

In her Journal Club, Caiati provides a cogent and fair review of arguments for and against monosynaptic GABA signaling at hippocampal mossy fiber (MF) synapses. Since GABA was found in MF terminals (Sandler and Smith, 1991) many studies have suggested that glutamatergic granule cells (GCs) also convey direct, monosynaptic GABA signals to CA3 pyramidal cells. Co-release of these two fast-acting neurotransmitters with functionally opposing actions could be specific to MF terminals and would be significant for information processing at this synapse. We would like to respond to some criticisms of our study and provide additional elements to the current debate.

Multiple preparations and experimental paradigms have been used to ask whether GABA is released from MF terminals. These include extracellular hilar stimulation in acute slices (Walker et al., 2001; Gutierrez et al., 2003; Safiulina et al., 2006), ³H-GABA release from isolated synaptosomes (Gomez-Lira et al., 2002) and, more recently, stimulation of putative single MF terminals contacting mechanically dissociated CA3 pyramidal cells (Beltran and Gutierrez, 2012). Like Caiati, we believe that the most convincing evidence probably comes from paired recordings of monosynaptically connected GCs and CA3 pyramidal cells. While a very low connectivity increases the difficulty of such experiments in acute slices, two studies have been done in slice cultures. One of them used rather mature slice cultures (Mori et al., 2004) where perhaps few GCs may still express GAD67. However in young slices, where a significant fraction of GCs express GAD67, our paired records provided no evidence for monosynaptic IPSCs (Cabezas et al., 2012). In our study, we focused on GAD67-expressing GCs identified by GAD67-driven GFP expression. Yet, monosynaptic, GABA-mediated IPSPs could not be identified. Although we agree with Caiati that organotypic slice cultures lack a number of afferents, it is unclear to us why and how this may specifically prevent the postsynaptic aggregation of GABAA receptors at MF synapses on CA3 pyramidal cells. In fact, in many years of synaptic physiology research on slice cultures, we are not aware of any differences in the properties of inhibitory signaling specific to this preparation.

Although our study confirms that GABA synthesized in MF may be released upon repetitive firing and activate presynaptic GABAB receptors, it is still unclear whether tonic firing releases GABA. In fact, several studies conclude that MF terminals do not express VIAAT (Sperk et al., 2003; Uchigashima et al., 2007). If not, GABA release presumably depends on a reversed action of the GAT-1 transporter that probably requires sustained firing (Wu et al., 2007). Furthermore, while immunogold staining (Bergersen et al., 2003) has shown postsynaptic GABAA receptors facing MF terminals, receptor density may not be sufficiently high to mediate detectable IPSCs. Postsynaptic clustering of GABAA receptors depends on gephyrin and other molecular scaffold components. None of the relevant molecules have been demonstrated to anchor GABAA receptors at this glutamatergic synapse. In their absence, GABAA receptors at MF synapses may originate from an extrasynaptic receptor fraction diffusing in the excitatory synaptic membrane, as observed in single particle tracking experiments (Renner et al., 2012). The density and dwell time of these exogenous receptors may not suffice to mediate synaptic currents.

Finally, we have established that GAD67 expression is not an age-dependent phenotype but instead reflects a maturation stage of GCs throughout ontogenesis (Cabezas et al., 2012). Caiati therefore suggests using retroviral transduction of GC precursors with channelrhodopsin to test responses induced in CA3 pyramidal cells by photoactivation. This important experiment was performed in 2008 by Toni and colleagues (Toni et al., 2008). Yet, photoactivation of the transduced GCs did not induce monosynaptic GABA responses in CA3 pyramidal cells.

In conclusion, our results and others show that GABA is clearly synthesized in and released from immature GCs. We agree with Caiati that it is hard to be certain whether GABA monosynaptically inhibits postsynaptic pyramidal cells. However, this GABA modulates MF excitability via presynaptic auto-receptors. Perhaps the role of GABA synthesis in maturing MFs is therefore primarily presynaptic. Its relevance to MF maturation and path finding offers an intriguing perspective that remains to be explored.

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