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Conditions and Constraints for Astrocyte Calcium Signaling in the Hippocampal Mossy Fiber Pathway

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SUMMARY

The spatiotemporal activities of astrocyte Ca^{2+} signaling in mature neuronal circuits remain unclear. We used genetically encoded Ca^{2+} and glutamate indicators as well as pharmacogenetic and electrical control of neurotransmitter release to explore astrocyte activity in the hippocampal mossy fiber pathway. Our data revealed numerous localized, spontaneous Ca^{2+} signals in astrocyte branches and territories, but these were not driven by neuronal activity or glutamate. Moreover, evoked astrocyte Ca^{2+} signaling changed linearly with the number of mossy fiber action potentials. Under these settings, astrocyte responses were global, suppressed by neurotransmitter clearance, and mediated by glutamate and GABA. Thus, astrocyte engagement in the fully developed mossy fiber pathway was slow and territorial, contrary to that frequently proposed for astrocytes within microcircuits. We show that astrocyte Ca^{2+} signaling functionally segregates large volumes of neuropil and that these transients are not suited for responding to, or regulating, single synapses in the mossy fiber pathway.

INTRODUCTION

Important progress has been made in understanding the roles of glia in the brain since their discovery over a century ago and following landmark physiological studies (Kuffler, 1967). Astrocytes (a subclass of glia) are known to display dynamic intracellular Ca^{2+} signals (Agulhon et al., 2008; Li et al., 2013), and it has recently been shown that astrocytes rapidly sense and regulate single synapses (Di Castro et al., 2011; Panatier et al., 2011). In these settings, astrocytes respond to single synapse glutamate release by exhibiting localized intracellular Ca^{2+} elevations in their main processes a few micrometers from the soma, implying that they are actively involved in microcircuit function (Di Castro et al., 2011; Panatier et al., 2011). However, abolishing one major form of intracellular Ca^{2+} signal within astrocytes was without obvious consequence for neuronal function (Agulhon et al., 2010; Fiacco et al., 2007; Petravicz et al., 2008). To understand these differences, one needs to explore when astrocytes become excited within neuronal circuits, but this has proven challenging. Unlike neurons, astrocytes are not electrically excitable and evaluations have had to rely on imaging. In particular, available imaging methods that use organic Ca^{2+} indicator dyes are not ideal for monitoring astrocyte branches, which are the primary sites for interactions with neurons (Reeves et al., 2011; Shigetomi et al., 2010, 2013a; Tong et al., 2013). As a result, the function of astrocyte Ca^{2+} signaling within neuronal circuits remains incompletely explored (Tong et al., 2013).

We have refined optical and genetic tools in order to study astrocyte branches and territories by building on recent progress with genetically encoded calcium indicators (GECIs) (Tian et al., 2012). GECIs are not a panacea, and they have effects such as Ca^{2+} buffering that are shared with organic dyes. However, under most circumstances they do not obviously perturb neurons or astrocytes and are complementary to other approaches (Chen et al., 2013; Shigetomi et al., 2013a, 2013b; Tian et al., 2009; Zariwala et al., 2012).

To image cytosolic and near-membrane Ca^{2+}, we used astrocyte-specific expression of cytosolic GCaMP3 or membrane-targeted Lck-GCaMP3, respectively (Shigetomi et al., 2010, 2011, 2013a; Tian et al., 2009). To directly image astrocyte cell-surface glutamate signals, we used a genetically encoded glutamate sensor (Marvin et al., 2013) (iGluSnFR). We also used GCaMP6f, a recent GECI with kinetics similar to the organic Ca^{2+} indicator dye OGB1-AM (Chen et al., 2013). In order to drive neurotransmitter release selectively from the mossy fiber pathway, we generated novel BAC transgenic "*SPRAE*" mice that express a drug-activated ion channel within the mossy fiber pathway. Using these optical and pharmacogenetic tools, we explored astrocyte signaling in the circuit formed by the dentate gyrus granule cell projection (the mossy fiber pathway) to the

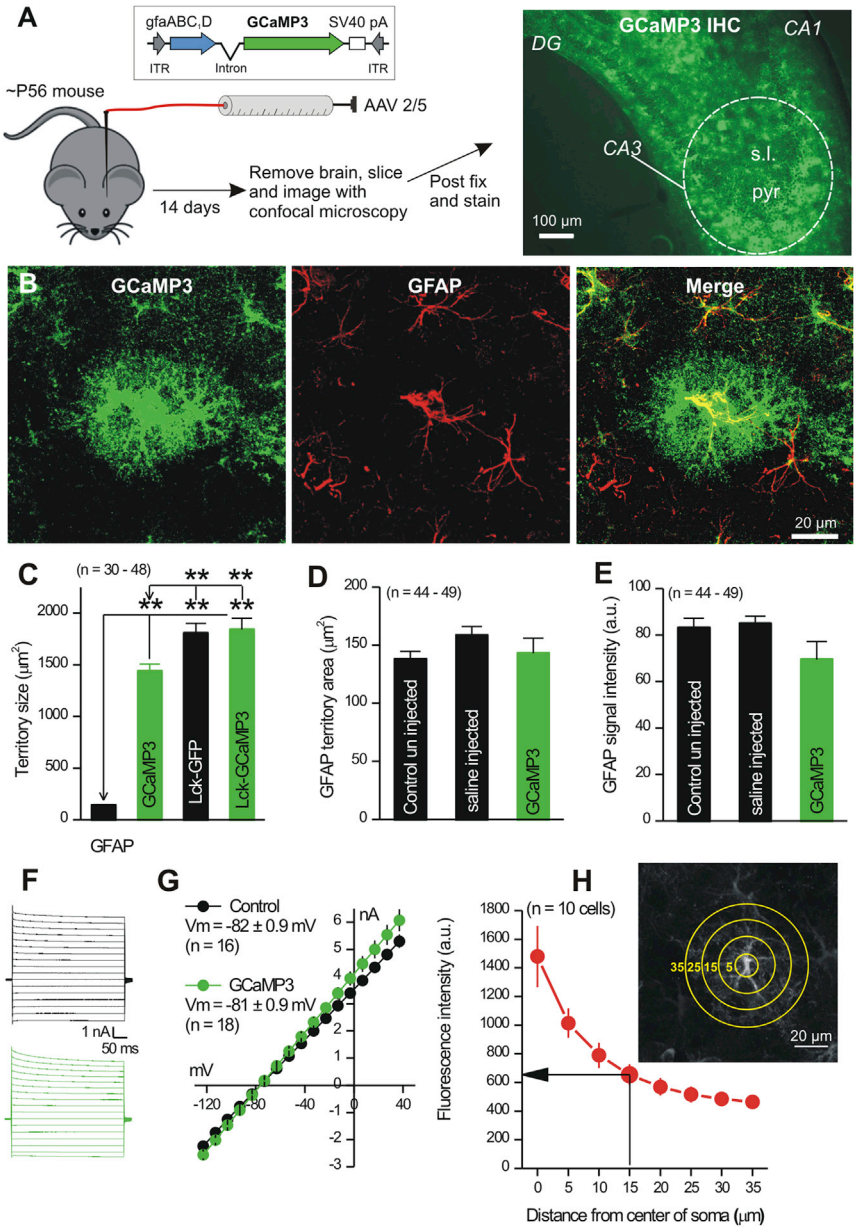

Figure 1. Expression of GCaMP3 in Astrocytes

(A) The cartoon illustrates the procedure to inject AAV2/5 capable of expressing GCaMP3 in s.l. astrocytes of P56 mice. The right hand image shows fluorescence signal for GCaMP3 detected by immunohisochemistry (IHC) in the CA3 region (DG indicates dentate gyrus; pyr indicates pyramidal cell layer).

(B) GCaMP3 and GFAP expression in an astrocyte from the s.l. region. The GCaMP3-expressing astrocyte was GFAP positive and 85% ± 2% of all GFAP-positive astrocytes in the s.l. expressed GCaMP3 (n = 4 mice).

(C) Maximal projection territory area for GFAP, GCaMP3, Lck-GCaMP3, and Lck-GFP.

(D and E) GFAP maximal projection territory area (D) and intensity (E) for astrocytes.

(F and G) Traces and average data for astrocyte current-voltage relationships (−120 to +40 mV) from control mice or those microinjected with GCaMP3.

(H) The image shows a representative astrocyte with circles drawn radially (5 µm spacing). Such circles were used to measure the intensity of GCaMP3 expression at increasing distances from the center of the soma (in the graph). The highest intensity was in the soma, which has the largest volume, and intensity fell with distance. Average data are shown as mean ±SEM.

CA3 region of the hippocampus (Amaral and Lavenex, 2007). We chose this circuit because mossy fibers are the only feed-forward excitatory input to the anatomically well-defined CA3 region of the stratum lucidum (s.l.) (Amaral and Lavenex, 2007; Ruiz and Kullmann, 2012; Spruston and McBain, 2007). Additionally, the anatomical relationship between mossy fibers and postsynaptic neuronal and astrocytic targets has been described by electron microscopy (Rollenhagen and Lübke, 2006; Rollenhagen et al., 2007; Wilke et al., 2013).

RESULTS

We deployed several imaging tools to study astrocytes located in the s.l. of the adult mouse hippocampus (~P70). The slices were ~300 µm thick, and the imaged astrocytes were located ~40 µm from the slice surface. The experiments were conducted at room temperature (~21°C) or close to mouse body temperature (34°C), as indicated. The imaging was performed using laser scanning confocal microscopy with a 40× objective lens with a numerical aperture of 0.8. The effective pixel size was 0.2 × 0.2 µm, which is larger than the size of astrocyte branchlets at <100 nm.

GCaMP3 Reveals Stratum Lucidum Astrocyte Branches and Territories

We could not reliably load hippocampal astrocytes from adult mice with organic Ca^{2+} indicator dyes. By extending tool development work that showed GCaMP3 is well suited to study astrocytes from adult mice (Shigetomi et al., 2013a, 2013b), we used it, Lck-GCaMP3, and GCaMP6f to explore s.l. astrocyte intracellular Ca^{2+} signals. For selective expression within astrocytes, we used adeno-associated viruses of the 2/5 serotype (AAV 2/5) and the astrocyte-specific *gfaABC$_1$D* promoter (Shigetomi et al., 2013a) (Figure 1). GECIs were innocuously expressed within large parts of s.l. astrocyte territories, including their branches. Furthermore, AAV2/5-mediated expression of fluorescent proteins does not alter spontaneous Ca^{2+} signals in astrocytes (Shigetomi et al., 2013a), which recalls and extends past work with neurons that reported little deleterious effect of GECI expression (Chen et al., 2013; Shigetomi et al., 2013b; Tian et al., 2009; Zariwala