Metabolic Interplay between Astrocytes and Neurons Regulates Endocannabinoid Action

Andreu Viader
The Scripps Research Institute

Jacqueline L. Blankman
Abide Therapeutics

Peng Zhong
Medical College of Wisconsin

See next page for additional authors

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Graphical Abstract

Highlights
- Genetic mouse models reveal cellular specificity of 2-AG metabolism
- Astrocytes and neurons collaborate to terminate endocannabinoid signaling
- Coordinated astrocytic-neuronal metabolism protects against CB_{1}R desensitization
- Astrocytes couple 2-AG hydrolysis to neuroinflammatory prostaglandin production

Authors
Andreu Viader, Jacqueline L. Blankman, Peng Zhong, ..., Dana E. Selley, Laura J. Sim-Selley, Benjamin F. Cravatt

In Brief
The endocannabinoid 2-arachidonoylglycerol (2-AG) is a retrograde lipid messenger that broadly modulates brain synapses, neurophysiology, and behavior. Viader et al. show that endocannabinoid signaling is regulated by the cooperative, transcellular metabolism of 2-AG, which is shuttled between neurons and astrocytes.

Correspondence
cravatt@scripps.edu
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Andreu Viader, Jacqueline L. Blankman, Peng Zhong, Xiaojie Liu, Joel E. Schlosburg, Christopher M. Joslyn, Qing-Song Liu, Aaron J. Tomarchio, Aron H. Lichtman, Dana E. Selley, Laura J. Sim-Selley, and Benjamin F. Cravatt

1The Skaggs Institute for Chemical Biology
2Department of Chemical Physiology
The Scripps Research Institute, La Jolla, CA 92037, USA
3Abide Therapeutics, San Diego, CA 92121, USA
4Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226, USA
5Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, CA 92037, USA
6Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298, USA

*Correspondence: cravatt@scripps.edu

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SUMMARY

The endocannabinoid 2-arachidonoylglycerol (2-AG) is a retrograde lipid messenger that modulates synaptic function, neurophysiology, and behavior. 2-AG signaling is terminated by enzymatic hydrolysis—a reaction that is principally performed by monoacylglycerol lipase (MAGL). MAGL is broadly expressed throughout the nervous system, and the contributions of different brain cell types to the regulation of 2-AG activity in vivo remain poorly understood. Here, we genetically dissect the cellular anatomy of MAGL-mediated 2-AG metabolism in the brain and show that neurons and astrocytes coordinately regulate 2-AG content and endocannabinoid-dependent forms of synaptic plasticity and behavior. We also find that astrocytic MAGL is mainly responsible for converting 2-AG to neuroinflammatory prostaglandins via a mechanism that may involve transcellular shuttling of lipid substrates. Astrocytic-neuronal interplay thus provides distributed oversight of 2-AG metabolism and function and, through doing so, protects the nervous system from excessive CB1 receptor activation and promotes endocannabinoid crosstalk with other lipid transmitter systems.

INTRODUCTION

Historical neuron-centric conceptualizations of brain function have given way to a deeper appreciation of the important roles played by additional brain cell types in regulating chemical transmission throughout the nervous system (McIver et al., 2013). Astrocytes are now recognized as integral components of synapses and central regulators of neurotransmission and interneuronal communication (Halassa and Haydon, 2010). Astrogial transporters clear neurotransmitters, such as glutamate and γ-amino butyric acid (GABA), from synaptic clefts to modulate the signaling activities of these chemical messengers and limit their toxic accumulation in the extracellular space (Danbolt, 2001). Metabolic pathways in astrocytes are also essential for the recycling and resupply of neurotransmitters needed to maintain sustained rounds of synaptic activity (Coulter and Eid, 2012). Moreover, defects in astrocytic functions that lead to alterations in the homeostasis of major neurotransmitters have been linked to a variety of mental and neurodegenerative disorders (Allaman et al., 2011; Halassa and Haydon, 2010). Microglia, which are resident immune cells of the nervous system, also contribute to neurotransmission through activity-dependent remodeling and pruning of synapses (Salter and Beggs, 2014; Schafer et al., 2012). Efforts to elucidate the contributions of glia to the regulation of synaptic function therefore stand to both enrich our mechanistic understanding of intercellular communication in the brain and provide new therapeutic avenues for the treatment of diverse neurological conditions.

The endogenous cannabinoid (endocannabinoid or eCB) 2-arachidonoylglycerol (2-AG) is an arachidonic acid (AA)-derived retrograde lipid messenger that broadly modulates synaptic function throughout the nervous system (Fowler et al., 2005; Kano et al., 2009; Pacher et al., 2006). 2-AG is synthesized by diacylglycerol lipases (DAGLs) (Reisenberg et al., 2012) and released in an activity-dependent manner from postsynaptic neurons to act on presynaptic G-protein-coupled cannabinoid receptors CB1 (CB1R) and CB2, which are also targets of the primary psychoactive component of marijuana (Δ9-tetrahydrocannabinol) (Mechoulam and Hanus, 2000). Principally through its activity on CB1Rs, 2-AG inhibits neurotransmitter release and regulates diverse neurophysiological processes, including mood, nociception, appetite, and memory (Pacher et al., 2006). Enzymatic hydrolysis of 2-AG, which is primarily mediated by monoacylglycerol lipase (MAGL) in the nervous system (Dinh et al., 2002; Ueda et al., 2013), terminates CB1R-dependent signaling (Chanda et al., 2010; Schlosburg et al., 2010; Zhong et al., 2011) and concomitantly provides a major source of AA transporters.
for brain eicosanoid synthesis (Nomura et al., 2011). The DAGL and MAGL enzymes thus tightly regulate 2-AG-mediated neurotransmission and have historically been viewed as doing so through their respective expression in the pre- and post-synaptic compartments of neurons (Di Marzo et al., 2015). Giall cells may also serve as cellular substrates for the modulation of 2-AG, however, as both astrocytes and microglia release this eCB and express CB1Rs (Navarrete and Araque, 2008, 2010; Stella, 2010; Walter et al., 2004; Witting et al., 2004). Furthermore, ectopic expression of MAGL in Bergmann glia has been shown to attenuate 2-AG-dependent retrograde synaptic suppression in cerebellar slices from MAGL-/- mice (Taniruma et al., 2012). Nonetheless, the relative contributions of neurons and glia to the termination of 2-AG-dependent synaptic and neurobehavioral functions, as well as the potential for crosstalk between these cell types to facilitate the recycling and homeostasis of eCB-eicosanoid pools, remain largely unexplored.

Global pharmacological or genetic disruption of MAGL robustly elevates brain 2-AG content (Chanda et al., 2010; Long et al., 2009; Schlosburg et al., 2010), alters CB1R-mediated signaling and behaviors (Chanda et al., 2010; Long et al., 2009; Schlosburg et al., 2010; Zhong et al., 2011), and impairs neuroinflammation through mechanisms that involve eCB-eicosanoid crosstalk (Nomura et al., 2011). With the goal of determining the relative contribution of different brain cell types to 2-AG metabolism and signaling in vivo, we report the generation and characterization of a conditional genetic mouse model used to ablate MAGL globally and specifically in neurons, astrocytes, and microglia. We discover that neuronal and astrocytic MAGL coordinately regulate brain 2-AG content, contribute to the termination of synaptic 2-AG signaling at CB1Rs, and, by doing so, maintain a balanced eCB tone that protects against desensitization caused by excessive signaling. Moreover, we show that astrocytic MAGL is principally responsible for generating 2-AG-derived arachidonate pools used for the synthesis of pro-inflammatory prostaglandins in the brain. Finally, we provide evidence for the transcellular shuttling of 2-AG and related metabolites between neurons and astrocytes as one mechanism by which these cells can coordinately regulate eCB-eicosanoid pathways in the nervous system. The distributed metabolism of 2-AG across both neurons and astrocytes thus affords the nervous system more refined control over dynamic features of eCB activity, including protection against desensitization and facilitating crosstalk with other lipid transmitter systems that are deregulated in disease states.

RESULTS

Cell-Type-Specific Deletion of MAGL in the Nervous System

To examine the contribution of different brain cell types to 2-AG metabolism and signaling in vivo, we generated a genetic mouse model for conditional deletion of the MAGL enzyme by flanking the catalytic exon of the Mgl gene (exon 4, containing catalytic S122) with loxP recombination sites (MAGLloxP, Figures 1A and S1A–S1C). The MAGLloxP line was initially validated by crossing to mice that ubiquitously express Cre recombinase (Rosa26-Cre; Otto et al., 2009) to produce total MAGL-/- mice (MAGL-TKO). Consistent with previous reports for other conventional MAGL-/- lines (Chanda et al., 2010; Schlosburg et al., 2010), MAGL-TKO mice lacked detectable MAGL expression and activity across different central and peripheral tissues and cell types (Figures 1B–1E and S1D). MAGL-TKO mice also exhibited marked (~90%) reductions in brain 2-AG hydrolysis (Figure 1F), as well as ~10-fold elevations in brain 2-AG content (Figure 1G).

We next crossed homozygous MAGLloxP mice to mice with restricted expression of Cre recombinase in neurons (Eno2-Cre; Frugier et al., 2000), astrocytes (GFAP-Cre; Tao et al., 2011), or microglia (LysM-Cre; Clausen et al., 1999) to generate mouse lines that lack MAGL specifically in these three brain cell types (MAGL-NKO, MAGL-AKO, and MAGL-MKO, respectively). Crossing of Eno2-, GFAP-, and LysM-Cre mice to a Cre-inducible Rosa26-tomato reporter line validated efficient Cre-mediated recombination throughout the brain of these animals, selectively in neurons, astrocytes, and microglia, respectively (Figure S1E). While the Eno2- and GFAP-Cre lines produced Cre-mediated recombination in the vast majority (80–90%) of neurons and astrocytes, respectively (with the GFAP-Cre line also showing low-level (~2%–6%) recombination in neurons, consistent with previous studies; Tao et al., 2011), the LysM-Cre line only appeared to promote Cre-mediated recombination in ~50%–60% of lba1-positive microglia in the brain. Cultured preparations of brain cells (Figure S1F and S1G), however, confirmed selective and near-complete loss of MAGL expression in microglia from MAGL-MKO mice, as well as in neurons (GABAergic and glutamergic; Figure S1H) and astrocytes from MAGL-NKO and MAGL-AKO, respectively (Figure 1B). Importantly, each MAGL-disrupted brain cell type also displayed significantly reduced 2-AG-hydrolytic activity, although the relative magnitude of this decrease was greater for neurons compared to astrocytes or microglia, possibly due to the presence of additional 2-AG hydrodases in glia (Marrs et al., 2010; Figure 1C).

Having established mouse models that display selective loss of MAGL in different brain cell types, we next examined how neurons, astrocytes, and microglia contribute to bulk 2-AG metabolism in the nervous system. Deletion of MAGL in both neurons and astrocytes, but not microglia, substantially reduced the total quantity of active enzyme in brain tissue, as measured by activity-based protein profiling (ABPP; Figures 1D and 1E). These data mirrored the effects on brain 2-AG hydrolysis, which was decreased by ~60% and 40% in MAGL-NKO and MAGL-AKO mice, respectively, but unaltered in MAGL-MKO mice (Figure 1F). Notably, both MAGL-NKO and MAGL-AKO mice displayed significant (~5- and 3-fold, respectively) increases in brain 2-AG (Figure 1G). No changes in brain 2-AG content were found in MAGL-MKO mice. Finally, none of the conditional MAGL-/- mouse lines displayed alterations in anandamide (AEA) (Figure 1H), a second major eCB that is principally hydrolyzed by fatty acid amid hydrolase (Cravatt et al., 1996). These data, taken together, indicate that both neuronal and astrocytic MAGL make substantial contributions to 2-AG metabolism in the brain.

Neuronal and Astrocytic MAGL Regulate eCB-Dependent Synaptic Plasticity

2-AG functions as a major retrograde synaptic messenger that suppresses neurotransmitter release after binding to presynaptic
CB₁Rs (Kano et al., 2009). Various forms of synaptic plasticity are regulated by retrograde eCB signaling, including depolarization-induced suppression of excitation (DSE) and inhibition (DSI) (Kano et al., 2009). Complete genetic or pharmacological ablation of MAGL activity significantly prolongs DSE/DSI (Pan et al., 2009; Straiker et al., 2009; Zhong et al., 2011), and the selective ablation of MAGL in cerebellar granule cells also extends DSE, but to a lesser degree than global ablation of the enzyme (Tanimura et al., 2012). To more clearly delineate the relative control that different brain cell types exert over 2-AG-dependent forms of synaptic plasticity, we initially examined DSE at parallel fiber (PF) to Purkinje cell (PC) synapses in acute cerebellar slices prepared from the different conditional MAGL-knockout lines.

A brief depolarization of PCs induced robust transient DSE at PF-PC synapses in MAGL +/+ cerebellar slices (Figures 2 A–2C). Consistent with previous reports (Tanimura et al., 2012; Zhong et al., 2011), global deletion of MAGL substantially prolonged the duration (Figure 2 A), but not magnitude (Figure S2 A), of DSE at PF-PC synapses in MAGL-TKO mice. A less dramatic but significant DSE extension was also observed in cerebellar slices from MAGL-NKO mice (Figure 2 B). Notably, cerebellar slices from MAGL-AKO mice also showed a significant prolongation in DSE (Figure 2 C) that was nearly identical in total duration to that observed in MAGL-NKO slices, as indicated by changes in the mean decay time constant (τ) (Figure 2 D).

No differences in DSE were evident in MAGL-MKO mice (Figure S2 B).

We further examined the contributions of neurons and astrocytes to CB₁R-mediated forms of synaptic plasticity by assessing DSI at CA1 pyramidal neuron synapses in hippocampal slices prepared from MAGL-TKO, MAGL-NKO, and MAGL-AKO, mice, as well as their wild-type littermates. DSI was readily induced in MAGL +/+ CA1 pyramidal neurons by applying a brief depolarization while evoking inhibitory postsynaptic currents (IPSCs) through stimulation of synaptic inhibitory inputs (see Experimental Procedures; Figures 2 E–2G). Consistent with previous
reports (Pan et al., 2011), the duration of hippocampal DSI in MAGL-TKO mice was substantially prolonged (Figure 2E). Significant, albeit less dramatic, extensions in DSI were also observed in hippocampal slices from MAGL-NKO and MAGL-AKO mice (Figures 2F and 2G), both of which displayed similar increases in $\tau$ (Figure 2H).

These findings, taken together, indicate that astrocytes and neurons both play prominent roles in terminating 2-AG signaling at synaptic terminals, and, as a result, dramatic increases in the duration of CB$_1$R activation are only observed when MAGL is deleted from both brain cell types.

Figure 2. Astrocytic and Neuronal MAGL Play Complementary Roles in Terminating Retrograde 2-AG Signaling

(A–C) Average time courses of PF-EPSCs in response to a brief depolarization in cerebellar slices prepared from MAGL-TKO (A), MAGL-NKO (B), and MAGL-AKO (C) mice and their wild-type littermates show prolonged DSE following global, neuron- or astrocyte-specific loss of MAGL. Sample traces are shown for MAGL-TKO mice. $n = 14–18$ cells from $n = 3$ mice per genotype.

(D) Time constant ($\tau$) of DSE in cerebellar slices prepared from MAGL-TKO (T), MAGL-NKO (N), and MAGL-AKO (A) mice and their wild-type littermates. $n = 14–18$ cells from $n = 3$ mice per genotype. Error bars represent SEM; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus MAGL+/+.

(E–G) Average time courses of IPSCs in CA1 pyramidal neurons in response to a brief depolarization in hippocampal slices prepared from MAGL-TKO (E), MAGL-NKO (F), and MAGL-AKO (G) mice and their wild-type littermates show prolonged DSI following global, neuron- or astrocyte-specific loss of MAGL. Sample traces are shown for MAGL-TKO mice. $n = 13–15$ cells from $n = 3$ mice per genotype.

(H) Time constant ($\tau$) of DSI in hippocampal slices prepared from MAGL-TKO (T), MAGL-NKO (N), and MAGL-AKO (A) mice and their wild-type littermates. $n = 13–15$ cells from $n = 3$ mice per genotype. Error bars represent SEM; *$p < 0.05$, **$p < 0.01$ versus MAGL+/+.

Neuronal and Astrocytic MAGL Regulate Functional CB$_1$R Adaptations In Vivo

Chronic, global inactivation of MAGL, achieved by either genetic disruption or repeated treatment with a MAGL inhibitor, has been shown to produce behavioral tolerance in pain models, cross-tolerance to exogenous cannabinoids, and desensitization and downregulation of brain CB$_1$Rs (Chanda et al., 2010; Schlosburg et al., 2010). In agreement with these earlier reports, we found that the antinociceptive and hypothermic effects of the CB$_1$R agonist WIN55,212-2 were substantially attenuated in MAGL-TKO mice compared to their wild-type littermates (Figures 3A and 3B). No evidence of cross-tolerance to WIN55,212-2 was observed in either MAGL-NKO or MAGL-AKO mice (Figures 3C and 3G). Correlating with these behavioral data, MAGL-TKO mice, but not MAGL-NKO or MAGL-AKO mice, showed a dramatic reduction in brain CB$_1$R function, as measured by CB$_1$R agonist (CP55,940)-stimulated [35S]-GTP$_{\gamma}$S binding (Figures 3G–3I). These results indicate that expression of MAGL in either neurons or astrocytes is sufficient to prevent excessive activation and corresponding desensitization of brain CB$_1$Rs in vivo, highlighting the contribution that
both cell types make to 2-AG metabolism and the maintenance of eCB signaling tone.

Astrocytes Are Critical Mediators of eCB-Eicosanoid Crosstalk

MAGL serves as a metabolic hub linking eCB and eicosanoid signaling pathways in the brain, where the hydrolysis of 2-AG provides a major source of AA substrate for prostaglandin synthesis (Nomura et al., 2011). MAGL blockade has been shown to coordinately elevate 2-AG and lower eicosanoid content in the nervous system under basal and neuroinflammatory states, including in disease models of parkinsonism and Alzheimer’s disease (Chen et al., 2012; Nomura et al., 2011; Piro et al., 2012). Despite these advances in our understanding of eCB-eicosanoid crosstalk, how this interplay is enacted at the cellular level in the nervous system remains unknown. We set out to address this important question by first assessing the integrity of the eCB-prostaglandin network in individual brain cell types. Cultured neurons lacking MAGL showed significant elevations in 2-AG and reductions in AA, but, interestingly, did not produce detectable levels of prostaglandins (Figures 4A–4C). In contrast, MAGL−/− astrocytes, while displaying a more modest relative increase in 2-AG compared to neurons, proved capable of translating their heightened eCB content into corresponding reductions in AA and prostaglandins (Figures 4A–4C). These findings indicate that individual brain cell types, despite expressing prostaglandin biosynthetic enzymes in culture (Hewett et al., 2000; Ikeda-Matsuo et al., 2005), differ in their capacity to autonomously convert eCBs to prostaglandins, raising the possibility that this process involves transcellular metabolism in vivo.

A broader analysis of brain lipids revealed that, as expected based on previous studies (Nomura et al., 2011), global inactivation of MAGL not only elevated 2-AG but also lowered AA and prostaglandins (Figure 4D). MAGL-TKO mice also displayed markedly blunted induction of prostaglandin synthesis following lipopolysaccharide (LPS) administration compared to their MAGL+/+ littermates (Figure 4D). In contrast, MAGL-NKO mice, despite showing ~5-fold elevations in brain 2-AG, exhibited negligible changes in AA or prostaglandin content under basal conditions or following LPS treatment (Figure 4E). MAGL-AKO mice, on the other hand, while exhibiting a more modest increase in brain 2-AG (~3-fold), showed significantly reduced brain AA content and attenuated prostaglandin production following LPS treatment (Figure 4F). Basal changes in brain prostaglandins were not observed in MAGL-AKO mice and the magnitude of decrease in these eicosanoids following LPS treatment was less substantial than that observed in MAGL-TKO mice (Figures 4D and 4F).

LPS treatment, in addition to inducing brain prostaglandin synthesis, stimulates an acute neuroinflammatory response as reflected in microglial activation and cytokine production. Consistent with previous findings, MAGL-TKO mice showed significant reductions in LPS-induced brain cytokines and microglial activation compared to MAGL+/+ littermates (Figure S3 A–S3D). These neuroinflammatory markers were, however, not altered in MAGL-NKO mice (Figures S3 E–S3H). MAGL-AKO mice showed an intermediate phenotype, with modest but significant reductions in LPS-induced brain cytokine and unaltered microglial activation compared to MAGL+/+ mice (Figures S3 I–S3L). Consistent with the absence of changes in bulk brain lipids in MAGL-MKO mice, these animals displayed no alterations in LPS-induced prostaglandin and cytokines production or microglial activation compared to MAGL−/− mice (Figures S3E–S3H). MAGL-AKO mice showed an intermediate phenotype, with modest but significant reductions in LPS-induced brain cytokine and unaltered microglial activation compared to MAGL−/− mice (Figures S3E–S3H). We did find, however, that cultured MAGL−/− microglia were impaired in producing prostaglandins and also displayed substantially reduced in vitro inflammatory responses (Figures S4E and S4F), consistent with recent reports (Pihlaja et al., 2015). It is possible that incomplete removal
of MAGL from all brain microglia in the MAGL-MKO mice (Figure S1E) prevented observation of a contribution from microglia-derived MAGL to neuroinflammatory responses in vivo.

Taken together, our in vitro and in vivo data provide strong evidence that eCB-eicosanoid crosstalk depends on the coordinated metabolic activities of neurons and astrocytes, with the former cell type providing robust production of 2-AG and the latter possessing the capacity to convert this eCB into prostaglandins. As such, robust anti-neuroinflammatory effects were only observed following global (versus cell-type-specific) deletion of MAGL, supporting a central role for neuronal-astrocytic crosstalk in the regulation of eCB-eicosanoid networks in the nervous system under pathological conditions.

Neuronal-Astrocytic Transcellular Shuttling of 2-AG and Related Metabolites

Bidirectional shuttling of conventional neurotransmitters (e.g., glutamate and GABA) and their metabolic products between neurons and glia is an established mechanism by which distinct cellular pools of these chemical messengers and their signaling activities can be regulated (Coulter and Eid, 2012). We postulated that 2-AG and/or 2-AG-derived metabolites might participate in a similar mode of intercellular transfer between neurons and astrocytes, which could provide a mechanism to rebalance bioactive lipid pools across these two cell types. To investigate this possibility, we measured 2-AG and related metabolites in MAGL+/+ and MAGL−/− neurons grown by themselves or cocultured with astrocytes in transwell dishes with polycarbonate membrane-permeable supports, which permitted free exchange of secreted metabolites in the absence of cell-cell contact. We found that, when cultured alone, MAGL−/− neurons accumulated and released higher amounts of 2-AG compared to MAGL+/+ neurons and experienced a corresponding depletion in intracellular AA content (Figures 5A and 5B). In the presence of MAGL+/+ astrocytes, however, MAGL−/− neurons showed smaller relative elevations in intracellular AA content (Figures 5A and 5B).
secreted 2-AG, and attenuated reductions in intracellular AA content (Figures 5A and 5B). Deletion of MAGL did not affect secreted AA, although the absolute quantity of this lipid was higher in the media of neuron/astrocyte co-cultures compared to isolated neurons (neuron MAGL \textsuperscript{+/+} = 128 ± 89; neuron MAGL \textsuperscript{-/-} = 125 ± 12; co-culture MAGL\textsuperscript{+/+} astrocytes/MAGL\textsuperscript{+/+} neurons = 326 ± 19; co-culture MAGL\textsuperscript{-/-} astrocytes/MAGL\textsuperscript{-/-} neurons = 298 ± 43 pmol/mg protein; Figure 5B). Together, these data indicated that astrocytes can take up 2-AG released from neurons and may, in turn, deliver 2-AG-derived metabolites (e.g., AA) back to neurons.

We next used metabolic labeling methods to examine in more depth the bidirectional shuttling of AA-derived metabolites between neurons and astrocytes. AA-\textsuperscript{d8} added to media was readily incorporated into intracellular lipids of either neurons or astrocytes in culture, including 2-AG (2AG-\textsuperscript{d8}) (Figure 5C) and other pools of neutral lipids and phospholipids (e.g., 18:0, 20:4 DAG-\textsuperscript{d8}, 18:0, 20:4 PS-\textsuperscript{d8}; d8-labeled prostaglandins were not detected, presumably due to their low abundance; Figures S5A and S5B). AA-\textsuperscript{d8}-labeled astrocytes and neurons were then co-cultured with "naïve" neurons or astrocytes, respectively, in transwell dishes (36 hr co-culture; see Methods), which resulted in extensive incorporation of AA-\textsuperscript{d8} in the naïve cells (Figures 5D, S5C, and S5D). Examination of media from these co-cultures revealed that AA-\textsuperscript{d8}-labeled neurons released higher amounts of 2AG-\textsuperscript{d8} compared to AA-\textsuperscript{d8}-labeled astrocytes, which preferentially secreted free AA-\textsuperscript{d8} (Figure 5E). Of note, AA-\textsuperscript{d8}-labeled MAGL\textsuperscript{-/-} neurons accumulated and released more 2AG-\textsuperscript{d8} than AA-\textsuperscript{d8}-labeled MAGL\textsuperscript{+/+} neurons (Figures 5C and 5E), resulting in higher uptake and metabolism of this eCB by neighboring "naïve" astrocytes (Figures 5D and SSD). Even without being directly labeled with AA-\textsuperscript{d8}, MAGL\textsuperscript{-/-} neurons also accumulated more 2AG-\textsuperscript{d8} than MAGL\textsuperscript{+/+} neurons when co-cultured with AA-\textsuperscript{d8}-labeled MAGL\textsuperscript{-/-} astrocytes (Figure 5D). These data thus provide evidence that transcellular shuttling from neurons to astrocytes contributes to the metabolism of 2AG, and, conversely, astrocyte-derived AA can be returned to neurons to rebalance the synthesis of arachidonoyl-containing lipids, including 2AG itself (Figure 6).
DISCUSSION

Some features of 2-AG metabolism and signaling were affected to similar degrees by deletion of MAGL in neurons and astrocytes. Selective loss of MAGL in neurons or astrocytes, for instance, comparably attenuated cerebellar DSE and hippocampal DSI in comparison to MAGL-TKO mice, suggesting complementary roles for these cell types in terminating retrograde 2-AG signaling. Our findings are also consistent with a recent study showing that heterologous expression of MAGL in Bergmann glia reverses the prolonged cerebellar DSE observed in global MAGL−/− mice (Tanimura et al., 2012) and extend this work in an important way by determining the function of endogenously expressed astrocytic MAGL in terminating 2-AG-mediated synaptic plasticity events in different brain regions. Perhaps most strikingly, we found that selective deletion of MAGL in either neurons or astrocytes, despite producing significant elevations in brain 2-AG (5- and 3-fold, respectively), averted the CB₁R desensitization and behavioral tolerance caused by global MAGL inactivation. The distributed metabolism of 2-AG across neurons and astrocytes thus protects the nervous system from adaptive changes caused by excessive eCB signaling. In this manner, 2-AG metabolism appears to differ from the cell-type-dominant inactivation observed for more conventional neurotransmitters (e.g., astrocytes primarily inactivate glutamate, while neurons mainly terminate GABA signaling; Schousboe et al., 2013). We should further note that the relative contributions of neuronal and astrocytic MAGL to 2-AG signal termination may differ at individual synapses, as not all CB₁-R-positive nerve terminals express MAGL (Uchigashima et al., 2011).

Our studies also uncovered distinct and complementary roles for neurons and astrocytes in 2-AG metabolism. MAGL deletion in neurons caused a more dramatic cellular increase in 2-AG, which was also efficiently secreted from these cells. Neurons in culture, however, exhibited no detectable capacity to convert 2-AG to prostaglandins. Similarly, MAGL-NKO mice, despite having substantially elevated brain 2-AG, did not show alterations in brain AA or prostaglandins under basal or neuroinflammatory conditions. Cultured astrocytes lacking MAGL, in contrast, coupled elevations in 2-AG with reductions in AA and prostaglandins. A similar lipid profile was observed for LPS-treated MAGL-AKO mice. These data, taken together, indicate that astrocytes play a major role in facilitating eCB-eicosanoid crosstalk in the nervous system, where they appear to convert both autonomously generated and neurally derived 2-AG into AA and eicosanoids, especially under conditions of neuroinflammation. While our studies did not provide evidence that microglia contribute to brain eCB-eicosanoid crosstalk in vivo, it is possible that bulk tissue measurements failed to detect more anatomically or temporally restricted roles for microglia in regulating eCB-eicosanoid networks. Likewise, other 2-AG metabolic enzymes (e.g., hydrolases [ABHD6, ABHD12; Blankman et al., 2007; Marrs et al., 2010] and oxygenases [COX2; Rouzer and Marnett, 2008]), as well as distinct enzymatic pathways for generating eicosanoids (e.g., PLA2G4A) (Burke and Dennis, 2009), constitute additional potential modes for metabolic regulation of eCB-eicosanoid pathways in the brain.

How might distinct pools of 2-AG in neurons and astrocytes be coordinately regulated and balanced? Our transwell co-culture studies indicate that both 2-AG and AA can shuttle between neurons and astrocytes, with neurons being the main producers of extracellular 2-AG and astrocytes converting this eCB into AA (and presumably AA-derived metabolites) that can be returned to neurons. We therefore propose a model in which neuronal 2-AG is hydrolyzed not only at synaptic terminals but also by neighboring astrocytes, which then transfer AA back to neurons to regenerate signaling pools of 2-AG (Figure 6). The

Figure 6. Distributed Oversight of 2-AG Metabolism and Function in Neurons and Astrocytes

Proposed model of astrocytic-neuronal transcellular shuttling and metabolism of 2-AG and AA. PIP2, phosphatidylinositol 4,5-biphosphate; PLC, phospholipase C; DAG, diacylglycerol; DAGLα, diacylglycerol lipase alpha; COX, cyclooxygenase.

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transcellular movement of 2-AG from neurons to astrocytes may be required to couple eCB and eicosanoid pathways under basal conditions in which selective deletion of MAGL in either neurons or astrocytes did not alter brain prostaglandins. In the context of neuroinflammation, however, some of the AA generated from autonomous and/or neuronal sources of 2-AG is diverted in astrocytes for prostaglandin synthesis (Figures 6 and S2E). The transcellular movement of AA and AA-derived metabolites to facilitate eicosanoid synthesis has also been shown to occur in select peripheral systems, such as between alveolar epithelial cells and macrophages or platelets and neutrophils (Folco and Murphy, 2006). Evidence has also accumulated to support the existence of lipid-binding proteins that regulate, at least in part, the uptake, transport, and release of 2-AG and AA; however, the identities and precise contributions of these proteins remain unclear (Brash, 2001; Fowler, 2013). Determining potential protein carriers and transporters that participate in neuron-astrocyte lipid shuttling represents a fertile topic for future research. Our model further shares some organizational similarity with the glutamate-glutamine cycle, in which synaptic glutamate is taken up by astrocytes, amidated into glutamine, and transferred back to neurons for re-synthesis of glutamate (Danbolt, 2001). Thus, neuron-astrocyte crosstalk appears to be a conserved mechanism for compartmentalizing and regulating the functions of diverse synaptic modulators that include both classical neurotransmitters and retrograde lipid messengers.

**EXPERIMENTAL PROCEDURES**

For an extended section, see Supplemental Experimental Procedures.

**Generation of Conditional MAGL<sup>loxP</sup>-/- Mice**

Mice carrying a loxP-flanked catalytic exon 4 Mgll allele for the conditional ablation of MAGL were generated using standard gene-targeting techniques. Homozygous MAGL<sup>loxPloxP</sup> mice were bred to Rosa26-Cre (Otto et al., 2009), Eno2-Cre (Fugger et al., 2000), GFAP-Cre (Tao et al., 2011), and LysM-Cre mice (Clausen et al., 1999) to generate MAGL-TKO, MAGL-NKO, MAGL-AKO, and MAGL-MKOs respectively.

**Biochemical Studies**

ABPP, substrate activity assays, and agonist-stimulated [35S]GTPγS binding assays were performed as previously described (Schlosburg et al., 2010) using cell membrane homogenates from 2-month-old conditional MAGL<sup>loxPloxP</sup>-/- mice and wild-type littermates or from primary neurons, astrocytes, and microglia.

**Electrophysiology**

DSE at PF to PC synapses and DSI at CA1 pyramidal neuron synapses were examined in cerebellar and hippocampal slices, respectively from MAGL<sup>loxPloxP</sup>-/- or MAGL<sup>loxPloxP</sup>-/- mice from the different MAGL conditional lines as previously described (Pan et al., 2009; Zhong et al., 2011).

**Behavioral Assays**

Two-month-old conditional MAGL<sup>loxPloxP</sup>-/- and wild-type littermates were evaluated for cannabinergic responses (an Inhibition of reinforcement) following injection with WIN55,212-2 using a cumulative dosing regimen as described elsewhere (Schlosburg et al., 2010).

**Liquid Chromatography-Mass Spectrometry Metabolite Profiling**

Tissue lipid levels in 2-month-old MAGL<sup>loxPloxP</sup>-/- or MAGL<sup>loxPloxP</sup>-/- mice from the different MAGL conditional lines were measured from organic-soluble brain extracts by targeted MRM methods (Nomura et al., 2011).

**Transwell Co-culture Studies**

Neurons and astrocytes were co-cultured in 10-cm transwell dishes with polycarbonate membrane permeable supports for 16 days prior to organic extraction of lipids from each cell type separately. For AA-d<sub>8</sub> labeling experiments, membrane permeable supports from 12 DIV co-cultures were temporarily transferred to a separate culture dish so that either neurons or astrocytes could be labeled with AA-d<sub>8</sub> conjugated to BSA (Kouzer et al., 2008). After 48 hr, cells were thoroughly washed and returned to the original co-culture for another 36 hr prior to organic extraction of lipids from each cell type separately.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, five figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.06.075.

**AUTHOR CONTRIBUTIONS**


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