An active role for astrocytes in synaptic plasticity?

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Key words: Astrocyte, neuron glia communication, gliotransmission, synaptic plasticity.

Acknowledgements: I would like to thank A. Tzingounis and D. Mulkey for their support and comments on the manuscript.
ABSTRACT

Recently, Henneberger et al. (2010) block hippocampal long-term synaptic potentiation (LTP) induction by “clamping” intracellular calcium concentration of individual CA1 astrocytes, suggesting calcium-dependant gliotransmitter release from astocytes plays a role in hippocampal LTP induction. However, using transgenic mice to manipulate astrocytic calcium Agulhon et al. (2010) demonstrate no effect on LTP induction. Until the question of how intracellular calcium causes gliotransmitter release is answered, the role of astrocytes in synaptic plasticity will be incompletely understood.
While glial cells were discovered over 100 years ago, an understanding of their role in brain physiology has come about relatively recently. From their original name “glia” it was apparent that they were thought to simply glue the brain together. Later, it was found that glia comprise a heterogeneous population of cells that include oligodendrocytes, astrocytes and microglia, each performing unique physiological functions in the nervous system (Somjen 1988). In particular, astrocytes are important for neuronal metabolism, synapse formation, transmitter reuptake and potassium buffering (Kimelberg 2010). As these roles for astrocytes became accepted amongst the scientific community newer, more active roles for astrocytes have been proposed, involving astrocytic release of ATP, glutamate and D-serine (Zhang and Haydon 2005). While there have been some detailed studies of “gliotransmitter” release from astrocytes in culture, there is little data supporting gliotransmission in intact brain tissue.

Henneberger et al (2010) test the hypothesis that astrocytic release of D-serine is essential for generation of long-term synaptic potentiation (LTP) at the Schaffer collateral (SC) – CA1 pyramidal cell (CA1) synapse of the hippocampus (Figure 1, left panel). This is a reasonable hypothesis based on three previously established findings. First, D-serine is an agonist of the glycine-binding site of N-methyl D-aspartate receptors (NMDARs) (Mothet 2000). Second, astrocytes in the CA1 are known to contain D-serine in their cytoplasm (Schell 1997). Third, astrocytes in culture are known to release D-serine in a calcium-dependant manner (Mothet 2005). Also, it was recently shown that D-serine is the co-agonist of NMDARs during LTP in the supraoptic nucleus of the hypothalamus, and strong evidence for astrocytic D-serine release was demonstrated (Panatier et al. 2006). Henneberger et al (2010) aim to clarify the role of astrocytic D-serine release in
The use of “calcium-clamp” on individual astrocytes is certainly the strongest aspect of the study by Henneberger et al (2010). Previous studies have used high concentrations of the calcium buffer EGTA (e.g. 10 mM) to suppress calcium transients in astrocytes. However, the use of exogenous calcium buffers can only inhibit rapid calcium transients but likely does not affect slow changes in free calcium due to homeostatic mechanisms (Henneberger et al. 2010). By making whole-cell patches with an internal solution that contained low concentration of calcium and EGTA Henneberger et al (2010) are able to eliminate slow and transient calcium fluctuations throughout individual astrocytes by effectively “clamping” internal calcium at 50 to 80 nM.

Previous evidence indicates that D-serine release from astrocytes is dependent on increased intracellular calcium (Mothet 2005). Thus, the authors clamp calcium to eliminate all calcium-dependant transmitter release from the patched astrocyte while simultaneously using standard protocols to induce and record LTP at the SC – CA1 synapse of the hippocampus (Figure 1, left panel). Utilizing this approach the authors demonstrate that LTP is induced in the region of an astrocyte that is patched using control internal solution (i.e. little calcium buffering capacity), but not when the astrocyte is calcium-clamped. Interestingly, the effect of LTP inhibition is spatially restricted to the region of the individual calcium-clamped astrocyte. The ability to induce LTP persisted when the pipette monitoring dendritic field potentials is moved away from the calcium clamped astrocyte. This coincides with the observation that individual astrocytes occupy domains that do not overlap with neighboring astrocytes. The reader is left with the impression that each astrocyte regulates synapses within its own local region of influence, with little overlap
from neighboring astrocytes.

The authors next attempt to address the mechanism by which astrocytes facilitate LTP, i.e. whether astrocytes release D-serine that co-agonizes the CA1 NMDARs and leads to LTP induction. To test this the authors first step is logical; rescue the loss of LTP with exogenous D-serine. Exogenous D-serine does rescue LTP near the calcium-clamped astrocyte, suggesting a lack of glycine-binding site activation blocks LTP induction during calcium-clamp experiments. The next step, in this reader's mind, is to demonstrate that D-serine is the co-agonist involved in LTP inductions. In my opinion, this is where the authors' experimental approach becomes problematic.

In an effort to suppress gliotransmission the authors use Fluoroacetate because it is considered a selective “gliotoxin”, and in so doing are able to block LTP. However, fluoroacetate is selective to astrocytes only because they take it up across their membranes more readily than their neuronal counterparts (Hassel et al. 2002). Possible effects on neurons aside, the effect of fluoroacetate on astrocytes will not be D-serine-specific. Fluoroacetate is a metabolic poison, and will certainly cause inhibition of membrane transport processes, most notably glutamate. Experiments blocking astrocytic glutamate transport may clarify the effect seen with fluoroacetate. As discussed below, relatively little is known about gliotransmitter release in vivo, so the authors use the most astrocyte-specific inhibitor available, however, with so many possible non-specific effects it is difficult to interpret the results of these experiments.

In more compelling experiments Henneberger et al (2010) use HOAsp, an inhibitor of D-serine production, loaded into the intracellular astrocyte pipette to block LTP
induction. However, HOAsp only effectively blocks LTP after high frequency stimulation in the presence of APV, an NMDAR antagonist, which they claim expels previously produced D-serine. In addition, HOAsp is not specific to D-serine production and can affect pyruvate metabolism, hence the general metabolism of the patched astrocyte (Strísovský et al. 2005). Both experiments utilizing fluoroacetate and HOAsp are not as informative as simply inhibiting D-serine activation of NMDARs using the antagonist DAOO, a serine-degrading enzyme, as was done in similar studies in hypothalamus slice preparation and hippocampal cultured neurons (Panatier et al. 2006, Yang et al. 2006). Thus, the authors leave the reader to wonder whether D-serine is the NMDAR co-agonist involved in LTP induction.

A different test of the hypothesis that calcium-induced release of gliotransmitter by astrocytes is required for LTP at the SC – CA1 synapse of the hippocampus comes from Agulhon et al (2010) who report contradictory results when utilizing a genetic approach to control astrocytic intracellular calcium. This study uses two transgenic mouse models. One line is an astrocyte-specific knockin of the MrgA1 receptor (MrgA1+); an exogenous receptor that acutely increases intracellular calcium, via a Gq GPCR, when activated by its peptide agonist, which is also exogenous to the CNS. Using MrgA1+ mice they demonstrate that increased intracellular calcium in astrocytes does not affect LTP in the hippocampus. While this experiment does not actually contradict the results of Henneberger et al (2010), as they never tested the consequence of increasing astrocytic intracellular calcium, it certainly is not very supportive of an astrocytic role in LTP induction.

The second mouse line utilized by Agulhon et al (2010) is a knockout of inositol triphosphate receptor 2 (IP3R2). IP3R2 is the receptor thought to be responsible for increasing intracellular calcium in astrocytes, whereas IP3R1 and IP3R3 are expressed in
neurons. The authors claim total elimination of astrocyte calcium transients, both spontaneous and those induced by synaptic activation. However, unlike the results obtained using pipette solution to calcium-clamp astrocytes (Henneberger et al. 2010), the IP3R2 knockout mice demonstrate both short- and long-term potentiation that is identical in amplitude to the wild type population.

When faced with contradictory results one often looks for explanation in the different methods used. Both models boast abolishment of astrocytic calcium transients. Henneberger et al (2010) use an internal solution that effectively buffers free calcium fluctuations, whereas the IP3R2 knockout model blocks calcium from entering the cytoplasm from intracellular stores. Could these two approaches have differential ability to block calcium dependant transmitter release? The answer is probably yes. Henneberger et al (2010) are able to clamp calcium, whereas Agulhon et al. (2010) block all calcium fluctuations due to IP3R2 activation. Unfortunately, exactly how increased intracellular calcium causes release of gliotransmitters is not certain (Figure 1, right panel). A recent review (Hamilton and Attwell 2010) points out that activation of PAR1, but not P2Y1, receptors causes transmitter release in astrocytes, although both result in increased intracellular calcium. Equally perplexing, the calcium influx generated by activation of MrgA1 in astrocytes of MrgA1+ mice (described above) does not mediate glutamate-induced inward currents in local neurons, while uncaging IP3 to increase intracellular calcium in the same mice did result in an increased frequency of neuronal mEPSCs.

It would appear that simply increasing intracellular calcium is not sufficient for gliotransmitter release. If calcium is truly required for transmitter release, then it may need to occur in specific nanodomains, where it is difficult to experimentally manipulate calcium
fluctuations. In this case, knocking out IP3R2 or clamping intracellular calcium may block
generalized astrocytic calcium oscillations, but may not effectively control calcium in
certain nanodomains. Calcium entry in these locations may occur via mechanisms other
than the IP3 pathway, including TRP or voltage gated calcium channels. Or perhaps the
knockout mice have elevated levels of D-serine present in the synaptic cleft, due to
membrane mechanisms altered by lack of IP3R2 function. Yet, it is also possible that
buffering intracellular calcium could have other unanticipated effects, besides eliminating
calcium fluctuations. However, the latter possibility still strongly implies individual
astrocytes play a role in the synaptic plasticity of the hippocampus.

The past two decades have seen a dramatic advance in the field of astrocyte
physiology, with evidence of astrocytes playing an active role appearing in many facets of
synaptic function. The present study suggests a new role for astrocytes in memory
processing. Indeed, altering astrocyte physiology affects LTP induction at the SC – CA1
synapse of the hippocampus, but because of lack of evidence that D-serine is required for
LTP induction and contradictory results when using a transgenic approach (Agulhon et al.
2010) exactly how this occurs remains unanswered. To further understand how astrocytes
actively regulate synaptic transmission a few questions must be addressed (Figure 1, right
panel). Is D-serine the co-transmitter involved in LTP? How does elevated astrocytic
intracellular calcium lead to transmitter release? Is calcium increase necessary? If so, what
proteins does it act on to cause transmitter release, and what is their cellular location? It is
important that the mechanism(s) of gliotransmitter release are reconciled in order to form
a more complete understanding of how astrocytes actively participate in hippocampal LTP.


Model of astrocyte regulation of long-term synaptic potentiation (LTP) in the hippocampus. The left panel depicts the experimental design utilized by Henneberger et al (2010) to clamp astrocyte intracellular calcium and record dendritic field potentials at the Schaffer collateral (SC) – CA1 pyramidal cell (CA1) synapse in the hippocampus. The right panel depicts a model for LTP. The currently accepted model of this hippocampal synaptic LTP involves glutamate being released from the presynaptic neuron and diffusing across the synaptic cleft to activate postsynaptic receptors. N-methyl D-aspartate (NMDA) receptor activation is necessary for LTP induction and requires both glutamate binding and activation of the glycine-binding site. Classically, the latter is thought to be accomplished by tonic levels of glycine but Henneberger et al (2010) propose that D-serine, released from astrocytes, activates the glycine-binding site. In this model glutamate activates mGluRs on astrocytes to increase intracellular calcium and cause D-serine release, which in turn binds the glycine-binding site of the NMDA receptor. By clamping internal calcium fluctuations of an astrocyte via a patch pipette Henneberger et al (2010) demonstrate elimination of LTP that supports this hypothesis, however another study (Agulhon et al. 2010) that uses a transgenic approach to block intracellular calcium transients finds opposite results. In order to more completely understand the role of astrocytes in hippocampal synaptic LTP three questions must be answer in vivo. What mechanism does increased intracellular calcium act on to promote D-serine release? How is D-serine released by astrocytes? And finally, is D-serine the endogenous co-transmitter involved in synaptic LTP of the hippocampus?