Neuropharmacology 58 (2010) 712–721

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

mGluR-mediated and endocannabinoid-dependent long-term depression in the hilar region of the rat dentate gyrus

Ben Nahir [b], Casie Lindsly [b], Charles J. Frazier [a,b,*]

[a] Department of Pharmacodynamics, College of Pharmacy, University of Florida, USA
[b] Department of Neuroscience, College of Medicine, University of Florida, USA

ARTICLE INFO

Article history:
Received 5 October 2009
Received in revised form
8 December 2009
Accepted 22 December 2009

Keywords:
Endocannabinoids
mGluRs
LTD
Dentate gyrus
Synaptic plasticity
Electrophysiology

ABSTRACT

We report that bath application of the group I mGluR agonist (*RS*)-3,5-dihydroxyphenylglycine (DHPG) causes acute inhibition of evoked IPSCs recorded from hilar mossy cells, and that significant long-term depression (LTD) of synaptic transmission remains following washout of DHPG. Subsequent experiments using minimal stimulation techniques revealed that expression of both acute and long-term effects of DHPG are restricted to a subset of GABAergic afferents that are also sensitive to depolarization-induced suppression of inhibition (DSI). Experiments with a selective CB1 antagonist and with transgenic animals lacking CB1 receptors indicate that all effects of DHPG, like DSI, depend on activation of CB1 receptors. Further work with selective mGluR antagonists suggests a direct involvement of mGluR1 receptors. Interestingly, we also report that induction of LTD under our experimental conditions does not require prior direct somatic depolarization via the patch pipette and does not appear to depend critically on the level of activity in incoming GABAergic afferents. Collectively, these results represent the first characterization of mGluR-mediated and endocannabinoid-dependent LTD in the hilar region of the dentate gyrus. The dentate gyrus is thus one of relatively few areas where this mechanism has clearly been demonstrated to induce long-term modulation of inhibitory synaptic transmission.

1. Introduction

Endogenous cannabinoids (endocannabinoids, eCB) regulate neurotransmission in the CNS primarily via retrograde activation of presynaptic metabotropic type 1 cannabinoid receptors (CB1Rs) (Kano et al., 2009; Katona et al., 1999; Kawamura et al., 2006; Wilson et al., 2001). Depending on a variety of factors, endocannabinoids can affect either a short-term or long-term synaptic depression. The most commonly observed forms of short-term plasticity, depolarization-induced suppression of inhibition (DSI) or excitation (DSE), are initiated by direct depolarization of postsynaptic neurons and depend heavily on subsequent calcium-dependent synthesis of eCBs. By contrast, eCB-mediated long-term depression (LTD) often requires activation of postsynaptic metabotropic glutamate receptors (and not just depolarization) as a key signal promoting eCB production. Metabotropic glutamate receptor-mediated, and CB1 receptor-dependent, forms of LTD have now been well characterized for excitatory synapses in the striatum and nucleus accumbens (Gerdeman et al., 2002; Robbe et al., 2002), and for inhibitory synapses in the hippocampus, basolateral amygdala, and dorsal striatum (Adermark and Lovinger, 2009; Azad et al., 2004; Chevaleyre and Castillo, 2003; Marsicano et al., 2002). In the hippocampus, this type of plasticity has been shown to have lower dependence on postsynaptic calcium influx and to clearly outlast the presence of a CB1 agonist. In addition to mGluR activation, other mechanisms potentially involved in enabling long-term as opposed to short-term eCB-mediated depression include the recent history of excitation in the postsynaptic neuron, the duration of time that agonist is available to CB1 receptors, and the level of activity of presynaptic neurons during that time (for examples from hippocampal studies see: Chevaleyre and Castillo, 2003; Edwards et al., 2008; Heifets et al., 2008). Overall, this field is intriguing in part because it is revealing what may be one of the major forms of postsynaptically induced yet presynaptically expressed long-term plasticity in the CNS, and in part because it has exciting potential roles in both synaptic recovery and metaplasticity (for recent and excellent review see, Chevaleyre et al., 2006; Lovinger, 2008).

Despite the likely importance of this mechanism relatively little is known about the potential for mGluR and eCB-dependent LTD in the dentate gyrus. While significant effort has gone into an examination of LTD as produced by low frequency stimulation of

* Corresponding author at: JHMHC Box 100487, 1600 S.W. Archer Road, Gainesville, FL 32610, USA. Tel.: +1 352 273 7686; fax: +1 352 273 7705.
E-mail address: cjfraz@ufl.edu (C.J. Frazier).

0028-3908/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2009.12.022

perforant path inputs to dentate granule cells (e.g. Huang et al., 1999; Naie et al., 2007; Trommer et al., 1996), a recent study suggests that eCB-dependent mechanisms in this area are likely restricted to mossy cell axon terminals in the inner molecular layer, and that even these lack eCB-dependent LTD as described in CA1 (Chiu and Castillo, 2008). Similarly, despite the fact that mossy cells also have been shown to produce robust DSI of CB1 positive GABAergic afferents in the hilus (Hofmann et al., 2006; Howard et al., 2007), the potential of CB1-dependent LTD has not previously been examined at these terminals. In fact, although DSI has been well characterized throughout the brain, to date mGluR-mediated and eCB-dependent LTD of GABAergic terminals has only been described at three distinct inhibitory synapses: stratum radiatum interneurons to CA1 pyramidal cells in the hippocampus (Chevaleyre and Castillo, 2003, 2004; Edwards et al., 2006, 2008; Heifets et al., 2008), interneurons to principal cells in the central and basolateral amygdala (Azad et al., 2004; Marsicano et al., 2002), and intrinsic afferents to medium spiny neurons in the striatum (Adermark and Lovinger, 2009). The present study provides an initial description of mGluR-induced and eCB-dependent LTD of CB1 positive GABAergic afferents to hilar mossy cells.

2. Materials and methods

2.1. Slice preparation

Male Sprague–Dawley (SD) rats (P18–25) received an intraperitoneal injection of ketamine hydrochloride (80–100 mg/kg) and were rapidly decapitated using a small animal guillotine. Horizontal slices (300 μm) were made in ice-cold artificial cerebral spinal fluid (ACSF) using a Pelco Series 3000 Vibratome (Pelco, Redding, CA). Slices were immediately transferred to ACSF maintained at 30–35 °C for 30 min and then allowed to equilibrate to room temperature. ACSF used for slice preparation and incubation was saturated with a 95% O_2–5% CO_2 gas mixture and consisted of (in mM): 124 NaCl, 2.5 KCl, 1.23 NaH_2PO_4, 2.5 $MgSO_4$, 10 D-glucose, 1 $CaCl_2$, and 25.9 $NaHCO_3$. Animal procedures were approved by the institutional animal care and use committee (IACUC) at the University of Florida and conformed to the animal welfare guidelines issued by the National Institutes of Health (NIH).

2.2. Whole cell recording

Slices were maintained at room temperature for up to 8 h prior to use. For whole cell recordings, slices were transferred to a recording chamber where they were submerged and constantly perfused at a rate of 2 ml/min with ACSF heated to 30 °C and saturated with 95% O_2–5% CO_2. ACSF for whole cell recordings contained (in mM): 126 NaCl, 3 KCl, 1.2 NaH_2PO_4, 1.5 $MgSO_4$, 11 D-glucose, 2.4 $CaCl_2$, and 25.9 $NaHCO_3$ (pH ≈ 7.3). The ionotropic glutamate receptor antagonists 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX, 20 μM), DL-2-Amino-5-phosphonovaleric acid (APV, 40 μM) were added to the ACSF several minutes after obtaining a whole cell recording. Slices were visualized in the recording chamber with infrared differential interference contrast microscopy (IR DIC) using an Olympus BX51WI microscope. Whole cell patch-clamp experiments were conducted using borosilicate glass pipettes pulled by a Flaming/Brown electrode puller (Sutter P-97, Sutter Instruments, Novato, CA). Patch electrode tip resistance was typically 3–6 MΩ when filled with an internal solution containing (in mM): 95 $CsCH_3SO_3$, 55 CsCl, 1 $MgCl_2$, 0.2 Cs-EGTA, 10 HEPES, 2 Na_2-ATP, 0.3 Na-GTP, and 5 lidocaine N-ethyl chloride (QX-314 Cl), pH adjusted to 7.3 using CsOH, and volume adjusted to a final osmolarity of 300–315 mOsm. Each day, sulforhodamine 101 (~75 μM) or alexa fluor 594 (50 μM) was added to the internal solution for epifluorescence imaging. Upon whole cell break in, access resistance was typically 10–20 MΩ. Although generally uncompensated, we continued to monitor access resistance (R_A) throughout each experiment. Any cell with R_A that exceeded 30 MΩ was rejected from analysis. Voltage clamp experiments were performed using an Axon Multiclamp 700A or 700B amplifier (Molecular Devices, Sunnyvale, CA). Data were sampled at 20 kHz, filtered at 2 kHz, and digitally recorded using either a Digidata 1322A or 1440 A/D converter and Clampex v. 9 or 10 (Molecular Devices). Chemicals used in these experiments were obtained from Sigma (St. Louis, MO) except for (*RS*)-3,5-dihydroxyphenylglycine (DHPG; group I mGluR agonist), 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP; selective mGluR5 antagonist), (*S*)-(+)-a-amino-4-carboxy-2-methylbenzeneacetic acid (LY367385; selective mGluR1 antagonist), and N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-phyrazole-3-carboxamide (AM-251; potent, selective CB1R antagonist/inverse agonist), which were obtained from Tocris Cookson (Ellisville, MO); Alexa Fluor 594 was obtained from Molecular Probes/Invitrogen (Eugene, OR).

For the experiments in Fig. 1, evoked IPSCs were generated at 0.33 Hz using a concentric bipolar stimulator (FHC, Bowdoin, ME) connected to a constant stimulus isolator (World Precision Instruments, Sarasota, FL). For all other experiments, minimally evoked IPSCs (meIPSCs) were generated using a monopolar stimulating electrode constructed from a patch pipette filled with ACSF. For these experiments, simulation frequency was 0.2 Hz, current intensity varied between 30 and 100 μA, and stimulation duration was 0.1 ms. Minimally evoked responses were identified as previously described in detail by our lab (for example, see illustrations in Hofmann et al., 2008; Nahir et al., 2007). In brief, for each stimulator placement, stimulation intensity was slowly increased from a subthreshold level. A minimally evoked response was one that appeared with a sharp current threshold, and that remained stable when stimulation intensity was increased an additional 10–15 μA (for a representative example from the current study see Fig. 2A).

Hilar mossy cells were identified using a wide range of physiological and anatomical criteria as outlined in several previous publications (Frazier et al., 2003; Hofmann et al., 2006, 2008; Nahir et al., 2007). In brief, the prototypical mossy cell studied here was clearly located in the hilus, appeared larger than other types of hilar cells when visualized under IR DIC, had large whole cell capacitance (193 ± 5.97 pF), moderate input resistance (191 ± 9.72 MΩ) and often displayed high frequency and at least some large amplitude (>100 pA) spontaneous postsynaptic currents when voltage clamped at −70 mV in the absence of glutamate receptor antagonists. While previous work has indicated that some hilar interneurons may have some of these features, no cell was considered a mossy cell unless it met all of the above criteria. Additionally, the vast majority of cells (~90%) were examined with fluorescence microscopy to confirm that they were both multipolar and spiny, with thorny excrescences being particularly prominent on the proximal dendrites.

$CB1R^{-/-}$ and $CB1^{+/+}$ C57BL6/J mice were obtained by crossing homozygous littermates derived from heterozygous parents (WT and $CB1^{-/-}$ mice were therefore cousins). Our original heterozygous breeders were descended from the line developed by Dr. A. Zimmer at NIMH and were a generous gift from Dr. Carl Lupica (NIDA).

2.3. Data analysis

The baseline period began a minimum of 15–20 min after the establishment of whole cell recording. This was due in part to time required to wash-in ionotropic glutamate receptor antagonists and to allow equilibration of the internal solution. However, it was often substantially later as variable time was required to isolate minimally evoked IPSCs. Initial drug applications of interest were delivered after a minimum of 6 min of stable meIPSC baseline recording, and experimental measurements were made 6–12 min after application began. LTD was routinely measured 14–20 min into the DHPG washout period; however it was also quantified for cells that survived at 39–45 min post-DHPG (for macroscopic responses in F1) and at 24–30 min post-DHPG (for minimally evoked IPSCs in all other figures). In all cases, DSI was induced by a 5 s depolarization from −70 mV to 0 mV, and quantified by dividing the average amplitude of the first two responses after the depolarization by the average amplitude of the 8 responses immediately preceding it. Typically 2–5 such 'sets' of DSI were recorded in each cell. A cell was considered to be DSI positive only if the evoked response during the test period fell outside of the 95% confidence bands for the mean of both the baseline and recovery periods. For experiments involving minimally evoked IPSCs, individual sweeps contaminated by spontaneous IPSCs were manually removed from analysis. Cells in which 50% or more of sweeps were contaminated were discarded. Statistical tests (Student's two tailed one-sample and two-sample *t*-tests, Pearson correlations, and linear regression analysis) were performed using OriginPro v. 8 (OriginLab, Northampton, MA). Error bars in all figures represent the standard error of the mean.

3. Results

3.1. DHPG induces both acute and long-term depression of evoked IPSCs recorded from hilar mossy cells

Hilar mossy cells were voltage clamped at −70 mV with a $CsCH_3SO_3$ based internal containing ~60 mM chloride. Evoked IPSCs were isolated in the presence of DNQX (20 μM) and APV (40 μM) using a concentric bipolar stimulator placed in the hilus. Under these conditions we observed that bath application of the group I mGluR agonist DHPG (50 μM) caused a robust acute inhibition of evoked IPSC amplitude (to 65.7 ± 3.36% of baseline, $n = 14$, $p < 0.001$), and also a clear long-term depression (LTD) that was apparent when measured at both 14–20 and 39–45 min into the DHPG washout period (14–20: 72.9 ± 2.62% of baseline, $n = 14$ and 39–45: 72.1 ± 7.94% of baseline, $n = 8$, respectively; $p < 0.001$, Fig. 1A–C). By contrast, prior studies have shown virtually complete recovery from the acute effects of bath applied DHPG on synaptic

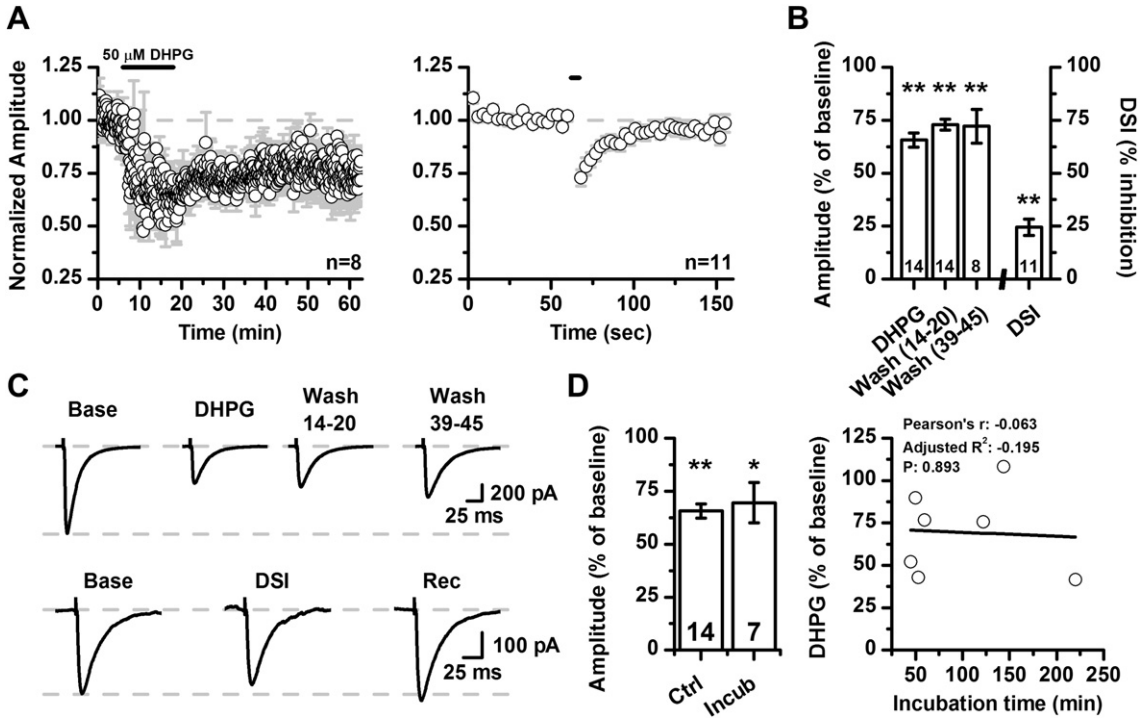

Fig. 1. Bath application of a group I mGluR agonist produces both acute and long-term inhibition of evoked IPSCs in hilar mossy cells. A) Application of the group I mGluR agonist DHPG (50 μM) caused an acute inhibition of eIPSCs to hilar mossy cells followed by a long-term depression. IPSCs were evoked using a bipolar stimulator placed in the hilus. DHPG sensitive evoked IPSCs (left) uniformly demonstrated robust DSI (right). Prior work from our lab has demonstrated that DSI as observed in hilar mossy cells is significantly enhanced by bath application of 3 μM CCh (Hofmann et al., 2006). In these experiments, CCh (3 μM) was bath applied a minimum of 20, and up to 45, minutes into the DHPG washout period, and DSI was induced with a 5-s depolarization from −70 mV to 0 mV. Several sets of DSI were collected for each cell and quantified as described in the methods. Thus in these experiments LTD was measured in the absence of CCh, and prior to checking for DSI, while by contrast DSI was measured only after LTD induction and in the presence of CCh. The left panel illustrates the 8 of 14 cells in which a full 45 min washout period was allowed, while the right panel illustrates the 11 of 14 cells in which DSI was successfully tested after DHPG washout and CCh wash-in. B) Summary graph illustrating the inhibitory effects of DHPG on eIPSCs and average DSI from the same population. Numbers on bars are *n* values. C) Averaged traces from a representative experiment in which DHPG produced robust acute and long-term depression and subsequent depolarization led to transient DSI. D) In separate experiments, 7 cells were incubated in ACSF containing 20 μM DNQX and 40 μM APV for a minimum of 45 min prior to DHPG application. Left panel: Summary plot showing no difference in the effect of DHPG between naïve and pre-incubated slices. Right panel: correlation plot demonstrating no relation between incubation time and DHPG effectiveness. Double asterisks indicate $p < 0.01$. Single asterisk indicates $p < 0.05$.

transmission in LTD negative preparations well within that time period (Chiu and Castillo, 2008; Galante and Diana, 2004; Ohno-Shosaku et al., 2002). Following washout of DHPG, and in the presence of stable LTD, 3 μM carbachol was bath applied and evoked IPSCs were tested for depolarization-induced suppression of inhibition (DSI) as previously described by Hofmann et al. (2006). In every case tested, LTD expressing eIPSCs were also found to be DSI positive (24.5 ± 3.86% inhibition, $n = 11$, $p < 0.001$, Fig. 1A–C).

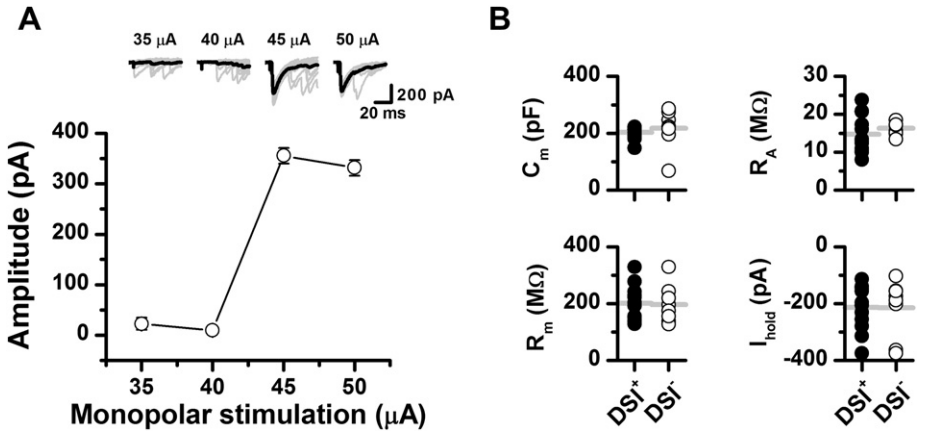

Fig. 2. Well-isolated DSI$^+$ and DSI$^-$ meIPSCs both innervate hilar mossy cells. A) Sample cell showing isolation of minimally evoked IPSCs. A threshold was defined as a switch from a majority of failures to a majority of successes over a small (i.e. ~5–10 μA) change in stimulator intensity followed by stable (unchanging) response amplitude over a further 10–15 μA increase in stimulation. Note that with the internal solution used here (containing ~60 mM Cl^-) the amplitude of unitary or near unitary IPSCs was often well in excess of 200 pA. Insets: individual (gray) and averaged (black) traces from each stimulation intensity. Averages are taken from a minimum of 10 sweeps for each condition. B) Summary plots showing no difference in intrinsic properties of postsynaptic cells between DSI$^+$ and DSI$^-$ groups, suggesting target cell specificity does not account for the differences in expression of DSI. Abbreviations: C_m – membrane capacitance; R_m – membrane resistance; R_A – access resistance (uncompensated); I_{hold} – holding current. Gray lines are averages for each parameter in each group. Closed circles represent cells with a DSI$^+$ meIPSC; open circles are cells with a DSI$^-$ input.

3.2. Both acute and long-term effects of DHPG are restricted to DSI positive afferents and require activation of CB1 receptors

In order to further investigate a potential role of endogenous cannabinoids in hilar mGluR-dependent LTD, we used minimal stimulation techniques (see Methods, Fig. 2A) to selectively test the effects of DHPG on both DSI negative and DSI positive GABAergic afferents to hilar mossy cells. In contrast to the experiments presented above, all minimally evoked IPSCs were tested for DSI prior to bath application of DHPG, and 3 μM CCh was bath applied at the beginning of each experiment rather than after DHPG application. CCh was used in this capacity because prior work from our lab has indicated that, while not absolutely required, its presence substantially enhances DSI of evoked IPSCs as observed in hilar mossy cells (Hofmann et al., 2006).

Our results indicated that DSI negative meIPSCs display neither acute nor long-term inhibition in response to bath application of DHPG. In fact, if anything, there was a trend towards slight potentiation of DSI negative meIPSCs during DHPG application, although this effect did not reach statistical significance (112 ± 8.4% of baseline, $n = 8$, $p = 0.18$, Fig. 3A, C). By sharp contrast, both the acute and long-term inhibition produced by DHPG on large amplitude evoked IPSCs in Fig. 1 were also clearly present on DSI positive afferents isolated with minimal stimulation techniques. Specifically, we found that bath application of 50 μM DHPG reduced the amplitude of DSI positive meIPSCs to 65.7 ± 3.44% of baseline, and that this effect had not recovered (77.5 ± 6.5% of baseline) when measured 14–20 min into the DHPG washout period ($n = 12$, $p < 0.001$, $p < 0.01$, respectively, Fig. 3B, D). However, it is important to point out that not all isolated DSI^+ meIPSCs underwent LTD following DHPG application. Specifically, three of the twelve DSI positive cells described above did in fact show complete recovery from the acute effects of DHPG when measured 14–20 min into the washout period (109.9 ± 3.69% of baseline, Fig. 3E, right). This result is important not only because it indicates the success rate for induction of mGluR-induced LTD is <100%, but also because it highlights the fact that in our experiments, as in prior studies, 20 min is clearly a sufficient period of time to washout DHPG from *in vitro* slices. For the remaining nine cells, LTD was quite robust when measured at this same time point (60.7 ± 4.21%, $p < 0.001$, $n = 9$) and was virtually unchanged when measured at 24–30 min into the washout period (74.5 ± 9.10% of baseline, $p = 0.034$, $n = 5$; Fig. 3E, left). Another detail worth highlighting is the observation that the acute inhibition produced by DHPG in these experiments on minimally evoked IPSCs was nearly identical to that reported in Fig. 1 for IPSCs evoked with a concentric bipolar stimulator (65.7 ± 3.44% vs. 65.7 ± 3.36% of baseline, from Figs. 1 and 3 respectively). That result is also important because it indicates that CCh (when applied before DHPG) does not substantially interact with or occlude the effects of mGluR activation. Collectively these results indicate that DSI positive afferents are required to observe DHPG-mediated acute inhibition of meIPSC amplitude in hilar mossy cells and that this inhibition progresses to robust LTD in approximately 75% of cases.

Previous work in other areas would strongly suggest that both acute and long-term effects of DHPG on DSI positive afferents are likely to depend on activation of CB1 cannabinoid receptors. In order to confirm this directly, we examined the effects of DHPG on minimally evoked IPSCs in slices that had been incubated in the CB1 receptor antagonist AM-251. Indeed, under these conditions, we observed no DSI and no apparent effect of DHPG (either acute or long-term) in 6 out 6 cells tested (Fig. 4). Like earlier minimal stimulation experiments, DHPG was tested in these cells in the presence of 3 μM CCh. Although very unlikely, we couldn't explicitly rule out the possibility that all 6 of these afferents would have been DSI negative even in the absence of AM-251. Therefore, we also performed a similar experiment in $CB1^{-/-}$ mice. As expected based on the AM-251 experiment, we found that $CB1^{-/-}$ mice lacked DSI (−2.9 ± 4.08% inhibition, $n = 13$, Fig. 5C) and showed minimal DHPG-induced inhibition (acute: 90.1 ± 3.44% of baseline, $p = 0.014$, $n = 7$; washout: 111.0 ± 16.39% inhibition, $p > 0.5$, $n = 7$; Fig. 5D, top). By contrast, $CB1^{+/+}$ controls (see Methods), showed both robust DSI (to 41.2 ± 6.11% of baseline, $n = 5$, $p < 0.001$), and clear acute inhibition in response to DHPG (to 68.3 ± 3.59% of baseline, $n = 5$, $p < 0.001$ relative to null, $p = 0.002$ relative to $CB1^{-/-}$, Fig. 5D, bottom). Interestingly, in contrast to the effects observed in SD rats, meIPSCs in $CB1^{+/+}$ mice largely recovered from the acute effects of DHPG during the washout period (to 91.5 ± 6.1% of baseline, $n = 5$, $p = 0.23$, Fig. 5D, bottom), with only one cell showing strong, long-lasting inhibition. While this may suggest a species difference in the induction of mGluR-mediated LTD, overall these data strongly support the conclusion that both the acute and long-term effects of DHPG in SD rats depends on activation of the CB1 receptor.

3.3. Target cell variability is not responsible for distinct effects of DHPG on DSI^+ and DSI^- GABAergic afferents

While the results described above clearly suggest that two distinct classes of inhibitory inputs to hilar mossy cells can be discriminated based on the susceptibility to mGluR-induced production of eCBs, two other possibilities should be considered. One possibility is that inadvertent patching of non-mossy hilar neurons could contribute to the DSI^- population. We consider this possibility extremely unlikely because all cells in this study met detailed physiological and anatomical criteria for identification as hilar mossy cells as described in the methods section and in several of our prior publications. Consistent with that conclusion we found no differences between DSI^+ and DSI^- cells in terms of membrane capacitance (203 ± 6.5 pF vs. 217 ± 23.9 pF), input resistance (201 ± 18.1 MΩ vs. 198 ± 23.5 MΩ), access resistance (14.7 ± 1.44 MΩ vs. 16.3 ± 0.65 MΩ), or holding current (−212 ± 23.2 pA vs. −214 ± 35.4 pA, $n = 12$ and 8, respectively; $p > 0.4$ in all cases; Fig. 2B). A more plausible possibility is that some cells that met criteria to be categorized as hilar mossy cells simply failed to release eCBs in response to mGluRs. However, we believe this is also quite unlikely because in two of the cells described in Fig. 3C–D we simultaneously recorded both a DSI^+ and a DSI^- input to a single hilar mossy cell, and in both cases, subsequent application of DHPG selectively inhibited only the DSI^+ afferent.

Finally, it is worth noting that we found the baseline amplitude to be larger on average for DSI^+ than for DSI^- meIPSCs (359.4 ± 54.18 pA vs. 167.2 ± 14.57 pA, respectively, $p = 0.011$, data not shown). This result may indicate that DSI negative inputs either release less transmitter or make more distal synaptic contacts with hilar mossy cells. However, we do not believe the smaller average amplitude of DSI^- meIPSCs is directly related to the lack of DHPG-mediated inhibition. That conclusion is based on the observation that there is no correlation between meIPSC amplitude and the effect of DHPG in the DSI^+ group (Pearson's r: −0.254, adjusted R^2: −0.029, $p = 0.425$, $n = 12$, data not shown), and further on the observation that the range of meIPSC amplitudes observed in the DSI^+ and mGluR sensitive group (99.6–649.4 pA) fully encompasses all responses observed in the DSI^- and mGluR insensitive population (123.1–230.2 pA).

3.4. mGluR1 but not mGluR5 is required for DHPG-mediated and endocannabinoid-dependent inhibition of minimally evoked IPSCs

Both mGluR1 and mGluR5 have been shown to elicit cannabinoid-dependent inhibition in other brain regions (Galante and

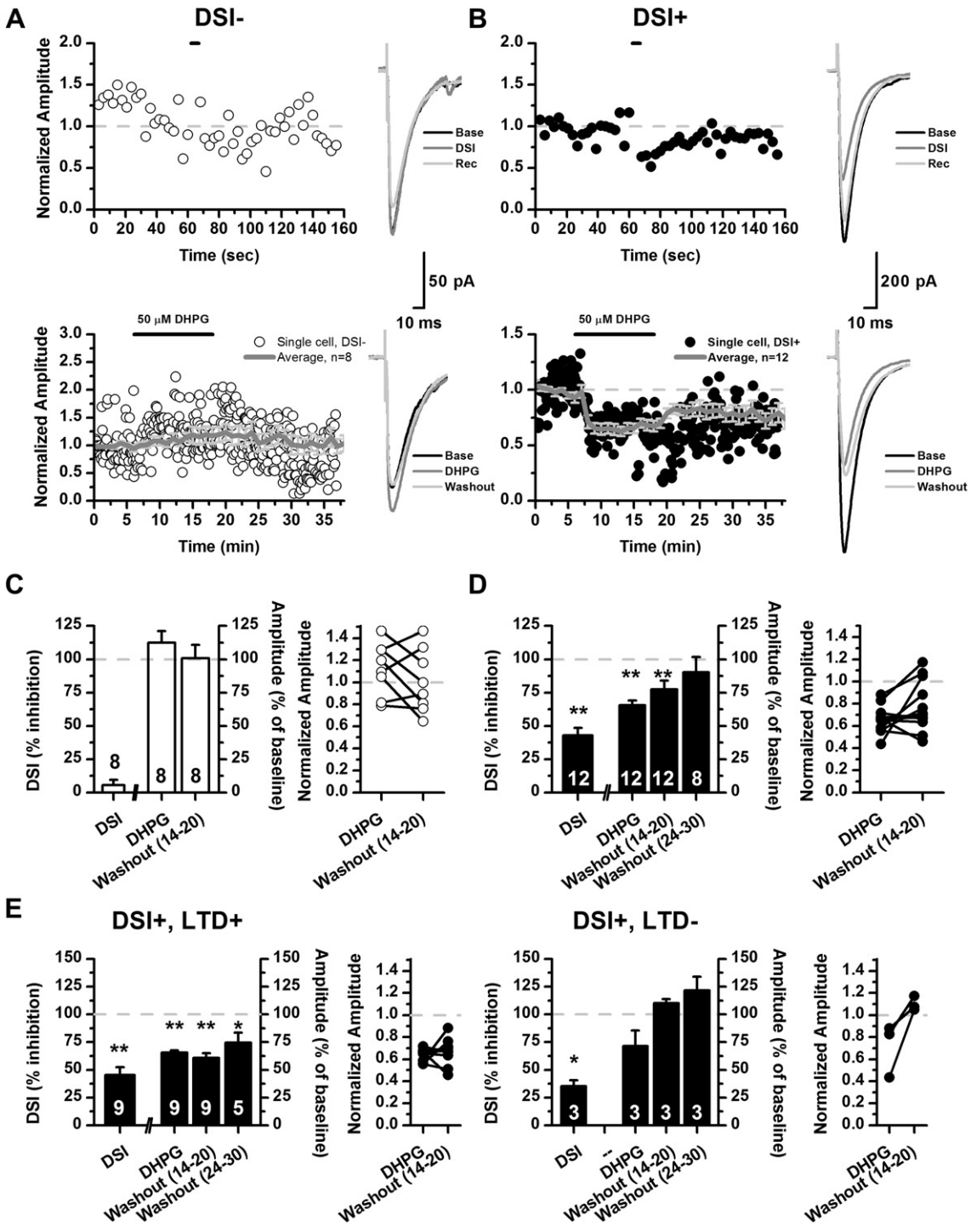

Fig. 3. Both acute and long-term effects of DHPG are restricted to DSI positive afferents to hilar mossy cells. Minimally evoked IPSCs were elicited using a monopolar electrode at 0.33 Hz for DSI, 0.2 Hz for DHPG application. In contrast to Figs. 1 and 3 μM CCh was bath applied early in the experiment and minimally evoked IPSCs were tested for DSI prior to being challenged with DHPG. A) Representative experiment from a minimally evoked IPSC that was DSI negative (top panel) indicates a corresponding lack of inhibition produced by DHPG (bottom panel). B) By contrast, a representative experiment on a minimally evoked IPSC that was DSI positive (top panel) shows robust acute and long-term inhibition induced by DHPG (bottom panel). Insets for both A and B are averaged sweeps from the cell shown in the corresponding scatter plot. Sweeps were averaged from measurement periods (Base, DSI, Recovery, etc.) as described in the methods. Solid gray lines in bottom panels of A and B represent the population average and standard errors for all cells that survived through the initial 20 min washout period. C) Summary of experiments from all cells where DSI negative afferents were isolated. Left panel: population means showing lack of DSI and corresponding lack of acute or long-term effects of DHPG. Right panel: individual cell responses to treatment with DHPG treatment during the measurement periods. D) Summary of experiments from all cells where DSI positive afferents were isolated. Left panel: population means showing significant DSI and corresponding robust acute and long-term inhibition produced by DHPG. Right panel: individual cell responses to treatment with DHPG treatment during the measurement periods. E) Summary of experiments from DSI positive data set, broken into LTD positive and negative subsets, respectively. Left panels: 9 of the 12 DSI positive meIPSCs showed robust acute and long-term inhibition in response to DHPG. Right panels: 3 DSI positive meIPSCs exhibited complete recovery following acute inhibition by DHPG. Numbers on the bars are *n* values. Double asterisks indicate $p < 0.01$. Single asterisk indicates $p < 0.05$.

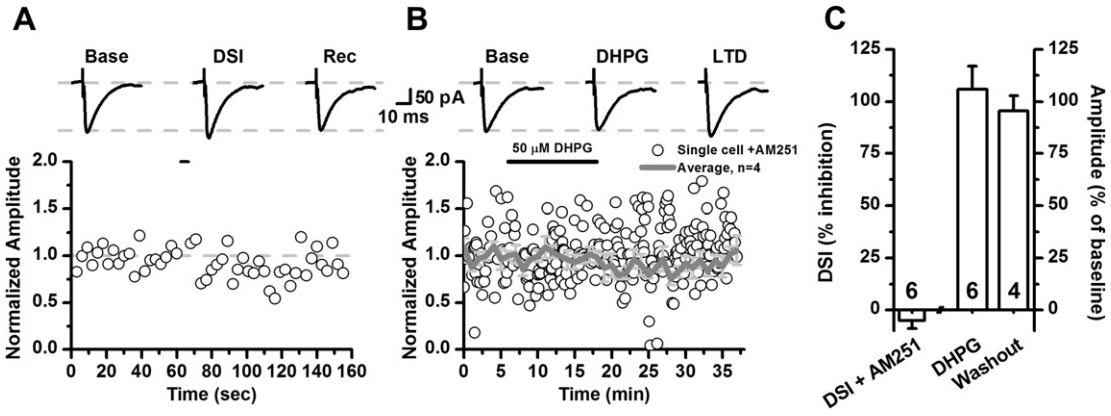

Fig. 4. Blockade of CB1Rs by AM-251 prevents both DSI and all effects of DHPG on meIPSC amplitude. For these experiments slices were incubated in ACSF containing 5 μM AM-251 (a CB1 receptor antagonist) for a minimum of 20 min prior to recording. A) Representative experiment indicating that minimally evoked IPSCs are DSI negative in the presence of AM-251. B) The DSI negative GABAergic afferent isolated in panel A is also completely unresponsive to bath application of 50 μM DHPG (open circles). The solid gray line is the population average and errors (light gray) for all cells that survived through the entire washout period. The insets in panels A and B are average traces from relevant time periods (as described in the methods) in this representative experiment. C) Summary graph from 6 cells where minimally evoked IPSCs were isolated in the presence of AM-251. None of the six cells tested were DSI positive or showed any acute effect of DHPG. Four of these six cells survived at least 20 min into the DHPG washout period, and indicated that long-term effects of DHPG are also absent in AM-251.

Diana, 2004; Ohno-Shosaku et al., 2002; Sergeeva et al., 2007). Therefore we tested the effects of DHPG on meIPSCs of DSI positive afferents in the presence of either the mGluR1 antagonist LY367385 (100 μM) or the mGluR5 antagonist MPEP (4 μM). Our results indicate that neither antagonist had a significant effect on basal meIPSC amplitude (MPEP: 102.5 ± 5.47% of baseline, $n = 8$; LY: 98.8 ± 5.83% of baseline, $n = 6$; $p > 0.05$; Fig. 6A, C), that both acute and long-term effects of DHPG were largely unaltered by MPEP (acute: 58.9 ± 3.30% of baseline, $p < 0.01$, $n = 8$; washout: 78.1 ± 10.31%, $p = 0.12$, $n = 4$; Fig. 6A, B), and that LY367385 provided near complete block of DHPG-mediated inhibition (acute: 92.5 ± 10.80% of baseline, $p > 0.05$, $n = 6$; washout: 99.1 ± 15.93%, $p > 0.5$, $n = 4$; Fig. 6C, D). Collectively, these results suggest that mGluR1 is likely necessary for both acute and long-term effects of DHPG in this system.

Although earlier data suggests that DHPG washes out well before the LTD measurement period (see Section 3.2), we confirmed that the observed LTD was not due to residual mGluR activation by applying LY367385 immediately after DHPG treatment. In seven cells, DHPG applied to DSI positive meIPSCs produced a robust acute inhibition similar to that observed in control experiments ($p > 0.1$) and application of LY367385 (100 μM) immediately following cessation of DHPG treatment did not block LTD (acute: 54.2 ± 7.30% of baseline, $p < 0.01$; washout: 70.6 ± 6.75% of baseline, $p < 0.01$, $n = 7$; Fig. 6E, F). This result indicates that while mGluR1 is responsible for the induction of

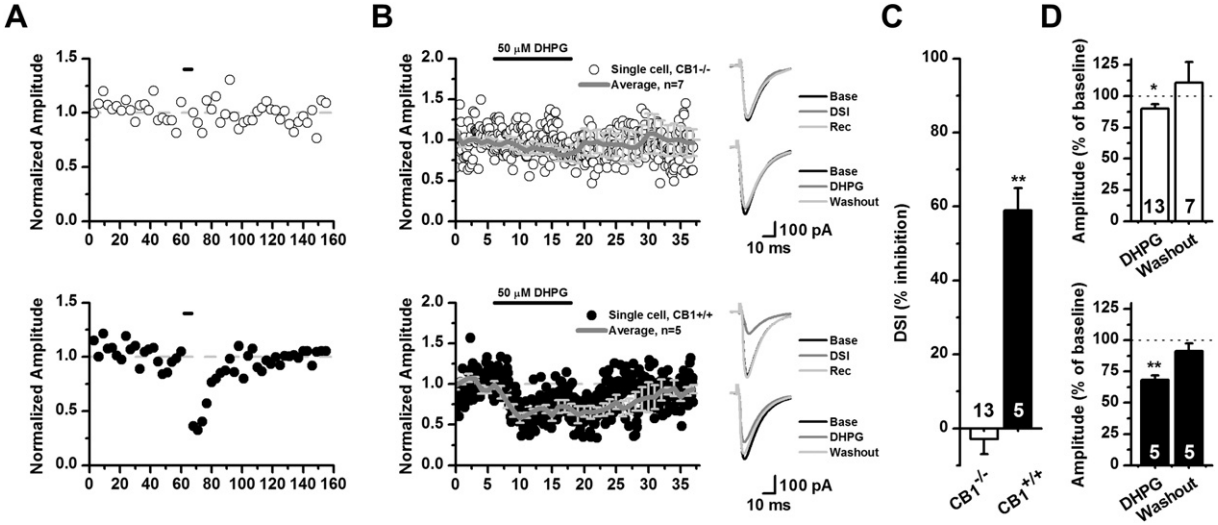

Fig. 5. DHPG has no effect on minimally evoked IPSC amplitude in CB1$^{-/-}$ mice. A) Representative data from a CB1R$^{-/-}$ mouse (top, open circles) and CB1R$^{+/+}$ mouse (bottom, filled circles) indicating a lack of DSI, and the presence of DSI, respectively. B) From the same cells shown in panel A, DHPG had no effect on meIPSCs in a CB1R$^{-/-}$ animal while mGluR activation induced an acute inhibition of meIPSCs in a CB1R$^{+/+}$ preparation. Insets show average traces from the corresponding runs for both DSI and DHPG treatments in the indicated time periods. Solid gray lines and errors (light gray) represent the population average for all cells that survived through the entire washout period. C) Population data for DSI experiments in CB1$^{-/-}$ and CB1$^{+/+}$ mice, again indicating an absence of DSI in CB1$^{-/-}$ animals. D) Summary graphs showing relative DHPG sensitivity of meIPSCs recorded form hilar mossy cells in CB1$^{-/-}$ (top, open bars) and CB1$^{+/+}$ (bottom, filled bars) mice. Overall there was a small but statistically significant inhibition of meIPSC amplitude produced by DHPG in CB1$^{-/-}$ mice that was not followed by any apparent LTD. By contrast, CB1$^{+/+}$ mice produced meIPSCs that were much more sensitive to DHPG and showed a mild but statistically insignificant trend towards LTD. Double asterisks indicate $p < 0.01$.

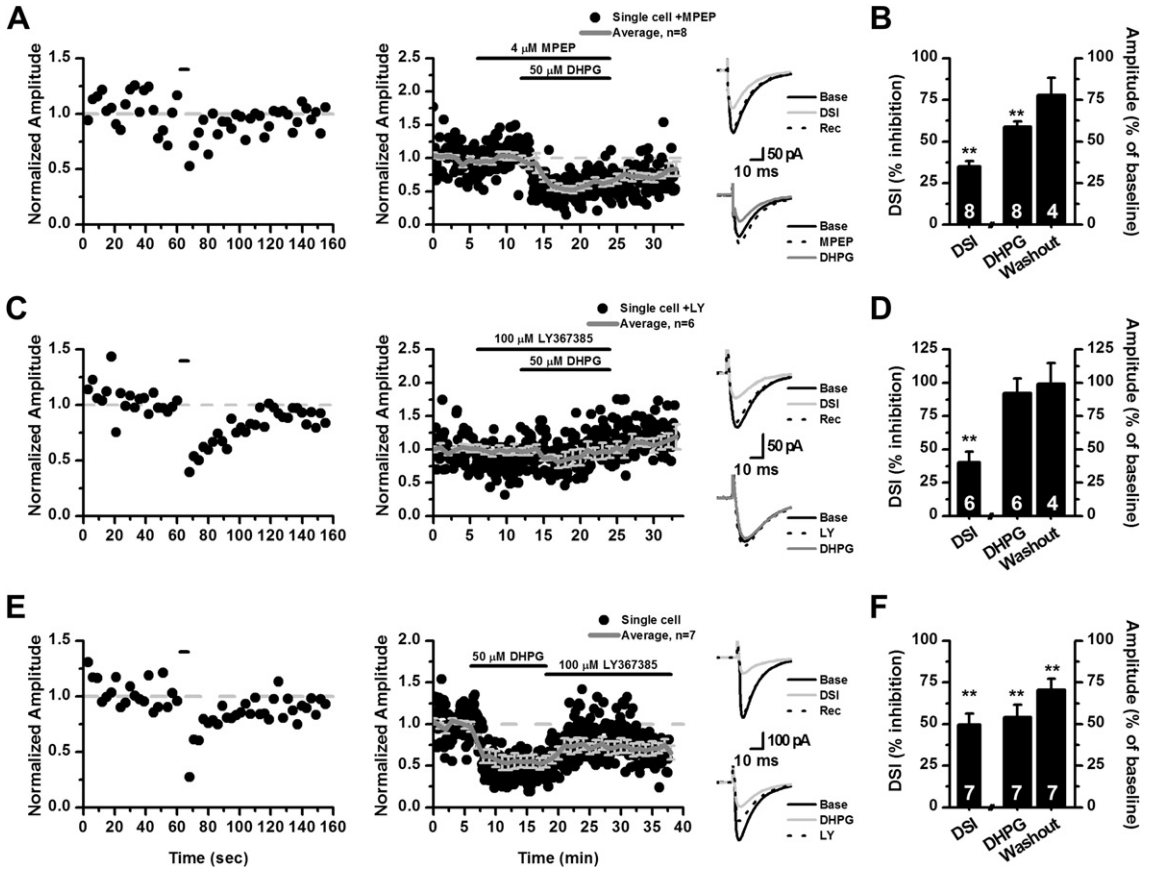

Fig. 6. Type I metabotropic glutamate receptors are required for the induction but not the maintenance of both acute and long-term effects of DHPG on meIPSCs recorded from hilar mossy cells. A) Treatment with the mGluR5 antagonist MPEP (4 μM) failed to block either the acute or long-term effects of DHPG on DSI positive afferents to hilar mossy cells. Left panel: Representative data from a sample cell showing robust DSI of a minimally evoked IPSC. Right panel: Data from that same cell indicating DHPG still produces robust acute and long-term inhibition of meIPSC amplitude in the presence of 4 μM MPEP. B) Population data from 8 cells where DSI positive afferents were tested with DHPG in the presence of MPEP. Four cells did not survive through the LTD measurement period. C) Unlike MPEP, the mGluR1 antagonist LY367385 (100 μM) prevented DHPG-mediated inhibition (both acute and long-term) of DSI positive meIPSCs. Left panel: Representative data from a sample cell showing robust DSI of a minimally evoked IPSC. Right panel: Data from that same cell indicating that DHPG fails to produce either acute or long-term inhibition of meIPSCs in the presence of LY367385. D) Population data from 6 cells where DSI positive afferents were tested with DHPG in the presence of LY367385. Two cells did not survive through the LTD measurement period. E) LY367385 (100 μM) applied coincident with the end of DHPG treatment had no effect on subsequent development of LTD ($p > 0.5$ compared to control). Left panel: Representative data from a sample cell showing robust DSI of a minimally evoked IPSC. Right Panel: Data from that same cell indicating robust acute inhibition by DHPG and a stable long-term depression even in the presence of an LY367385 chase. F) Population data from 7 cells where DSI positive afferents were treated with LY367385 immediately after DHPG application. Solid lines in panels A, C, and E represent the population average for all cells. Right panels in A and C are truncated to 33 min because some cells did not survive entire washout period. Insets in panels A, C, and E are average traces from the indicated time periods. Double asterisks indicate $p < 0.01$.

DHPG-mediated inhibition, its prolonged activation is not necessary for LTD maintenance.

3.5. mGluR-induced and eCB-dependent LTD in the hilus does not appear to depend critically on presynaptic activity or postsynaptic priming

To date, this form of mGluR-induced and eCB-dependent LTD of inhibitory transmission has only been described in the amygdala (Azad et al., 2004; Marsicano et al., 2002), area CA1 of the hippocampus (Chevaleyre and Castillo, 2003, 2004), and the dorsolateral region of the striatum (Adermark and Lovinger, 2009). In CA1, recent studies have suggested at least two requirements for the successful expression of iLTD. These include presynaptic activity in CB1 positive GABAergic afferents (Heifets et al., 2008) and postsynaptic priming via direct depolarization of the cell prior to application of mGluR agonists (Edwards et al., 2008). Therefore we first asked whether any features of spontaneous IPSCs observed in hilar mossy cells were able to predict the acute or long-term effects of DHPG application on DSI positive afferents. In the baseline condition average sIPSC frequency was 4.7 ± 0.77 Hz (range: 1.5–10.6 Hz), average sIPSC amplitude was 28.5 ± 1.63 pA (range: 20.7–42.9 pA), and average sIPSC area was 272.6 ± 23.02 pA msec (range: 134.3–455.3 pA msec), and these values were typically increased during bath application of DHPG (e.g. frequency to 15.3 ± 1.37 Hz, amplitude to 37.4 ± 3.74 pA, and area to 459.1 ± 60.20 pA msec; $p < 0.05$ for all conditions). However, overall, we could find no statistically significant correlation between any of these parameters of spontaneous IPSCs (either prior to or during DHPG application) and the acute or long-term inhibition of evoked IPSCs produced by DHPG ($p > 0.1$ in all cases, $n = 14$ and 12 respectively, data not shown). This result suggests either that there is no requirement for presynaptic activity for induction of eCB-dependent LTD in the hilus, or that the required level of activity is so low that all afferents tested were sufficiently active.

We next questioned whether prior postsynaptic depolarization was required for observing mGluR-induced and eCB-dependent LTD in the hilus. As noted earlier, we found that DHPG-mediated inhibition proved robust in the hilus even when DSI was explicitly tested *after* the drug treatment (Fig. 1). Since mossy cells often

receive a large amount of spontaneous afferent activity (Scharfman, 1993; Strowbridge et al., 1992), we hypothesized that endogenous activation of ionotropic glutamate receptors might preclude the need for postsynaptic priming by direct somatic depolarization. In order to test that hypothesis, slices were incubated for an average of 99.1 ± 24.95 min (range: 45–220 min) in ACSF containing 20 μM DNQX and 40 μM APV to minimize endogenous excitatory synaptic transmission prior to testing for LTD. Ensuing application of DHPG still produced robust acute inhibition (to 69.5 ± 9.47% of baseline, $n = 7$, $p = 0.018$, Fig. 1D) and LTD (measured at 14–20 min post-DHPG: 79.4 ± 6.91% of baseline, $n = 7$, $p = 0.024$, data not shown), neither of which differed significantly from control values ($p > 0.3$ for both). Furthermore, no correlation was observed between the incubation length and the acute effects of DHPG (Pearson's r: = 0.063, Adjusted R^2: −0.195, $p > 0.5$, Fig. 1D). Collectively, these results indicate that neither direct somatic depolarization nor robust endogenous excitatory transmission is explicitly required to prime hilar mossy cells for expression of DHPG-induced and eCB-dependent LTD.

4. Discussion

In the present manuscript, we report that bath application of the group I mGluR agonist DHPG causes an acute inhibition of evoked IPSCs recorded from hilar mossy cells through activation of an LY367385 sensitive and MPEP insensitive metabotropic glutamate receptor. Further, we find that clear long-term depression of evoked IPSC amplitude remains after washout of DHPG. This form of LTD was measured up to 45 min after cessation of DHPG application in experiments that involved bulk stimulation techniques, and was assessed up to 30 min post-DHPG in experiments involving unitary or near unitary responses. In the later group, LTD was successfully induced in 75% of trials, while rapid recovery from the acute effects of DHPG was observed in the remaining 25% of cases. Both the acute and long-term forms of DHPG-induced inhibition were observed exclusively in DSI positive afferents, and were absent in slices pretreated with the CB1 receptor antagonist AM-251. Further, $CB1^{-/-}$ mice lacked both acute and long-term effects of DHPG, while wild type controls showed strong acute inhibition and mild (not quite statistically significant) long-term depression. Overall, these results clearly indicate that mGluR-induced and eCB-dependent LTD modulates a subset of inhibitory inputs to hilar mossy cells that express CB1 receptors. Although mossy cells are clearly innervated by both cholecystokinin immunoreactive and parvalbumin immunoreactive interneurons, several lines of evidence from other groups suggest that it is most likely the CCK positive interneurons express the CB1 receptor and thus provide the DSI and LTD positive inputs studied here (Acsady et al., 2000; Katona et al., 1999; Tsou et al., 1999). Further, we find that spontaneous activity in inhibitory afferents has no clear correlation to either acute or long-term effects of DHPG, and that direct depolarization of the postsynaptic cell prior to DHPG application is not required for induction of LTD in this system.

Although eCB-LTD has been reported for both inhibitory and excitatory synapses in several other areas of the brain (Adermark and Lovinger, 2009; Azad et al., 2004; Chevaleyre and Castillo, 2003; Kreitzer and Malenka, 2007; Robbe et al., 2002; Soler-Llavina and Sabatini, 2006), it has become apparent that the presence of all necessary synaptic machinery (both pre and post) does not guarantee functional expression of eCB-LTD (e.g. see Chiu and Castillo, 2008). Moreover, a number of studies, in an attempt to mimic eCB-LTD absent mGluR activation, have demonstrated that extended CB1R activation alone, either directly with an exogenous CB1 agonist or through an alternate means of eCB production, is generally insufficient to induce LTD (Edwards et al., 2006; Pan et al., 2008). In the present study it is clear that DHPG-induced and eCB-dependent inhibition clearly persists well after DHPG washes out of the slice, and thus represents a long-term form of synaptic plasticity. This is evident not only because LTD was observed for up to 45 min after cessation of DHPG application, but also because much more rapid washout of DHPG from acute brain slices was observed in a subset of LTD negative afferents, a phenomenon routinely documented in other studies (e.g. Chevaleyre and Castillo, 2003; Galante and Diana, 2004). Further, application of the mGluR1 antagonist LY367385 immediately after DHPG treatment failed to prevent LTD onset. What is less clear at this point is whether mGluR-induced and eCB-dependent LTD as observed in the hilus is mediated by long-term changes in eCB tone, or by presynaptic changes that become independent of both mGluRs and CB1Rs. Experiments that involve application of CB1 antagonists after DHPG may be helpful in this area, however preliminary efforts have indicated that such experiments will be complicated significantly by the presence of a variable (and possibly activity dependent) cannabinoid tone that is present in the hilus under basal recording conditions.

Throughout the CNS, a wide variety of receptor systems have been implicated in modulation of eCB production. These include not only mGluRs, but also muscarinic acetylcholine receptors (mAChRs), type 2 dopamine receptors (D_2Rs), and type 2 serotonin receptors (Chevaleyre and Castillo, 2003; Edwards et al., 2006; Hashimotodani et al., 2005; Kreitzer and Malenka, 2005; Melis et al., 2004; Wang et al., 2006). In addition, in certain areas multiple receptor systems have been shown to act synergistically to produce eCB-LTD. For example, D_2R activation is known to enhance mGluR-dependent eCB-LTD of glutamatergic synapses in the striatum (Kreitzer and Malenka, 2005). More recently, it was suggested that dopamine achieves this augmentation indirectly by reducing cholinergic drive of postsynaptic type 1 mAChRs (Wang et al., 2006). In that context it is interesting to note that we found no detectable difference between eCB-LTD of GABAergic transmission in the presence and absence of the general mAChR agonist carbachol. That result, combined with previous studies from our lab (Hofmann et al., 2006), suggests that carbachol is likely to selectively modulate activity dependent (e.g. depolarization-induced), as opposed to mGluR-mediated, pathways for eCB production in hilar mossy cells.

Throughout the CNS there is also likely to be a variety of distinct mechanisms available for presynaptic modulation of the effects of cannabinoids on CB1 positive terminals. For example, presynaptic NMDARs are necessary for eCB-LTD induction at glutamatergic terminals in the visual and somatosensory cortices (Bender et al., 2006; Rodriguez-Moreno and Paulsen, 2008; Sjostrom et al., 2003), although NMDARs are not required in the striatum (Singla et al., 2007). Further, a number of non-receptor based manipulations can also affect the induction of eCB-LTD. In the hippocampus, both presynaptic activity coincident with mGluR-induced eCB signaling and the recent history of the postsynaptic cell heavily affect whether eCB-LTD will occur. In area CA1 of the hippocampus, presynaptic activity leading to Ca^{2+} influx into the terminal is thought to critically determine whether eCB-LTD is expressed at the synapses of *stratum radiatum* interneurons onto CA1 pyramidal cells (Heifets et al., 2008). Interestingly, we found no correlation between spontaneous activity in the hilar inhibitory network and the efficacy of DHPG, either acute or long-term, using both bulk and minimal stimulation protocols.

Edwards et al. (2008) have recently presented evidence that eCB-LTD at interneuron synapses as observed in area CA1 requires a postsynaptic priming step that is effectively induced by direct somatic depolarization. They further suggest that such a requirement may have been missed in previous studies that routinely

tested for DSI prior to evaluating mGluR-induced and eCB-dependent inhibition (e.g. Chevaleyre and Castillo, 2003; Varma et al., 2001). Interestingly, we found that mGluR-induced eCB-LTD is quite obvious in afferents to hilar mossy cells even in the absence of both direct somatic depolarization and CCh, and without any other obvious form of artificial postsynaptic priming (Fig. 1). Since mossy cells receive unusually high levels of spontaneous excitatory activity it seemed possible that they might be intrinsically primed for DHPG-induced and eCB-dependent LTD. However, we found that prolonged (up to ~4 h) reduction of excitatory drive to hilar mossy cells (via incubation in the presence of ionotropic mGluR antagonists) failed to prevent DHPG-induced inhibition. While this suggests that mossy cells do not in fact require postsynaptic priming for induction of eCB-LTD, we cannot explicitly rule out an effect of the patch clamping procedure itself.

In prior studies patterned stimulation protocols have sometimes been used to produce endogenous mGluR-dependent eCB signaling and subsequent LTD. Successful protocols for induction of eCB-LTD include low frequency stimulation (LFS) in the amygdala (Azad et al., 2004; Marsicano et al., 2002), theta burst stimulation (TBS) in the hippocampus (Chevaleyre and Castillo, 2003) and visual cortex (Huang et al., 2008), high frequency stimulation (HFS) in the hippocampus (Yasuda et al., 2008), striatum (Gerdeman et al., 2002), and superior colliculus explants (Henneberger et al., 2007), and varying other stimulus patterns as well (Pan et al., 2008; Soler-Llavina and Sabatini, 2006). Additionally, eCB-LTD has shown significant spike-timing dependence at certain synapses (Kreitzer and Malenka, 2005; Sjostrom et al., 2003; Tzounopoulos et al., 2007). Several factors complicate use of electrical stimulation techniques to induce mGluR-dependent LTD in the hilus. As noted above, hilar mossy cells naturally have a high level of spontaneous EPSCs, indicative of significant activity in their glutamatergic afferents; yet even now, the source of this activity remains highly controversial. Mossy fiber axons from granule cells have generally been considered the most likely source of this activity (Scharfman, 1993). On the other hand, granule cells have a fairly negative resting membrane potential and do not often spontaneously fire action potentials *in vitro*. Further, glutamatergic innervation of the hilus from the granule cell layer is diffuse and not well suited to bulk activation from a single stimulation site. As such, initial attempts in our laboratory to induce eCB-LTD in the hilus by tetanizing the granule cell layer have been unsuccessful. Other potential sources of glutamatergic afferents to hilar mossy cells include CA3 pyramidal cells (Nahir et al., 2007; Scharfman, 1994), the newly discovered semilunar granule cells (Williams et al., 2007), and potentially other mossy cells, although mossy cell-mossy cell synapses have been reported to be extremely rare in *in vitro* slices (Larimer and Strowbridge, 2008). These other pathways are also not readily amenable to broad activation using conventional electrical stimulation techniques. However, regardless of the source, the clearly high level of spontaneous activity in many glutamatergic afferents to hilar mossy cells raises the intriguing possibility that such activity could prove (even absent exogenous stimulation) to be a robust endogenous regulator of both eCB tone and mGluR-induced LTD. Thus, in our view, future studies should carefully evaluate the relationship between spontaneous excitatory activity in the hilar network and the induction of mGluR-dependent and eCB-mediated forms of synaptic plasticity.

Acknowledgements

This work was supported by NIDA R01 DA019576. We thank Drs. David Edwards and Ben Strowbridge for constructive comments on an earlier version of the manuscript.

References

Acsady, L., Katona, I., Martinez-Guijarro, F.J., Buzsaki, G., Freund, T.F., 2000. Unusual target selectivity of perisomatic inhibitory cells in the hilar region of the rat hippocampus. J. Neurosci. 20, 6907–6919.
Adermark, L., Lovinger, D.M., 2009. Frequency-dependent inversion of net striatal output by endocannabinoid-dependent plasticity at different synaptic inputs. J. Neurosci. 29, 1375–1380.
Azad, S.C., Monory, K., Marsicano, G., Cravatt, B.F., Lutz, B., Zieglgansberger, W., Rammes, G., 2004. Circuitry for associative plasticity in the amygdala involves endocannabinoid signaling. J. Neurosci. 24, 9953–9961.
Bender, V.A., Bender, K.J., Brasier, D.J., Feldman, D.E., 2006. Two coincidence detectors for spike timing-dependent plasticity in somatosensory cortex. J. Neurosci. 26, 4166–4177.
Chevaleyre, V., Castillo, P.E., 2003. Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. Neuron 38, 461–472.
Chevaleyre, V., Castillo, P.E., 2004. Endocannabinoid-mediated metaplasticity in the hippocampus. Neuron 43, 871–881.
Chevaleyre, V., Takahashi, K.A., Castillo, P.E., 2006. Endocannabinoid-mediated synaptic plasticity in the CNS. Annu. Rev. Neurosci. 29, 37–76.
Chiu, C.Q., Castillo, P.E., 2008. Input-specific plasticity at excitatory synapses mediated by endocannabinoids in the dentate gyrus. Neuropharmacology 54, 68–78.
Edwards, D.A., Kim, J., Alger, B.E., 2006. Multiple mechanisms of endocannabinoid response initiation in hippocampus. J. Neurophysiol. 95, 67–75.
Edwards, D.A., Zhang, L., Alger, B.E., 2008. Metaplastic control of the endocannabinoid system at inhibitory synapses in hippocampus. Proc. Natl. Acad. Sci. U.S.A. 105, 8142–8147.
Frazier, C.J., Strowbridge, B.W., Papke, R.L., 2003. Nicotinic receptors on local circuit neurons in dentate gyrus: a potential role in regulation of granule cell excitability. J. Neurophysiol. 89, 3018–3028.
Galante, M., Diana, M.A., 2004. Group I metabotropic glutamate receptors inhibit GABA release at interneuron-Purkinje cell synapses through endocannabinoid production. J. Neurosci. 24, 4865–4874.
Gerdeman, G.L., Ronesi, J., Lovinger, D.M., 2002. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nat. Neurosci. 5, 446–451.
Hashimotodani, Y., Ohno-Shosaku, T., Tsubokawa, H., Ogata, H., Emoto, K., Maejima, T., Araishi, K., Shin, H.S., Kano, M., 2005. Phospholipase Cbeta serves as a coincidence detector through its Ca^{2+} dependency for triggering retrograde endocannabinoid signal. Neuron 45, 257–268.
Heifets, B.D., Chevaleyre, V., Castillo, P.E., 2008. Interneuron activity controls endocannabinoid-mediated presynaptic plasticity through calcineurin. Proc. Natl. Acad. Sci. U.S.A. 105, 10250–10255.
Henneberger, C., Redman, S.J., Grantyn, R., 2007. Cortical efferent control of subcortical sensory neurons by synaptic disinhibition. Cereb. Cortex 17, 2039–2049.
Hofmann, M.E., Nahir, B., Frazier, C.J., 2006. Endocannabinoid-mediated depolarization-induced suppression of inhibition in hilar mossy cells of the rat dentate gyrus. J. Neurophysiol. 96, 2501–2512.
Hofmann, M.E., Nahir, B., Frazier, C.J., 2008. Excitatory afferents to CA3 pyramidal cells display differential sensitivity to CB1 dependent inhibition of synaptic transmission. Neuropharmacology 55, 1140–1146.
Howard, A.L., Neu, A., Morgan, R.J., Echegoyen, J.C., Soltesz, I., 2007. Opposing modifications in intrinsic currents and synaptic inputs in post-traumatic mossy cells: evidence for single-cell homeostasis in a hyperexcitable network. J. Neurophysiol. 97, 2394–2409.
Huang, L., Rowan, M.J., Anwyl, R., 1999. Induction of long-lasting depression by (+)-alpha-methyl-4-carboxyphenylglycine and other group II mGlu receptor ligands in the dentate gyrus of the hippocampus in vitro. Eur. J. Popul. 366, 151–158.
Huang, Y., Yasuda, H., Sarihi, A., Tsumoto, T., 2008. Roles of endocannabinoids in heterosynaptic long-term depression of excitatory synaptic transmission in visual cortex of young mice. J. Neurosci. 28, 7074–7083.
Kano, M., Ohno-Shosaku, T., Hashimotodani, Y., Uchigashima, M., Watanabe, M., 2009. Endocannabinoid-mediated control of synaptic transmission. Physiol. Rev. 89, 309–380.
Katona, I., Sperlagh, B., Sik, A., Kafalvi, A., Vizi, E.S., Mackie, K., Freund, T.F., 1999. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J. Neurosci. 19, 4544–4558.
Kawamura, Y., Fukaya, M., Maejima, T., Yoshida, T., Miura, E., Watanabe, M., Ohno-Shosaku, T., Kano, M., 2006. The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. J. Neurosci. 26, 2991–3001.
Kreitzer, A.C., Malenka, R.C., 2005. Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. J. Neurosci. 25, 10537–10545.
Kreitzer, A.C., Malenka, R.C., 2007. Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 445, 643–647.
Larimer, P., Strowbridge, B.W., 2008. Nonrandom local circuits in the dentate gyrus. J. Neurosci. 28, 12212–12223.
Lovinger, D.M., 2008. Presynaptic modulation by endocannabinoids. Handb. Exp. Pharmacol., 435–477.

Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., Hofmann, C., Zieglgansberger, W., Di Marzo, V., Lutz, B., 2002. The endogenous cannabinoid system controls extinction of aversive memories. Nature 418, 530–534.

Melis, M., Pistis, M., Perra, S., Muntoni, A.L., Pillolla, G., Gessa, G.L., 2004. Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. J. Neurosci. 24, 53–62.

Nahir, B., Bhatia, C., Frazier, C.J., 2007. Presynaptic inhibition of excitatory afferents to hilar mossy cells. J. Nephrol. 97, 4036–4047.

Naie, K., Tsanov, M., Manahan-Vaughan, D., 2007. Group I metabotropic glutamate receptors enable two distinct forms of long-term depression in the rat dentate gyrus in vivo. Eur. J. Neurosci. 25, 3264–3275.

Ohno-Shosaku, T., Shosaku, J., Tsubokawa, H., Kano, M., 2002. Cooperative endocannabinoid production by neuronal depolarization and group I metabotropic glutamate receptor activation. Eur. J. Neurosci. 15, 953–961.

Pan, B., Hillard, C.J., Liu, Q.S., 2008. Endocannabinoid signaling mediates cocaine-induced inhibitory synaptic plasticity in midbrain dopamine neurons. J. Neurosci. 28, 1385–1397.

Robbe, D., Kopf, M., Remaury, A., Bockaert, J., Manzoni, O.J., 2002. Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. Proc. Natl. Acad. Sci. U.S.A. 99, 8384–8388.

Rodriguez-Moreno, A., Paulsen, O., 2008. Spike timing-dependent long-term depression requires presynaptic NMDA receptors. Nat. Neurosci. 11, 744–745.

Scharfman, H.E., 1993. Characteristics of spontaneous and evoked EPSPs recorded from dentate spiny hilar cells in rat hippocampal slices. J. Nephrol. 70, 742–757.

Scharfman, H.E., 1994. Evidence from simultaneous intracellular recordings in rat hippocampal slices that area CA3 pyramidal cells innervate dentate hilar mossy cells. J. Neurophysiol. 72, 2167–2180.

Sergeeva, O.A., Doreulee, N., Chepkova, A.N., Kazmierczak, T., Haas, H.L., 2007. Long-term depression of cortico-striatal synaptic transmission by DHPG depends on endocannabinoid release and nitric oxide synthesis. Eur. J. Neurosci. 26, 1889–1894.

Singla, S., Kreitzer, A.C., Malenka, R.C., 2007. Mechanisms for synapse specificity during striatal long-term depression. J. Neurosci. 27, 5260–5264.

Sjostrom, P.J., Turrigiano, G.G., Nelson, S.B., 2003. Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors. Neuron 39, 641–654.

Soler-Llavina, G.J., Sabatini, B.L., 2006. Synapse-specific plasticity and compartmentalized signaling in cerebellar stellate cells. Nat. Neurosci. 9, 798–806.

Strowbridge, B.W., Buckmaster, P.S., Schwartzkroin, P.A., 1992. Potentiation of spontaneous synaptic activity in rat mossy cells. Neurosci. Lett. 142, 205–210.

Trommer, B.L., Liu, Y.B., Pasternak, J.F., 1996. Long-term depression at the medial perforant path-granule cell synapse in developing rat dentate gyrus. Brain Res. Dev. Brain Res. 96, 97–108.

Tsou, K., Mackie, K., Sanudo-Pena, M.C., Walker, J.M., 1999. Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. Neuroscience 93, 969–975.

Tzounopoulos, T., Rubio, M.E., Keen, J.E., Trussell, L.O., 2007. Coactivation of pre- and postsynaptic signaling mechanisms determines cell-specific spike-timing-dependent plasticity. Neuron 54, 291–301.

Varma, N., Carlson, G.C., Ledent, C., Alger, B.E., 2001. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. J. Neurosci. 21, RC188.

Wang, Z., Kai, L., Day, M., Ronesi, J., Yin, H.H., Ding, J., Tkatch, T., Lovinger, D.M., Surmeier, D.J., 2006. Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. Neuron 50, 443–452.

Williams, P.A., Larimer, P., Gao, Y., Strowbridge, B.W., 2007. Semilunar granule cells: glutamatergic neurons in the rat dentate gyrus with axon collaterals in the inner molecular layer. J. Neurosci. 27, 13756–13761.

Wilson, R.I., Kunos, G., Nicoll, R.A., 2001. Presynaptic specificity of endocannabinoid signaling in the hippocampus. Neuron 31, 453–462.

Yasuda, H., Huang, Y., Tsumoto, T., 2008. Regulation of excitability and plasticity by endocannabinoids and PKA in developing hippocampus. Proc. Natl. Acad. Sci. U.S.A. 105, 3106–3111.