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Astrocytic Endocannabinoids Mediate Hippocampal Transient Heterosynaptic Depression

Nathan A. Smith^{1,2,3}, Lane K. Bekar⁴, Maiken Nedergaard¹

¹Division of Glia Disease and Therapeutics, Center for Translational Neuromedicine, Dept. of Neurosurgery, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

²Center for Neuroscience, Children's Research Institute, Children's National Medical Center, Washington DC 20010, USA.

³George Washington University School of Medicine and Health Sciences, Washington DC 20052, USA.

⁴Department of Anatomy, Physiology and Pharmacology, University of Saskatchewan, Saskatoon, SK S7N 5E5, Canada.

Abstract

Astrocytes are highly dynamic cells that modulate synaptic transmission within a temporal domain of seconds to minutes in physiological contexts such as Long-Term Potentiation (LTP) and Heterosynaptic Depression (HSD). Recent studies have revealed that astrocytes also modulate a faster form of synaptic activity (milliseconds to seconds) known as Transient Heterosynaptic Depression (tHSD). However, the mechanism underlying astrocytic modulation of tHSD is not fully understood. Are the traditional gliotransmitters ATP or glutamate released via hemichannels/vesicles or are other, yet, unexplored pathways involved? Using various approaches to manipulate astrocytes, including the Krebs cycle inhibitor fluoroacetate, connexin 43/30 double knockout mice (hemichannels), and inositol triphosphate type-2 receptor knockout mice, we confirmed early reports demonstrating that astrocytes are critical for tHSD. We also confirmed the importance of group II metabotropic glutamate receptors (mGluRs) in astrocytic modulation of tHSD using a group II agonist. Using dominant negative SNARE mice, which have disrupted glial vesicle function, we also found that vesicular release of gliotransmitters and activation of adenosine A1 receptors are not required for tHSD. As astrocytes can release lipids upon receptor stimulation, we asked if astrocyte-derived endocannabinoids are involved in tHSD. Interestingly, a cannabinoid receptor 1 (CB1R) antagonist blocked and an inhibitor of the endogenous endocannabinoid 2-arachidonoyl glycerol (2-AG) degradation potentiates tHSD in hippocampal slices. Taken together, this study provides the first evidence for group II mGluR-mediated astrocytic endocannabinoids in transiently suppressing presynaptic neurotransmitter release associated with the phenomenon of tHSD.

Keywords

endocannabinoids; astrocytes; transient heterosynaptic depression

Introduction

Over the last few decades, astrocytes have gained recognition as being active participants in synaptic activity rather than mere bystanders with a role in metabolic support. Previous studies have shown that astrocyte receptor-mediated Ca^{2+} increases lead to release of 'gliotransmitters' such as glutamate (Parpura et al., 1994; Bezzi et al., 1998; Kang et al., 1998), *D*-serine (Mothet et al., 2000; Yang et al., 2003), or ATP (Coco et al., 2003). Gliotransmission is thought to be critical in calcium wave propagation by ATP-dependent astrocytic paracrine signaling (Cotrina et al., 1998; Guthrie et al., 1999), and may also modulate synaptic activity by signaling to neurons (Kang et al., 1998; Mothet et al., 2005; Verkhratsky and Nedergaard, 2018). However, the extent of and the circumstances under which gliotransmitters modulate synaptic activity is still under debate (Agulhon et al., 2010; Hamilton and Attwell, 2010; Nedergaard and Verkhratsky, 2012; Verkhratsky and Nedergaard, 2018).

Transient (<1 second) post-burst depression of transmitter release, often termed tHSD, is a rapid form of intersynaptic communication in which an active synapse transiently decreases the efficacy of a neighboring inactive synapse (Andersson et al., 2007). Although the role of astrocytes in tHSD, is well-established (Andersson et al., 2007; Andersson and Hanse, 2010; Andersson and Hanse, 2011), details surrounding 'gliotransmission' remain ambiguous. Andersson et al. (2007) describe a role for astrocytes, GABA_B receptors, and group II/III mGluRs in tHSD, and propose a model whereby glutamate- and/or GABA-induced astrocyte glutamate release is responsible for the synaptic suppression. However, evidence for astrocyte-derived glutamate release is not provided, leaving the mechanistic role of astrocytes yet to be characterized. More recently, Andersson and Hanse (2010) go on to demonstrate that disruption of astrocyte Ca^{2+} via patch pipette delivery of the calcium chelator BAPTA completely abolished tHSD but note that the mechanism responsible for synaptic depression remains to be determined. In this study, we explore endocannabinoids, known for their role in inhibition of presynaptic neurotransmitter release (Szabo et al., 2006), as a candidate mechanism for astrocyte-mediated induction of tHSD.

Although astrocytes have been shown to release endocannabinoids in a calcium-dependent manner (Walter and Stella, 2003; Stella, 2004; Walter et al., 2004), there is some evidence that endocannabinoids can also undergo calcium-independent release (Dinh et al., 2002b; Sugiura et al., 2002; Bisogno et al., 2003). In this study, we demonstrate that tHSD is dependent on group II mGluR receptor-mediated astrocyte calcium using select pharmacology and inositol triphosphate type-2 receptor knock-out mice ($\text{IP}_3\text{R}_2^{-/-}$) where astrocyte calcium signaling is disrupted. Furthermore, using dnSNARE or double connexin knockout mice we demonstrate that tHSD is not dependent on the traditional slow gliotransmitter release mechanisms involving vesicular exocytosis or hemichannel opening, respectively. Finally, we demonstrate that the transient suppression of synaptic activity is lost

in the presence of a type-1 cannabinoid receptor (CB1) antagonist and potentiated when metabolism of the endogenous cannabinoid 2-arachidonyl glycerol (2-AG) is inhibited.

MATERIALS AND METHODS

Animals

Connexin 43/30^{-/-} mice were bred as described previously (Wallraff et al., 2006), along with homozygotic knockout mice and their wildtype littermates. IP₃R₂^{-/-} mice were generated as described previously (Sharp et al., 1999; Holtzclaw et al., 2002; Hertle and Yeckel, 2007), and dominant negative SNARE mice were bred as described previously (Pascual et al., 2005), which were used with and without doxycycline (DOX) treatment. C57BL/6J mice were obtained from Charles River Laboratories. All studies were conducted with animals aged 18 to 30 days. The Animal Care and Use Committee of the University of Rochester approved all animal experiments.

Slice preparation

Hippocampal slices were prepared from C57BL/6J mice. After decapitation under deep anesthesia with isoflurane, brains were quickly removed to oxygenated, ice-cold cutting solution containing (in mM) 230 sucrose, 1 KCl, 1.25 NaH₂PO₄, 0.5 CaCl₂, 10 MgSO₄, 0.04 sodium ascorbate, 1 kynurenic acid, 26 NaHCO₃, and 10 glucose. Transverse hippocampal slices 400 μm thick were cut into this solution with a vibratome and transferred to oxygenated artificial cerebrospinal fluid (ACSF) that contained (in mM) 124 NaCl, 2.5 KCl, 2 CaCl₂, 2 MgSO₄, 26 NaHCO₃, 1.25 NaH₂PO₄ and 10 glucose. Slices were kept at 30–32 °C in ACSF saturated with 95% O₂ and 5% CO₂. During the recordings, slices were placed in a perfusion chamber and superfused at a rate of 2 ml/min with ACSF saturated with 95% O₂ and 5% CO₂ at 30–32 °C. Picrotoxin (100 μM) was always present in the perfusion fluid to block GABA_A receptor-mediated activity.

Electrophysiological Recording and Analysis

Electrical stimulation of Schaffer collateral-commissural axons and recording of synaptic responses were carried out in the stratum radiatum of the CA1 region. Stimuli consisted of biphasic constant current pulses (Master 8, AMPI, Israel) delivered through tungsten wires (~0.1 MΩ). A pair of stimulation electrodes was positioned in the stratum radiatum at a separation of about 500 μm and were activated every 10 s with the duration of the activation between the inputs being 5s (long interval) or 0.5s (short intervals) (Figure 1A). One input was used as a test input while the other was used as a conditioning input and activated using a 3-pulse train at 50 Hz. Test stimuli were compared under the two conditions, long and short-intervals, to measure the extent of synaptic suppression. The long-interval was used as control, while the short interval was used to induce tHSD. Field evoked post-synaptic potentials (EPSPs) were recorded using a glass micropipette filled with 1 M NaCl (resistance 1–2 MΩ) placed between the two stimulation electrodes. The field EPSPs were sampled at 10 kHz with an AxoPatch 200B amplifier (Axon Instrument) and filtered at 1 kHz. Evoked responses were analyzed off-line using pClamp 10 (Molecular Devices). Data analyzed were the peak amplitude of the baseline fEPSPs and of the fEPSPs evoked after

application of the conditioning pulses. fEPSP amplitudes were expressed as percentage of baseline values to control for differences in absolute magnitude across slices and conditions.

Pharmacological agents used in slice experiments

Slice studies were undertaken with the addition to the medium of: adenosine 5'-triphosphate (ATP, 100 μ M); *trans*- (1S, 3R)-1-amino-1, 3-cyclopentanedicarboxylic acid (*t*-ACPD, 100 μ M); (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid disodium salt (LY341495 20 μ M, Tocris); *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4 methyl-1*H*-pyrazole-carboxamide (AM251, 4 μ M, Tocris); 4-[*bis*(1,3-benzodioxol-5-yl)hydroxymethyl]-1-piperidinecarboxylic acid 4-nitrophenyl ester (JZL184, 1 μ M, Tocris); 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 1 μ M, Tocris) and rhod-2 AM (4.5 mM, Invitrogen). All chemicals were from Sigma unless otherwise noted.

Statistical analysis

All values are expressed as means $\pm$ S.E.M. Normality of the data was evaluated with Shapiro-Wilk test with $\alpha = 0.05$. Differences between two means were assessed by Student's *t* test (unpaired and unpaired; Mann-Whitney post-hoc test) and multiple means were assessed by one-way analysis of variance (ANOVA) with Kruskal-Wallis post-hoc test. Statistical significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) were calculated using GraphPad Prism 6.0 (GraphPad Software, La Jolla, Ca).

RESULTS

Astrocytes are necessary for tHSD

Using the stimulation protocol necessary to induce tHSD (Figure 1A), we first tested the previous finding that fluoroacetate (FAC), which inhibits astrocytic metabolism (Fonnum et al., 1997), blocks tHSD. We observed a 25–30% suppression with tHSD under control conditions, which was reduced to a 5% suppression with the addition of FAC (10 mM) to the ACSF (Figure 1B, $n=4$), thus confirming previous findings. To further test the role of astrocytes in tHSD, we assessed tHSD in separate experiments in brain slices prepared from connexin 43/30 (gap junctions specific to astrocytes) double knockout mice (Wallraff et al., 2006). These animals are conditional knockouts that have connexin43 specifically ablated in astrocytes, driven by inactivation of floxed *Cx43* by Cre recombinase. The human GFAP promoter drives the expression in the conditional KO mice, while *Cx30* is a global KO (Theis et al., 2003; Wallraff et al., 2006; Lin et al., 2008). tHSD was significantly attenuated in hippocampal slices prepared from connexin 43/30^{-/-} mice compared to WT mice (Figure 1C, $n=8$), thus confirming previous findings that used pharmacological methods to disrupt gap junction connexin function (Andersson et al., 2007). Although significant, the attenuation of tHSD in knockout mice was far from complete. As Ca^{2+} is critical for release of gliotransmitters, the observed inhibition in the connexin knockout may be a result of reduced Ca^{2+} wave propagation and signaling (Naus et al., 1997; Scemes et al., 1998) as opposed to removal of a potential gliotransmission pathway.

To examine the role of intracellular Ca^{2+} in tHSD, we undertook tHSD studies in hippocampal slices prepared from IP_3R_2 ^{-/-} mice, in which the astrocytic isoform of the

intracellular IP₃ receptors are ablated as a consequence of global deletion of IP₃R₂ (Sharp et al., 1999; Holtzclaw et al., 2002; Hertle and Yeckel, 2007). Astrocytes from these mice are thus unable to respond with rises in intracellular Ca²⁺ upon IP₃ receptor stimulation. Consistent with previous studies (Li et al., 2005; Petravic et al., 2008; Wang et al., 2012), we observed that ATP-mediated increases in intracellular Ca²⁺ in slices from wildtype mice (IP₃R₂^{+/+}) did not occur in slices from IP₃R₂^{-/-} mice (Figure 1D, upper panel). Interestingly, tHSD was largely inhibited in the IP₃R₂^{-/-} mice but remained intact in the wildtype mice (Figure 1D, n=4–6), thus supporting the notion that astrocytic Ca²⁺ signaling plays an essential role in tHSD.

Group II mGluRs are necessary for tHSD

We assessed effects of direct activation of group II mGluR on synaptic activity in the stratum radiatum of the CA1 region by pressure ejection of trans-1-amino cyclopentane-1, 3-dicarboxylic acid (tACPD), a specific group II mGluR agonist (Figure 2A left panel). Application of 50 μM tACPD evoked a significant depression of synaptic activity (Figure 2B, n=6). Because local application of tACPD could potentially activate neuronal mGluRs (Pacelli and Kelso, 1991), we next employed electrical stimulation with a selective antagonist in order to reveal the role of group II mGluRs activated by endogenous glutamate on tHSD (Figure 2A right panel). In the presence of 20 μM LY341495, a specific antagonist for group II mGluR, we observed blockade of tHSD (Figure 2B, n=6), thus confirming previous findings (Andersson et al., 2007). Notably, there was no effect on baseline field potentials, indicating that blockage of group II mGluR does not inhibit basic synaptic transmission.

Astrocytic vesicular release is not involved in Group II mGluR-mediated tHSD

We investigated the possible mechanism behind group II mGluR-mediated tHSD in SNARE mice (Figure 3A). We performed a beta galactosidase (β-Gal) expression assay to confirm the appropriate expression profile of the dominant negative SNARE protein. In the presence of DOX, there was no β-Gal expression, indicating that the functional snare proteins were expressed (Figure 3A, right panel top), whereas in the absence of DOX, β-Gal expression was evident (Figure 3A, right panel bottom). In the dominant negative mice with disrupted vesicular trafficking, tHSD was still present, suggesting that vesicular release of gliotransmitters is not essential for tHSD (Figure 3A, n=6). Furthermore, we induced normal tHSD despite blockade with 8-cyclopentyl-1, 3, dipropylxanthine (DPCPX; 1 μM), an antagonist of the adenosine A1 receptors, which are known to be important in non-transient heterosynaptic depression (Grover and Teyler, 1993) (Figure 3B, n= 4).

CB1R antagonists block Group II mGluR-mediated tHSD

Endocannabinoids can inhibit neurotransmitter release by activating CB1R on presynaptic terminals. In the presence of the CB1R antagonist AM251 (4 μM), tHSD was significantly depressed (Figure 4A, n=6). To further establish the role of the CB1R in mGluR-induced astrocyte-mediated synaptic depression we pressure ejected tACPD with or without slice perfusion with AM251 for comparison. AM251 demonstrated a near complete block of tACPD-mediated synaptic depression that was reversible with washout (Figure 4B, n=5–7) supporting the notion that group II mGluRs do not directly mediate the suppression on the

pre-synapse. To identify the specific endocannabinoid involved, we applied JZL184, a specific inhibitor of monoacyl glycerol lipase (MGL), which inactivates 2-AG (Dinh et al., 2002b; Dinh et al., 2002a). Treatment of the slice with JZL184 (1 μ M) resulted in a significantly enhanced tHSD (Figure 4C, n=6), endorsing the role of 2-AG. Data presented here provide strong evidence for astrocytic group II mGluR-induced release of 2-AG in modulating tHSD (Figure 4D).

DISCUSSION

Although there is a well-established role for astrocytes in tHSD, our understanding of the exact mechanism by which they modulate this depression remains incomplete (Andersson et al., 2007; Andersson and Hanse, 2010; Andersson and Hanse, 2011). Previous work proposed that activation of group II mGluR causes release of glutamate from astrocytes, which in turn activates presynaptic neuronal group II mGluRs, inducing tHSD (Andersson et al., 2007). This was an attractive hypothesis as glutamate, indeed, inhibits neurotransmitter release from presynaptic terminals by activating group II mGluR in various brain regions (Bushell et al., 1996; Glitsch et al., 1996; Macek et al., 1996; Yokoi et al., 1996; Scanziani et al., 1997; Dube and Marshall, 2000; Kew et al., 2001). However, based on autoradiography and histological studies, the majority of group II mGluR expression is localized to astrocytic processes rather than presynaptic neuronal terminals within the CA1 stratum radiatum region of the hippocampus (Poncer et al., 1995; Petralia et al., 1996; Tamaru et al., 2001; Sun et al., 2013), limiting the possibility for neuronal group II mGluR participation in tHSD. We validate and extend previous studies on tHSD by demonstrating for the first time an additional step involving astrocyte-dependent release of endocannabinoids. The fact that the CB1R antagonist also blocked the exogenous group II mGluR agonist in this study is consistent with group II mGluRs not being involved at the pre-synapse. This begs the question, how many studies showing group II mGluR-mediated reduction in neurotransmitter release can be attributed to astrocyte mGluR-mediated release of endocannabinoids?

The group II metabotropic glutamate receptor (mGluR) family consists of mGluR2 and mGluR3, which are Gi-coupled receptors that negatively regulate adenylate cyclase (Pin and Duvoisin, 1995). The most prominent metabotropic glutamate receptors found in astrocytes are mGluR3 and mGluR5 (Testa et al., 1994; Petralia et al., 1996; Balazs et al., 1997; Sun et al., 2013). The main focus has been placed on studies of the astrocytic mGluR5, since it is a Gq-coupled receptor directly linked to polyphosphoinositide (PI) hydrolysis, and is known to stimulate intracellular Ca^{2+} increases (Nakahara et al., 1997). However, Gi-coupled receptors such as mGluR3 can also provoke rises in intracellular Ca^{2+} (Tang et al., 2015; Chai et al., 2017), which may be due to cross talk with Gq receptors (Werry et al., 2003) or through the $\beta\gamma$ subunit (Taussig and Zimmermann, 1998). A recent study revealed that these receptor types undergo developmental regulation in astrocytes whereby mGluR5 is down-regulated with age, while mGluR3 persists throughout adulthood (Sun et al., 2013). In addition, anatomical studies localize the majority of mGluR3 expression seen in the hippocampal CA1 region to astrocytic processes (Petralia et al., 1996; Tamaru et al., 2001). Furthermore, hippocampal astrocytes associated with synaptic terminals in the CA1 region

respond to neuronal release of glutamate (Tang et al., 2015), providing a possible mechanism for neuron/astrocyte communication.

Synaptobrevin 2 and syntaxin, which are core components of the SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors) complex, are expressed in astrocytes (Parpura et al., 1995). The dominant negative SNARE mice are conditional knockouts that express a disrupted synaptobrevin 2 protein in astrocytes when the animals are withdrawn from doxycycline (DOX), thus blocking astrocytic vesicular transmitter release (Pascual et al., 2005). However, it is worth noting that the dnSNARE transgene has some expression in cortical neurons (Fujita et al., 2014). We here assessed β -Gal expression, but concede that this does not exclude neuronal expression, because the neuronal and glial transgenes operate separately, resulting in independent expression levels. Nevertheless, the present results, in conjunction with the adenosine A1 receptor blockade, do not indicate an important role of vesicular gliotransmitter (ATP) release in tHSD.

A few studies have shown that endocannabinoids are released from post-synaptic neuronal membranes in response to high frequency stimulation through the activation of NMDA receptors (Ohno-Shosaku et al., 2007), but astrocytes are also capable of releasing endocannabinoids in both a Ca^{2+} -dependent and independent manner (Walter and Stella, 2003; Stella, 2004; Walter et al., 2004). Endocannabinoids are unique lipid modulators that suppress neuronal activity by acting on their receptors located on presynaptic terminals (Mechoulam et al., 1996; Stella et al., 1997; Di Marzo et al., 1998; Freund et al., 2003). Established endocannabinoid-mediated modulation of synaptic activity entails retrograde signaling of endocannabinoids from the postsynaptic neuronal membrane, which is only inducible by high frequency depolarization or direct activation of NMDA or mGluR1 receptors (Maejima et al., 2001; Ohno-Shosaku et al., 2001; Varma et al., 2001; Zhu, 2006). However, the possibility of retrograde neuronal signaling of endocannabinoids seems unlikely in the current study. First, tHSD is independent of NMDA receptor activation but dependent on astrocytic group II mGluR signaling (Andersson et al., 2007). Furthermore, establishing tHSD does not require the high frequency stimulation required for neuronal release of endocannabinoids (10 pulses at 100 Hz), but is evoked by the moderate physiological stimulation used in the present study (3 pulses at 50 Hz). An equally important consideration is that astrocytes can also release endocannabinoids upon receptor stimulation (Walter and Stella, 2003; Stella, 2004; Walter et al., 2004).

We initially thought that because tHSD occurs on a millisecond time-scale, and that a number of studies have demonstrated receptor-mediated increases in intracellular Ca^{2+} in astrocytes occurs on a time-scale of seconds (Cornell-Bell et al., 1990; Wang et al., 2006; Srinivasan et al., 2015), it could be due to Ca^{2+} -independent release of astrocytic endocannabinoids. However, loss of tHSD with patch dialysis of a calcium chelator (Andersson and Hanse, 2010) as well as in the $\text{IP}_3\text{R}_2^{-/-}$ transgenic mice presented in these studies, supports involvement of astrocyte Ca^{2+} on a sub-second time-scale. It is worth noting that a recent study has shown that the $\text{IP}_3\text{R}_2^{-/-}$ mice are not completely devoid of Ca^{2+} signaling especially in their processes which could explain why we don't see a complete abolishment of tHSD in these mice (Srinivasan et al., 2015). Equally important, several studies have shown that astrocytes can exhibit fast Ca^{2+} signaling, notably in relation

to sub-second blood flow responses (Winship et al., 2007; Nimmerjahn et al., 2009; Lind et al., 2013), a time-frame consistent with that in the present study. Thus, these findings were significant because they provided first hand evidence of sub-second Ca^{2+} signaling in astrocytes that can potentially affect many CNS functions.

In conclusion, the present findings conceptually extend ‘gliotransmitter’ signaling to include the endocannabinoid system that can function on a sub-second time-scale. These are the first studies to present evidence for astrocytic lipid modulation of fast synaptic activity in the adult brain.

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Figure 1: Astrocytes are necessary for tHSD

A.) A schematic illustration showing the placement of stimulating electrodes for conditioning and testing in the stratum radiatum of the CA1 region. tHSD is induced by stimulating one group of synapses with a short burst (3 pulses at 50Hz: conditional stimulation), which elicits a depression of fEPSPs at another synapse stimulated by a test stimulation at a short interval of 0.5s. Lower panel illustrates depression of the test fEPSP during tHSD. B.) Bar graph comparing tHSD before and after exposure to the astrocytic toxin fluoroacetate (10 mM) (FAC) in hippocampal slices from wild-type mice (*P<0.05, unpaired t-test, Mann-Whitney post-hoc). C.) Bar graph comparing tHSD in Connexin 43/30^{+/+} and Connexin 43/30^{-/-} mouse hippocampal slices (*P<0.05, unpaired t-test). D.) Application of ATP (100 μ M) failed to increase Ca²⁺ release in Rhod-2AM (4.5 μ M) loaded slices prepared from IP₃R₂^{-/-} mice (left panel), whereas ATP (100 μ M) induced increased Ca²⁺ release in a slice prepared from IP₃R₂^{+/+} mice. Lower panel, Bar graph comparing tHSD in IP₃R₂^{+/+} and IP₃R₂^{-/-} mice hippocampal slices (**P<0.001, unpaired t-test). Displayed are means $\pm$ S.E.M.

Figure 2: Group II mGluR activation is necessary for tHSD

A.) A schematic illustration that shows the placement of picospritzer and test stimulating electrode in the stratum radiatum of the CA1 region. This experimental approach induced depression of fEPSP at local synapses (test stimulation) upon picospritzing 50 μ M of tACPD, a group II mGluR agonist. (right panel) A schematic illustration showing the placement of stimulating electrodes for conditioning and testing in the stratum radiatum of the CA1 region. tHSD is induced by stimulating one group of synapses with a short burst (three pulses at 50 Hz, with conditional stimulation at 0.5 or 5 seconds prior to test stimulation), which elicits depression of fEPSP at another group of synapses (test stimulation). B.) Bar graph comparing local application via picospritzer of tACPD-mediated depression to electrical evoked tHSD before and after exposure to the mGluR group II antagonist LY341495 (20 μ M), in wild-type mouse hippocampal slices (* P < 0.05, ** P < 0.001, one-way ANOVA, Kruskal-Wallis post-hoc test). Displayed are means $\pm$ S.E.M.

Figure 3: Astrocytic vesicular release is not involved in Group II mGluR mediated tHSD

A.) Bar graph comparing tHSD in hippocampal slices of dnSNARE mice treated with and without the inducer doxycycline (DOX) ($P = 0.4177$, unpaired t-test, Mann Whitney post-hoc test). Right panel, image of X-Gal staining of brain tissue from dnSNARE mice with or without doxycycline (DOX) treatment. B.) Bar graph comparing tHSD in wild type mouse hippocampal slices before and after the adenosine A1 receptor antagonist DPCPX (1 μ M). ($P = 0.4286$, paired t-test, Mann Whitney post-hoc test). Displayed are means $\pm$ S.E.M.

Figure 4: CB1R antagonist blocks Group II mGluR-mediated tHSD

A.) Bar graph comparing tHSD before and after exposure to the CB1 receptor antagonist AM251 (4 μ M) in wild-type mouse hippocampal slices (** P < 0.0001, unpaired t-test). B.) Bar graph comparing the effects of group II mGluR agonist mediated depression before and after AM251 exposure in wild-type mice hippocampal slices (** P < 0.001, *** P < 0.0001, one-way ANOVA, Kruskal-Wallis post-hoc test). C.) Bar graph comparing tHSD before and after exposure to the MGL inhibitor JZL184 (1 μ M) (** P < 0.001, unpaired t-test, Mann-Whitney post-hoc). D.) Schematic illustration of one active synapse (glutamatergic) and one inactive synapse (glutamatergic) with an interposed astrocyte endfoot. The astrocyte presents Group II mGluRs, activation of which can increase intracellular Ca^{2+} release leading to the production of 2AG. This in turn activates CB1Rs on nearby presynaptic terminals, resulting in decreased release of vesicular glutamate. Displayed are means $\pm$ S.E.M.