection (Wang et al., 2006), indicating that this Ca^{2+}-based excitability is a fundamental aspect of astrocyte biology in situ. While such signaling could initiate morphological changes or alter gene expression, events more or less constrained to astrocytes, the intimate relationship between astrocytes and neurons has raised the possibility that such events could also influence neuronal activity on a rapid timescale.

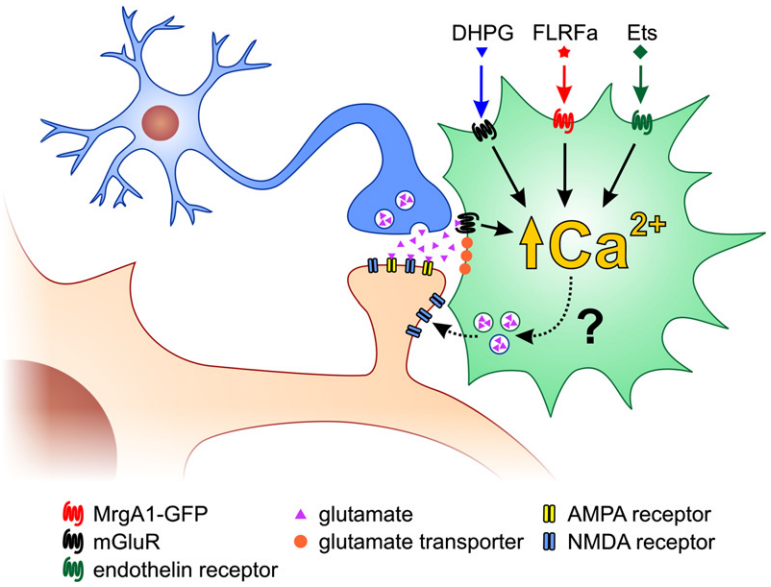

Figure 1. Neuromodulation by Astrocytes
A presynaptic neuron (blue) and a postsynaptic neuron (beige) form an excitatory synapse that is contacted by an astrocyte (green). Astrocytes express metabotropic receptors that can elevate $[Ca^{2+}]_i$. Previous studies have reported that this rise in astrocyte Ca^{2+} induces glutamate release and activation of neuronal NMDA receptors. Here, Fiacco et al. use transgenic mice that express a foreign metabotropic receptor, MrgA1, to ensure selective stimulation of astrocytes. Activation of MrgA1 did not lead to NMDA receptor activation in neurons, suggesting that elevation of astrocyte Ca^{2+} is not sufficient to trigger glutamate release. DHPG, agonist of group I metabotropic glutamate receptors; FLRFa, phe-leu-arg-phe amide, agonist of MrgA1; Ets, endothelins (1 and 3).

Astrocytes maintained in culture are capable of releasing glutamate into the media through various means, including volume-sensitive anion channels, gap junction hemichannels, and fusion of glutamate-filled secretory vesicles. Induction of astrocyte Ca^{2+} transients in astrocyte-neuron cocultures using metabotropic agonists, brief touch, or photolysis of caged Ca^{2+}, causes the appearance of an NMDA receptor-dependent slow inward current (SIC) in nearby neurons and an increase in the frequency of mEPSCs (Araque et al., 1998). These effects appear to require vesicle secretion from astrocytes, as it can be prevented by toxins that cleave SNARE proteins or by overexpression of dominant-negative SNAREs in astrocytes. Ca^{2+}-dependent exocytosis has been confirmed in astrocytes by monitoring whole-cell capacitance and destaining of amphipathic FM dyes using total internal reflection microscopy (Haydon and Carmignoto, 2006). However, these cultured cells bear little resemblance to astrocytes, notably lacking the highly ramified appearance of astrocytes within intact gray matter, raising concern that they may also exhibit physiological properties that are not representative of astrocytes in vivo. Nevertheless, essential aspects of this astrocyte-neuron signaling have been substantiated in acute brain slices. In some astrocytes, elevation of Ca^{2+} through stimulation of G_q-coupled metabotropic receptors, photolysis of caged IP_3 or caged Ca^{2+} directly infused into an astrocyte, or repetitive depolarization of the astrocyte membrane results in glutamate-dependent effects on neurons in the vicinity, including activation of presynaptic (Fiacco and McCarthy, 2004; Jourdain et al., 2007; Kang et al., 1998) and postsynaptic ionotropic receptors (Fellin et al., 2004; Parri et al., 2001). Thus, several groups have reported that elevation of Ca^{2+} in astrocytes in brain slices triggers the release of glutamate at a concentration sufficient to activate receptors on surrounding neurons (Figure 1).

However, concerns have been raised that the mechanisms that were used to induce Ca^{2+} elevations in astrocytes were either nonphysiological (global elevation of Ca^{2+} or IP_3, extreme depolarization) or nonselective (bath application of agonists of unknown specificity). As metabotropic receptors appear to provide the primary link between neuronal activity and astrocyte Ca^{2+} signaling in vivo, what is needed is a way to unambiguously activate only metabotropic receptors in astrocytes in situ. Fiacco and colleagues accomplished this feat by creating a line of mice in which a "foreign" G_q-coupled receptor is selectively expressed in astrocytes using a portion of the human GFAP promoter. To ensure specificity, they chose to express the MrgA1 receptor, a receptor that is only expressed in peripheral sensory neurons involved in nociception (Dong et al., 2001). By crossing *hGFAP-tTA* mice with *tetO-MrgA1-GFP* mice they were able force expression of MrgA1 in 80% of astrocytes in the hippocampus; selective expression of MrgA1 in astrocytes was confirmed through colocalization of GFP and GFAP. Although the endogenous ligand of MrgA1 has not been identified, this receptor can be activated by the phe-leu-arg-phe amide (FLRFa) peptide. Accordingly, application of FLRFa to acute hippocampal slices elicited a rise in Ca^{2+} in a majority of astrocytes. The amplitudes of these responses were comparable to those observed in response to mGluR activation, although FLRFa-induced Ca^{2+} transients were typically longer lasting. As expected, FLRFa did not alter astrocyte or neuron Ca^{2+} levels in *MrgA1*$^-$ (*hGFAP-tTA*$^+$) mice, confirming that *MrgA1*$^+$ mice provide a means to probe the consequences of astrocyte Ca^{2+} signaling in acute tissue.

While Ca^{2+} signaling in astrocytes has many potential consequences, Fiacco et al. focused their studies on NMDA receptor-dependent SICs. Unfortunately, however, application of FLRFa did not result in the appearance of SICs in CA1 pyramidal neurons in hippocampal slices from *MrgA1*$^+$ mice; FLRFa also did not affect the amplitude or kinetics of mEPSCs or induce Ca^{2+} transients in the dendrites of these neurons. Based on these findings,

they conclude that a metabotropic receptor-induced rise in Ca^{2+} is not sufficient to trigger glutamate release from astrocytes. Could the constitutive expression of the MrgA1 receptor have caused some unanticipated disruption in the ability of Ca^{2+} to trigger glutamate secretion? This seems less likely, as Fiacco et al. were able to replicate their own published findings (Fiacco and McCarthy, 2004), showing that photorelease of IP_3 within astrocytes caused a small increase in the frequency of spontaneous AMPA receptor-mediated EPSCs in *MrgA1*$^+$ mice, an effect that was previously shown to be blocked by group I mGluR antagonists (Fiacco and McCarthy, 2004). Nevertheless, these findings do not completely remove the possibility that expression of this foreign G_q-coupled receptor alters an aspect of astrocyte physiology that is critical for Ca^{2+}-dependent glutamate release near postsynaptic NMDA receptors.

What about mice in which MrgA1 has not been expressed? In a surprising twist, the authors also were unable to induce SICs in hippocampal pyramidal neurons in wild-type mice following bath application of endothelins or photorelease of Ca^{2+} in astrocytes, manipulations that produced reliable Ca^{2+} transients in astrocytes. However, NMDA receptor-dependent events reminiscent of SICs were observed in CA1 pyramidal neurons when *MrgA1*$^+$ hippocampal slices were exposed to hypotonic solution containing 20% less NaCl; these events were blocked by APV but were unaffected by pretreatment with bafilomycin A, an inhibitor of the proton ATPase that is required to load glutamate into secretory vesicles, suggesting that they do not arise through fusion of glutamate-loaded vesicles. To avoid the possibility that hypotonic solution induced glutamate release by acting indirectly though a G protein-mediated signaling pathway, the authors made use of a third line of transgenic mice in which the type 2 IP_3 receptor has been knocked out (Li et al., 2005). The type 2 IP_3 receptor appears to be the predominant isoform expressed by astrocytes, as application of a variety of metabotropic receptor agonists, including DHPG, to hippocampal slices from *IP3R2*$^{-/-}$ mice failed to elevate $[Ca^{2+}]_i$ in astrocytes. However, hypotonic solution reliably induced SIC-like events in these mice, indicating that there is a mode of glutamate release that does not require IP_3-dependent release of Ca^{2+} from astrocyte internal stores. While these experiments do not directly address why Ca^{2+} elevation was insufficient to trigger glutamate release in their hands, the findings suggest that cell swelling can induce slow NMDA receptor-mediated transients. In this regard, it will be important for those who reliably observe DHPG-induced SICs to assess whether this phenomenon is preserved in mice in which astrocytes express a dominant-negative SNARE (Pascual et al., 2005).

How are we to reconcile these findings with evidence from other laboratories that support the release of glutamate from astrocytes? In any physiological study where conflicting observations are made, there is a tendency to focus on technical minutiae. However, in this case a simple manipulation is all that is required to induce the phenomenon—bath application of a drug (e.g., DHPG) while looking for the appearance of relatively large, slow inward currents. Thus, it seems unlikely that recording conditions or technical expertise could provide an explanation. McCarthy and colleagues reported recently that constitutive expression of a different foreign receptor, in this case one that is coupled to G_i, led to hydrocephalus, despite the fact that the transgenic animals were never exposed to an agonist for this receptor. This finding highlights the potential problems associated with expressing a foreign receptor to achieve cell-specific manipulation. However, because Fiacco et al. were unable to elicit SICs in wild-type mice using even more forceful methods than metabotropic receptor activation to induce a rise in astrocyte $[Ca^{2+}]_i$, this cannot be the only explanation.

A consistent observation has been that SICs are observed in less than one-third of hippocampal CA1 pyramidal neurons following DHPG application (Fellin et al., 2004). Thus, excluding all other sources of variability, Fiacco et al. should have observed SICs in three of the nine CA1 pyramidal cells in *MrgA1*$^+$ mice that were exposed to DHPG in their study; although this is a small sample size, DHPG also failed to elicit neuronal Ca^{2+} transients in nine other neurons in *MrgA1*$^+$ mice. To have not observed the phenomenon raises the possibility that different groups have unknowingly selected different populations of pyramidal neurons for their recordings. Regardless, it appears that there is a yet unidentified variable that critically impacts glutamate signaling from astrocytes. In this regard, one has to consider that work in brain slices always involves a mix of physiology and pathology, particularly with regard to glial cells, which respond rapidly to tissue damage. Perhaps some aspect of astrocyte behavior changes with injury, either to allow or to prevent glutamate release in response to metabotropic receptor activation.

Caution should be exercised in extrapolating these results to all potential glutamate receptor-dependent phenomena. It is possible that more subtle glutamate signaling was induced following astrocyte Ca^{2+} signaling in *MrgA1*$^+$ mice. In addition, it is important to note that glutamate is only one potential mediator of rapid neuron-glia interactions. In particular, there is an emerging consensus that astrocyte-derived ATP is able to alter synaptic efficacy and neuronal excitability (Gordon et al., 2005; Newman and Zahs, 1998; Pascual et al., 2005). While there is not yet a consensus regarding the mechanisms responsible for glutamate release from astrocytes, these two mouse lines—the type 2 IP_3 receptor knockout and the astrocyte-specific expression of MrgA1$^+$—are powerful tools that have the potential to reveal the physiological consequences of astrocyte Ca^{2+} signaling within the intact nervous system.

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Is the Movement Representation in the Motor Cortex a Moving Target?

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In redundant neural networks, many different combinations of connection weights will produce the same output, thereby providing many possible solutions for a given computation. In this issue of *Neuron*, Rokni et al. propose that the arm movement representations in the cerebral cortex act like redundant networks that drift randomly between different synaptic configurations with equivalent input-output behavior because of random noise in the adaptive learning mechanism.

The arm and hand contain a few dozen muscles innervated by a few thousand spinal motor neurons, but tens of millions of neurons in several motor cortical areas contribute to their control. Because of this redundancy, it is theoretically possible for the response properties of single cortical neurons to change with time while the global cortical output signal continues to produce the same movements. Rokni et al. (2007) present evidence that the motor representations in the primary motor cortex (M1) and the supplementary motor area (SMA) of monkeys vary with time at the single-neuron level. They then show that a simple redundant neural network with a noisy learning mechanism will form a motor representation that is variable but still produces accurate movements and adapts to external forces.

During neural recordings, one sometimes observes gradual changes in the activity of a neuron, such as its baseline tonic rate or its response to a stimulus, over the course of minutes or hours. The origin of these changes is usually unknown, but the analytical complications they present can often be managed by such strategies as randomized task designs. In some studies, however, gradual drifts in neural activity over time are a problem. Suppose that one wants to study how neural activity changes while an animal learns a new stimulus-response rule. If neural responses are inherently stable, then one can assume that all observed activity changes are related to the learning process. However, if neural responses are not stable, an indefinable part of the observed changes are not learning related, which confounds their interpretation.

A case in point was a recent series of studies of neural activity in M1 and SMA of monkeys while they adapted to external forces during arm movements (Li et al., 2001; Padoa-Schioppa et al., 2004). During each daily learning session, monkeys made reaching movements in different directions in three consecutive blocks of 160 trials: (1) a *baseline* block with no external forces; (2) an *adaptation* block during which a velocity-dependent "viscous-curl" field pushed on the arm orthogonal to the direction of movement; (3) a *washout* block without forces. The monkeys quickly adapted to the curl field to straighten out their hand paths during the adaptation block and then readapted to the absence of forces in the washout block.

Neurons in M1 and SMA have broad directional tuning curves centered on