An Active Role for Astrocytes in Synaptic Plasticity?

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Recently, Henneberger and colleagues blocked hippocampal long-term synaptic potentiation (LTP) induction by “clamping” intracellular calcium concentration of individual CA1 astrocytes, suggesting calcium-dependent gliotransmitter release from astrocytes plays a role in hippocampal LTP induction. However, using transgenic mice to manipulate astrocytic calcium, Agulhon and colleagues demonstrated 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.

Although glial cells were discovered over 100 years ago, an understanding of their role in brain physiology has come about only 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 includes 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 uptake, and potassium buffering (Kimelberg 2010). As these roles for astrocytes became accepted among the scientific community newer, more active roles for astrocytes have been proposed, involving astrocytic release of adenosine 5′-triphosphate, glutamate, and d-serine (Zhang and Haydon 2005). Although there have been some detailed studies of “gliotransmitter” release from astrocytes in culture, there are few data supporting gliotransmission in intact brain tissue.

Henneberger et al. (2010) tested 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 (Fig. 1, left). 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-dependent manner (Mothet 2005). Also, it was recently shown that d-serine is the coagonist of NMDARs during LTP induction (Fig. 1, left). Using this approach the authors demonstrated 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. 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 coagonizes 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 coagonist 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 that of glutamate. Experiments blocking astrocytic glutamate transport may clarify the effect seen with fluoroacetate. As discussed in the following text, relatively little is known about gliotransmitter release in vivo, so the authors use the most astrocyte-specific inhibitor available; however, with so...
many possible nonspecific effects it is difficult to interpret the results of these experiments. In more compelling experiments Henneberger et al. (2010) used l-erythro-3-hydroxyaspartate (HOAsp), an inhibitor of D-serine production, loaded into the intracellular astrocyte pipette to block LTP induction. However, HOAsp effectively blocks LTP after high-frequency stimulation only in the presence of 2-amino-5-phosphonovaleric acid (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, and thus the general metabolism of the patched astrocyte (Strˇ asovský et al. 2005). Both experiments using fluoroacetate and HOAsp are not as informative as simply inhibiting D-serine activation of NMDARs using the antagonist D-amino acid oxidase, 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 coagonist 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 using a genetic approach to control astrocytic intracellular calcium. The authors claim total elimination of LTP that supports this hypothesis, although another study (Agulhon et al. 2010) that uses a transgenic approach to block intracellular calcium transients finds opposite results. To more completely understand the role of astrocytes in hippocampal synaptic LTP three questions must be answered in vivo. 1) What mechanism does increased intracellular calcium act on to promote D-serine release? 2) How is D-serine released by astrocytes? 3) Is D-serine the endogenous cotransmitter involved in synaptic LTP of the hippocampus?

an exogenous receptor that acutely increases intracellular calcium, via a Gq G protein-coupled receptor, 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. Although this experiment does not actually contradict the results of Henneberger et al. (2010), in that 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 used 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 potentiations that are identical in amplitude to those of 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) used 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-dependent 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 (Fig. 1, right). 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 earlier) does not mediate glutamate-induced inward currents in local neurons, whereas uncaging IP3 to increase intracellular calcium in the same mice did result in an increased frequency of neuronal miniature excitatory postsynaptic currents.

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 transient receptor potential 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. Nonetheless, 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. Although evidence of astrocytes actively participating in hippocampal LTP is not reconciled to form a more complete understanding of how astrocytes actively participate in hippocampal LTP.

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REFERENCES


