Systems/Circuits

2-AG-Mediated Control of GABAergic Signaling Is Impaired in a Model of Epilepsy

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Repeated seizures result in a persistent maladaptation of endocannabinoid (eCB) signaling, mediated part by anandamide signaling deficiency in the basolateral amygdala (BLA) that manifests as aberrant synaptic function and altered emotional behavior. Here, we determined the effect of repeated seizures (kindling) on 2-arachidonoylglycerol (2-AG) signaling on GABA transmission by directly measuring tonic and phasic eCB-mediated retrograde signaling in an *in vitro* BLA slice preparation from male rats. We report that both activity-dependent and muscarinic acetylcholine receptor (mAChR)-mediated depression of GABA synaptic transmission was reduced following repeated seizure activity. These effects were recapitulated in sham rats by preincubating slices with the 2-AG synthesizing enzyme inhibitor DO34. Conversely, preincubating slices with the 2-AG degrading enzyme inhibitor KML29 rescued activity-dependent 2-AG signaling, but not mAChR-mediated synaptic depression, over GABA transmission in kindled rats. These effects were not attributable to a change in cannabinoid type 1 (CB1) receptor sensitivity or altered 2-AG tonic signaling since the application of the highly selective CB1 receptor agonist CP55,940 provoked a similar reduction in GABA synaptic activity in both sham and kindled rats, while no effect of either DO34 or of the CB1 inverse agonist AM251 was observed on frequency and amplitude of spontaneous IPSCs in either sham or kindled rats. Collectively, these data provide evidence that repeated amygdala seizures persistently alter phasic 2-AG-mediated retrograde signaling at BLA GABAergic synapses, probably by impairing stimulus-dependent 2-AG synthesis/release, which contributes to the enduring aberrant synaptic plasticity associated with seizure activity.

Key words: **electrophysiology; endocannabinoids; epilepsy; GABA; kindling; synaptic plasticity**

Significance Statement

The plastic reorganization of endocannabinoid (eCB) signaling after seizures and during epileptogenesis may contribute to the negative neurobiological consequences associated with seizure activity. Therefore, a deeper understanding of the molecular basis underlying the pathologic long-term eCB signaling remodeling following seizure activity will be crucial to the development of novel therapies for epilepsy that not only target seizure activity, but, most importantly, the epileptogenesis and the comorbid conditions associated with epilepsy.

Introduction

Seizure activity results in aberrant neuronal plasticity (Colangeli et al., 2017; Farrell et al., 2020), which manifests as learning and memory impairments (Farrell et al., 2017). However, the pathophysiological underpinnings of the aberrant rewiring of brain circuits following seizures remain elusive. A large body of evidence supports the view that the endocannabinoid (eCB) system plays an important role in the pathophysiology of epilepsy (Soltesz et al., 2015; Sugaya and Kano, 2018; Colangeli et al., 2021; Sugaya and Kano, 2021). As a general rule, activation of the cannabinoid type 1 (CB1) receptor typically protects against seizure activity by producing a net reduction in overall neuronal

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excitability (Marsicano et al., 2003; Sugaya et al., 2016; Colangeli et al., 2017). Conversely, both acute (Farrell et al., 2021) and persistent (Chen et al., 2003, 2007; Colangeli et al., 2020) maladaptation of eCB signaling following seizure activity may account for some of the negative neurobiological consequences of seizures.

eCBs are lipid molecules that modulate synaptic function to regulate homeostatic excitatory/inhibitory balance and thereby finely tune neuronal excitability (Kano et al., 2009; Castillo et al., 2012). eCB signaling subserves both tonic and phasic control of synaptic activity in several brain regions (Kano et al., 2009), including the basolateral amygdala (BLA; Azad et al., 2004; Yoshida et al., 2011; Colangeli et al., 2020; Yasmin et al., 2020). The BLA is a common epileptogenic temporal lobe structure, principally involved in seizure generation and expression (Engel, 2001) and, for this reason, is often targeted in kindling studies (Teskey, 2020).

In the BLA, phasic eCB signaling encompasses transient and long-lasting changes in presynaptic transmission (Marsicano et al., 2002; Yoshida et al., 2011). eCB-mediated transient changes in neurotransmitter release occur following postsynaptic depolarization with a subsequent rise in Ca^{2+}, which triggers eCB production (Kano et al., 2009). eCB mobilization can also occur on activation of G_q-coupled GPCRs to induce inhibitory long-term depression (LTD; Wettschureck et al., 2006; Heifets and Castillo, 2009). In addition to the phasic regulation of synaptic activity in the BLA, tonic eCB signaling also contributes to the homeostatic control of neurotransmitter release (Hill and Tasker, 2012). We have previously reported that repeated seizures in the amygdala cause a persistent maladaptive downregulation of anandamide (AEA) signaling in the BLA, which drives aberrant excitatory synaptic function and altered emotional behavior (Colangeli et al., 2020). Here, we determined the effect of repeated amygdala seizures on 2-arachidonoylglycerol (2-AG) signaling on GABA transmission in the BLA. We provide evidence that repeated seizure activity in the amygdala is associated with enduring alterations of both transient and long-term GABAergic synaptic plasticity in the BLA; these synaptic alterations were mimicked by the disruption of 2-AG signaling in sham rats by inhibiting 2-AG synthesis. Inhibition of the 2-AG degrading enzyme MAGL completely rescued transient but not long-term GABAergic plasticity following kindling.

Materials and Methods

Animals. One hundred eight male Hooded Long Evans rats (weight, 300–350 *g*) at the start of electrophysiological experiments (Charles River) were housed in a temperature-controlled (23 ± 1°C) vivarium room and maintained under specified pathogen-free conditions in a 12 h light/dark cycle (lights on, 7:00 A.M. to 7:00 P.M.). Food and water were available *ad libitum*. All experimental procedures complied with protocols approved by the University of Calgary Animal Care Committee and guidelines from the Canadian Council on Animal Care. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Surgery. Rats were anesthetized with a 5% isoflurane in O_2, maintained between 1% and 2%, and then positioned in a stereotaxic frame (David Kopf Instruments). One Teflon-coated stainless steel bipolar electrode (A-M Systems) was chronically implanted into the right BLA (coordinates: anteroposterior, −2.8 mm, and mediolateral, −4.8 mm from bregma; dorsoventral, −8.0 mm from dura) and anchored to the skull using dental cement and three stainless steel screws. One of the three screws served as a ground electrode. Rats were given buprenorphine, housed individually, and allowed to recover from surgery for 1 week before the start of kindling procedures.

Kindling procedure. The electrical kindling procedure was performed as previously reported (Colangeli et al., 2020). Briefly, 20 twice-daily electrical stimulations of the right amygdala were elicited using standard kindling stimulation (1 s train of 1 ms biphasic square wave pulses at 60 Hz) through a stimulator (Grass S88, Natus). Seizure duration and stage were recorded, and seizure score was classified as follows: class 1, immobility; class 2, orofacial automatisms with head nodding; class 3, unilateral forelimb clonus; class 4, rearing with bilateral forelimb clonus; and class 5, rearing with bilateral forelimb clonus followed by falling. Using this classification system, rats are considered "kindled" after three consecutive class 5 convulsions (Teskey, 2020). Sham rats were treated identically, but no current was delivered.

Electrophysiology. One week after the last evoked sham or kindled seizure, rats were decapitated under deep anesthesia with isoflurane and transcardially perfused with ice-cold slicing solution containing the following (in mM): 87 NaCl, 2.5 KCl, 25 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgCl_2$, 1.25 NaH_2PO_4, 25 D-glucose, and 75 sucrose. Coronal brain slices of the BLA (300 μm) were prepared with a slicer (model VT1200S, Leica) in ice-cold slicing solution. Slices were incubated in a holding chamber with oxygenated aCSF containing the following (in mM): 126 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.5 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 D-glucose, at pH 7.4 for 30 min at 32°C. Slices were then incubated for at least 45 min in regular aCSF or vehicle (DMSO), DO34 (1 μM), or KML29 (1 μM) containing aCSF, depending on the experimental design. We applied drugs directly to the slices, rather than perform intraperitoneal systemic injection, to narrow the potential extra-amygdala effects of eCB signaling manipulation in other brain regions, such as hippocampus (Segev et al., 2018) and frontal cortex (Morena et al., 2014; Vogel et al., 2016; Marcus et al., 2020), which may, in turn, affect eCB-mediated control of synaptic transmission and plasticity in the BLA. After incubation, slices were placed in the recording chamber and continuously superfused with regular aCSF or vehicle, DO34 or KML29, containing aCSF at a flow rate of 1.5 ml/min. All experiments were performed at room temperature (22–25°C; Azad et al., 2004; Chevaleyre et al., 2007). Whole-cell patch-clamp recordings were performed on BLA principal neurons. $GABA_A$ receptor-mediated IPSCs were recorded by using glass electrodes (3–5 MΩ) filled with a solution containing the following (in mM): 90 $CsCH_3SO_3$, 50 CsCl, 1 EGTA, 10 HEPES, 4.6 $MgCl_2$, 0.1 $CaCl_2$, 5 QX314, 0.3 Na-GTP, and 4 Mg-ATP, at pH 7.3 adjusted with CsOH. IPSCs were isolated by adding to the bath 40 μM D-AP-5 [D(−)-2-amino-5-phosphonopentanoic acid] and 20 μM DNQX (6,7-dinitroquinoxaline-2,3-dione) to block glutamatergic transmission. To elicit evoked IPSCs (eIPSCs), putative GABAergic fibers were stimulated using monopolar aCSF-filled patch-type pipettes (1 MΩ) placed within the BLA. For depolarization-induced suppression of inhibition (DSI) studies, stimuli were applied at 0.2 Hz. The magnitude of DSI was determined as the reduction from the mean amplitude of 12 successive eIPSCs evoked just before a depolarization (5 s at 0 mV) of pyramidal cell [baseline (BL)], compared with the mean amplitude of three successive eIPSCs taken just after the pulse (DSI) and the mean amplitude of the last five eIPSCs (90–120 s; recovery). Whenever possible, two to four DSI trials were averaged to obtain the mean DSI in each condition. For muscarinic acetylcholine receptor (mAChR)-induced eCB mobilization experiments and CB1 sensitivity, evoked simulations were elicited every 15 s with four stimulations being averaged to obtain one data point per minute. mAChR-driven synaptic suppression was assessed by a 10 min bath application of carbachol (CCh; 10 μM) after a 5 min BL recording, and the magnitude of LTD was calculated for 10 min (from 30 to 40 min after the end of CCh application) as a percentage of BL responses. Two neurons insensitive to CCh effect during its application were excluded from the LTD analysis. CB1 sensitivity was assessed by bath application of the CB1 receptor agonist CP55,940 (3 μM) and calculated as the average of CB1-induced eIPSC amplitude depression 10–15 min from agonist application. eIPSCs were analyzed with Clampfit 10.7 software. The amplitudes and frequencies of spontaneous IPSCs (sIPSCs) were detected (and confirmed by visual inspection) by continuous recording over 300 s by using Mini Analysis 6.0.7 software (Synaptosoft) using thresholds of five times rms noise levels. Cell capacitance and access resistance (initial value, $<$30 MΩ) were

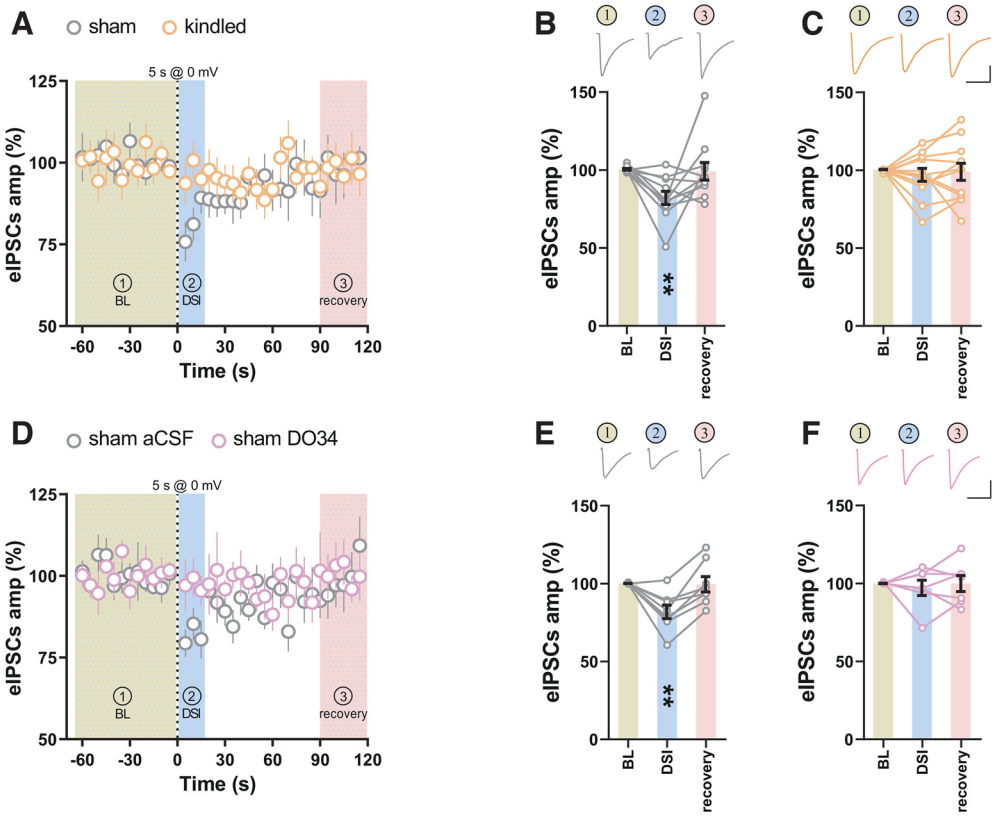

Figure 1. DSI is impaired in kindled rats, an effect mimicked by the inhibition of 2-AG synthesis. ***A***, Time course data showing a transient inhibition of eIPSC amplitude following postsynaptic depolarization (vertical dashed line) in sham and kindled rats. ***B***, Top, "BL" (1) trace represents the average of 12 stimulations before depolarization, "DSI" (2) trace represents the average of three stimulations immediately after the depolarization, and "recovery," (3) trace represents the average of the last five stimulations (95–115 s) corresponding to the indicated regions in ***A***. ***B***, ***C***, Bottom, Summary data corresponding to shaded regions in ***A*** showing that DSI is induced in sham rats (***B***) but not in kindled rats (***C***). Significance is reported with respect to the preceding shaded BL region. ***D–F***, Preincubating and superfusing slices from sham rats with the DAGL inhibitor DO34 1 μM recapitulated the effect of seizures on DSI as shown in the time course graph (***D***) and in the summary data graph (***E***, ***F***), respectively. Calibration: 200 pA, 40 ms. *Post hoc* versus BL *p*-values are shown as follows: $^{**}p < 0.01$. Data are shown as the mean ± SEM.

monitored during experiments, and recordings were accepted for analysis if either variable did not change by >20%. Signals were collected via an Axopatch 200B amplifier (Molecular Devices). The pCLAMP 9 software (Molecular Devices) was used for data acquisition.

Enzyme activity assays. Brain structures were excised on ice, and samples were then immediately snap frozen and stored at −80°C. Brain tissues were homogenized, and membrane fractions were isolated as described previously (Vecchiarelli et al., 2021). The monoacylglycerol lipase (MAGL) activity was measured as the conversion of [^{3}H]−2-oleoylglycerol to [^{3}H]-glycerol. The maximal hydrolytic activity (V_{max}) of MAGL and the binding affinity (K_m) of 2-AG for MAGL were determined by fitting the data to the Michaelis–Menten equation.

Statistical analyses. Data were analyzed using Prism 8 (GraphPad; RRID:SCR_002798). Differences between groups were analyzed using Student's *t* test for unpaired or paired data, two-way, or repeated-measures (RM) ANOVA, followed by Tukey's *post hoc* test for multiple comparisons. Significance was accepted at $p < 0.05$. Statistical design for each experiment is detailed in the Results section.

Results

Enduring loss of activity-dependent 2-AG retrograde signaling at GABA synapses after repeatedly evoked seizures

To determine whether repeated seizure activity persistently alters 2-AG actions at BLA GABAergic synapses 1 week after the last evoked seizure, we used the most well established form of 2-AG-mediated retrograde eCB signaling expressed widely throughout the CNS: the DSI. DSI has been reliably reported in the BLA (Zhu and Lovinger, 2005; Patel et al., 2009; Yoshida et al., 2011) and, consistent with those data, we found that the depolarization of BLA pyramidal neuron from −70 to 0 mV for 5 s resulted in a transient depression of the amplitude of the $GABA_A$-mediated eIPSCs, which was followed by a complete recovery of inhibitory activity (RM ANOVA: $F_{(2,32)} = 6.304$, $p = 0.017$; DSI: 82.07 ± 4.20% of BL, $p = 0.004$; recovery: 99.17 ± 5.63% of BL, $p > 0.05$; $n = 11$ neurons, 9 rats; Fig. 1*A*,*B*). In kindled rats, depolarization of pyramidal neurons did not induce a significant decrease of eIPSC magnitude (RM ANOVA: $F_{(2,35)} = 0.260$, $p > 0.05$; DSI; 96.44 ± 4.152% of BL, $p > 0.05$; recovery: 98.38 ± 5.526% of BL, $p > 0.05$; $n = 12$ neurons, 9 rats; Fig. 1*A*,*C*). In the brain, DSI has been shown to be exclusively dependent on 2-AG retrograde signaling as it is completely absent in DAGL KO mice that are unable to synthesize 2-AG (Tanimura et al., 2010) and is abolished by the DAGL inhibitor DO34 (Ogasawara et al., 2016). Here, we preincubated brain slices from sham rats with either vehicle or DO34 (1 μM; Ogasawara et al., 2016) and then tested DSI. We found that DO34 completely abolished DSI, thus mimicking what was observed in kindled rats in the absence of DO34. Indeed, while in vehicle-treated slices of sham rats, depolarization of pyramidal neurons induced a significant transient suppression of eIPSC (RM ANOVA: $F_{(2,23)} = 12.26$, $p = 0.002$; DSI: 81.79 ± 4.309% of BL, $p = 0.009$; $n = 8$ neurons, 5 rats), which eventually recovered to BL values (recovery: 99.49 ± 4.894%, $p > 0.05$ of BL; Fig. 1*D*,*E*), the transient suppression of eIPSCs after the depolarization step was absent in slices treated with DO34 (RM ANOVA: $F_{(2,20)} = 0.219$, $p > 0.05$; DSI: 97.37 ± 4.903% of BL; recovery: 100.1 ± 5.104%; $n = 7$ neurons, 6 rats; Fig. 1*D*,*F*).

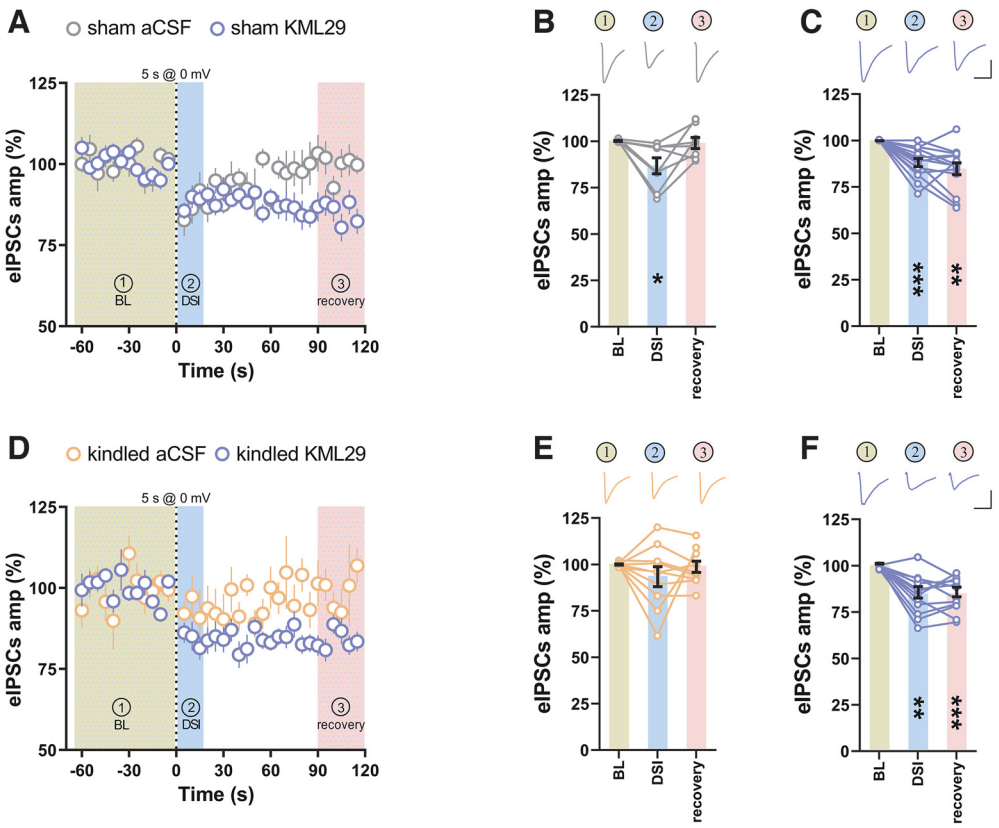

Figure 2. Inhibition of 2-AG degradation with KML29 prolongs DSI in sham rats and restores DSI in kindled rats. ***A***, Summary time course of eIPSC amplitude normalized to the BL period obtained from slices of sham rats treated with either vehicle or the MAGL inhibitor KML29 (1 μM). ***B***, ***C***, Representative traces and summary data corresponding to shaded regions in ***A*** in slices from sham rats treated with either vehicle (***B***) or the MAGL inhibitor KML29 (***C***), showing that KML29 did not affect maximum DSI but significantly prolonged the duration of DSI. Significance is reported with respect to the preceding shaded BL region. ***D***, Time course data showing the effect of vehicle and KML29 containing aCSF on DSI in kindled rats. ***E***, ***F***, Representative traces and summary data corresponding to shaded regions in ***D*** in slices from kindled rats treated with either vehicle (***E***) or KML29 (***F***) showing that DSI is restored by KML29 treatment in kindled rats. Significance is reported with respect to the preceding shaded BL region. Calibration: 200 pA, 40 ms. *Post hoc* versus BL *p*-values are shown as follows: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. Data are shown as the mean ± SEM.

2-AG restoration normalizes DSI in kindled rats

We then determined whether blocking 2-AG deactivation and thereby amplifying its actions at CB1 receptors could rescue the DSI impairment observed in kindled rats. We first assessed the effect of KML29 (1 μM), an inhibitor of the 2-AG degrading enzyme MAGL, on DSI in slices obtained from sham rats. As shown in Figure 2, depolarization of postsynaptic neurons caused a similar transient suppression of eIPSC amplitude in slices treated with either vehicle (RM ANOVA: $F_{(2,23)} = 6.64$, $p = 0.015$; DSI: 86.94 ± 4.29% of BL, $p = 0.040$; $n = 8$ neurons, 7 rats; Fig. 2*A*,*B*), or KML29 (RM ANOVA: $F_{(2,41)} = 17.36$, $p < 0.001$; DSI: 88.26 ± 2.17% of BL, $p < 0.001$; $n = 14$ neurons, 5 rats; Fig. 2*A*,*C*), indicating that occlusion did not occur at the drug concentration used in these experiments. In addition, KML29 significantly increased the late component of DSI (recovery: 84.91 ± 3.17% of BL, $p = 0.01$; Fig. 2*C*), thereby significantly prolonging the suppression of IPSC amplitude following postsynaptic depolarization. In the kindled group, preincubating slices with KML29 rescued DSI. Specifically, the transient suppression of eIPSC amplitude following depolarization step could be induced in KML29-treated slices (RM ANOVA: $F_{(2,35)} = 22.45$, $p < 0.001$; DSI: 84.24 ± 3.09% of BL, $p = 0.001$; $n = 12$ neurons, 6 rats; Fig. 2*D*,*F*), but not in vehicle-treated slices (RM ANOVA: $F_{(2,29)} = 1.03$, $p = 0.36$; DSI: 93.36 ± 5.40% of BL; $n = 10$ neurons, 7 rats, Fig. 2*D*,*E*) of kindled rats. Moreover, as observed in sham rats, KML29 significantly prolonged DSI in kindled rats (recovery: 84.43 ± 2.58% of BL, $p < 0.001$; Fig. 2*D*,*F*).

Repeated seizure activity attenuated mAChR-dependent depression on inhibitory transmission in the BLA

In addition to the strong depolarization of neurons, eCB release can be triggered by the activation of Gq-coupled receptors such as group I metabotropic glutamate receptors (mGluR1s) and mAChRs (Maejima et al., 2001; Fukudome et al., 2004) through the phospholipase C (PLC)– diacylglycerol lipase (DAGL) cascade (Hashimotodani et al., 2005; Maejima et al., 2005). In the BLA, mGluR1 appears to stimulate AEA production, rather than 2-AG, independently of the PLC–DAGL activity (Azad et al., 2004). On the other hand, mAChR activation triggers PLC-dependent eCB release in many brain regions (Kim et al., 2002; Ohno-Shosaku et al., 2003; Neu et al., 2007) but has not yet been demonstrated in the BLA. Therefore, we first asked whether prolonged mAChR activation would also result in an LTD in the BLA and whether this form of plasticity is dependent on the CB1 receptor. To activate mAChRs, after a 5 min BL period, CCh (10 μM) was bath applied for 10 min and eIPSCs were recorded for 40 min in BLA pyramidal neurons from control non-operated rats (Fig. 3*A*). We found that CCh induced a transient depression followed by a long-lasting depression that remained on washout ($n = 9$ neurons, 7 rats; paired *t* test: $t_{(8)} = 4.36$, $p < 0.02$ vs BL; Fig. 3*B*). The long-lasting depression after CCh washout was still

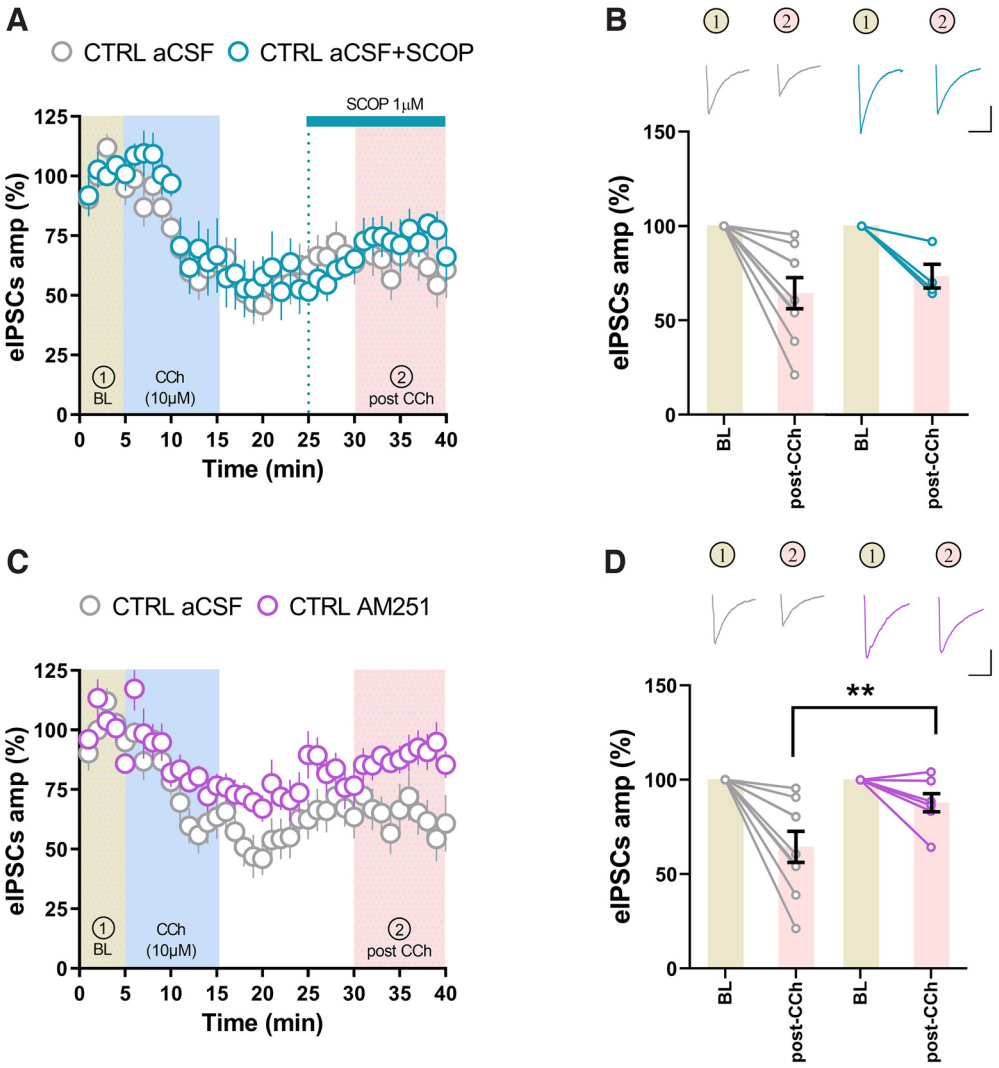

Figure 3. BLA pyramidal neurons display mAChR-dependent long-term synaptic depression on inhibitory transmission, which is mediated by CB1 receptor activation. ***A***, Time course graph showing the effect of mAChR activation on eIPSC amplitude on naive rats (CTRL). eIPSCs were evoked every 15 s and then averaged to obtain a value per minute. CCh (10 μM) was bath applied for 10 min and then washed out to assess long-term suppression of eIPSCs. In a subset of cells, SCOP (1 μM) was bath applied during CCh washout to demonstrate that the synaptic depression is not attributable to a lasting effect of CCh that has not been completely washed out of slices. ***B***, Bar graphs quantifying the percentage of control of eIPSC amplitude by CCh 10–20 min post-application (2) with respect to the BL period (1) in aCSF and SCOP treated slices. ***C***, AM251 treatment (2 μM) in neurons of CTRL rats completely blocked LTD after CCh washout. ***D***, Bar graphs quantifying the percentage of control eIPSC amplitude by CCh recorded 10–20 min postapplication (2) with respect to the BL period (1) in aCSF-treated and AM251-treated slices from CTRL rats. Calibration: 200 pA, 40 ms. *Post hoc* CTRL aCSF versus CTRL AM251 *p*-values: $^{**}p < 0.01$. Data are shown as the mean ± SEM. CTRL aCSF data are repeated in the graphs as they belong to the same experimental design.

present when the mAChRs were blocked by scopolamine (SCOP; $n = 4$ neurons, 4 rats; RM two-way ANOVA: SCOP × CCh interaction: $F_{(1,11)} = 0.46$, $p > 0.05$; CCh effect: $F_{(1,11)} = 22.33$, $p < 0.001$; SCOP effect: $F_{(1,11)} = 0.46$, $p > 0.05$; Fig. 3*B*), while it was reversed when slices were continuously superfused with the CB1 receptor inverse agonist AM251 ($n = 7$ neurons, 7 rats; RM two-way ANOVA: AM251 × CCh interaction: $F_{(1,14)} = 5.18$, $p = 0.039$; CCh effect: $F_{(1,14)} = 21.93$, $p < 0.001$; AM251 effect: $F_{(1,14)} = 5.18$, $p = 0.039$; Fig. 3*C*,*D*). Collectively, these data provide evidence that BLA pyramidal neurons undergo CB1-dependent, mAChR-induced LTD. We then determined whether this form of plasticity is altered by seizure activity. Similar to control non-operated rats, both sham and kindled rats displayed CCh-induced LTD (Fig. 4*A*); however, RM two-way ANOVA followed by Tukey's *post hoc* test revealed that the reduction in GABA synaptic efficacy after CCh washout was significantly more pronounced in sham rats ($n = 8$ neurons, 7 rats) compared with kindled rats ($n = 6$ neurons, 4 rats; RM two-way ANOVA: seizures × CCh interaction: $F_{(1,12)} = 12.87$, $p = 0.004$; CCh effect: $F_{(1,12)} = 111.22$, $p < 0.001$; seizure effect: $F_{(1,12)} = 12.87$, $p = 0.004$; Fig. 4*B*). Having established that inhibitory synapses in the BLA can undergo CB1-mediated, mAChR-dependent LTD and this form of plasticity is attenuated in kindled rats, we next asked whether it requires 2-AG signaling. To this aim, we preincubated slices from sham rats with the 2-AG synthesis inhibitor, DO34 ($n = 4$ neurons, 4 rats) and then bath applied CCh (Fig. 4*C*). In these conditions, the long-lasting depression recorded after CCh washout was significantly attenuated in the presence of DO34 (RM two-way ANOVA: DO34 × CCh interaction effect: $F_{(1,10)} = 18.00$, $p = 0.002$; CCh effect: $F_{(1,10)} = 112.55$, $p < 0.001$; DO34 effect: $F_{(1,10)} = 18.00$, $p = 0.002$; Fig. 4*D*), thus resembling the mAChR-LTD obtained in slices from kindled rats superfused with aCSF only. These findings support the

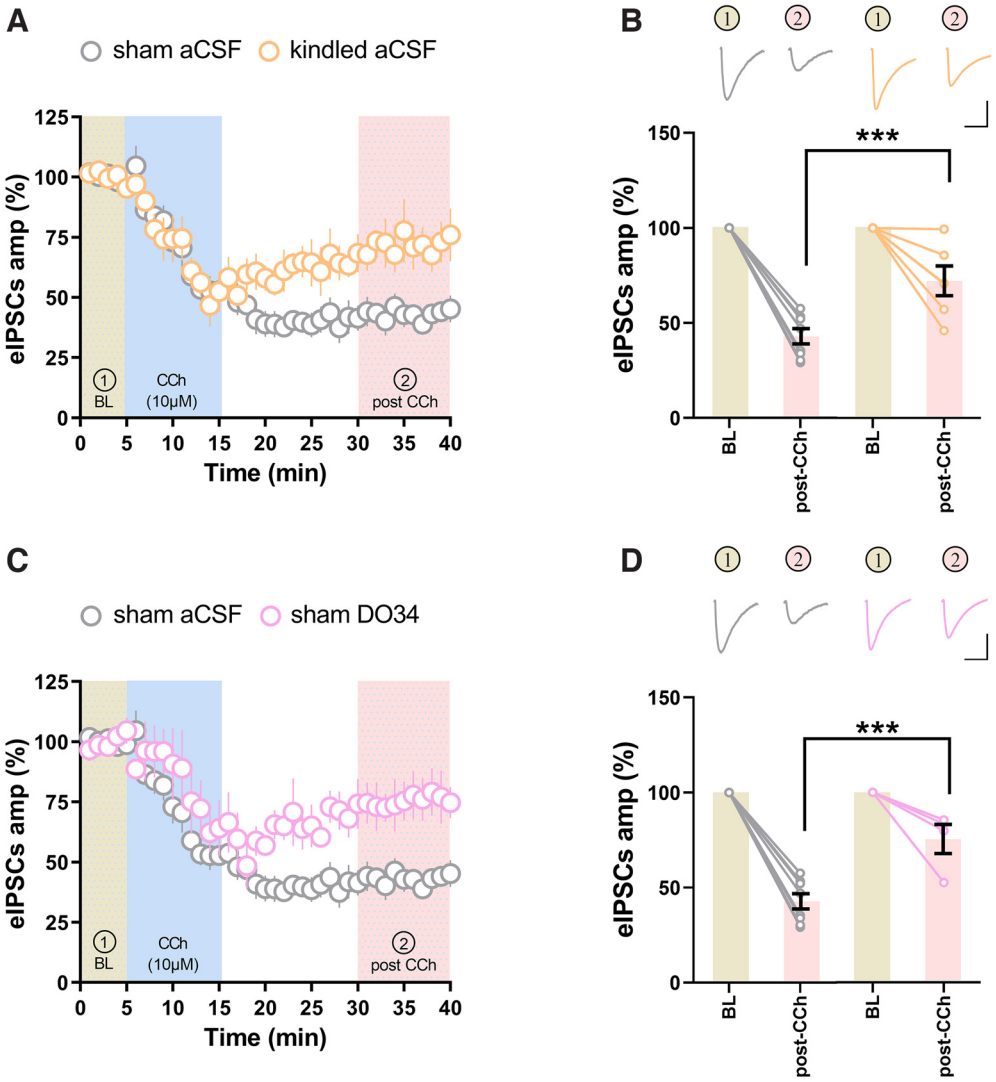

Figure 4. mAChR-dependent synaptic depression on inhibitory transmission in the BLA is attenuated in the kindled rats, and this effect is mimicked by application of the 2-AG synthesis inhibitor DO34. ***A***, Time course graph showing the effect of acute mAChR activation on eIPSC amplitude on sham and kindled rats. eIPSCs were evoked every 15 s and then averaged to obtain a dot per minute. CCh (10 μM) was bath applied for 10 min and then washed out to assess long-term suppression of eIPSCs. ***B***, Bar graphs quantifying the percentage inhibition of eIPSC amplitude by CCh 10–20 min postapplication (2) with respect to the BL period (1) in sham and kindled rats. Kindled rats display a significant attenuation of LTD after CCh washout. ***C***, Time course graph showing the effect of mAChR activation on eIPSC amplitude on slices from sham rats, treated with vehicle or DO34. ***D***, Bar graphs quantifying the percentage inhibition of eIPSC amplitude by CCh recorded 10–20 min postapplication (2) with respect to the BL period (1) in sham and DO34-treated slices. DO34 did not affect acute suppression of eIPSC amplitude during CCh application but attenuated LTD after CCh washout, thus mimicking the impaired mAChR-induced LTD observed in kindled rats. Calibration: 200 pA, 40 ms. *Post hoc* sham versus kindled *p*-values, ***$p < 0.001$; *post hoc* sham versus DO34 *p*-values, ***$p < 0.001$. Data are shown as the mean ± SEM. Sham aCSF data are repeated in the graphs as they belong to the same experimental design.

possibility that 2-AG signaling, at least in part, subserves mAChR-LTD in the BLA and that repeated seizures negatively impacted 2-AG-mediated plasticity in the BLA.

Inhibition of the 2-AG-degrading enzyme does not restore mAChR-dependent depression on inhibitory transmission in the BLA

We then asked whether inhibiting 2-AG degradation might normalize mAChR-LTD in kindled rats by amplifying 2-AG activity at GABA terminals. To test this possibility, brain slices were treated with the MAGL inhibitor KML29 (1 μM) before bath application of CCh. We first tested KML29 in slices from sham rats ($n = 5$ neurons, 4 rats; Fig. 5*A*). In contrast with what we expected, KML29 reduced, rather than enhanced mAChR-LTD in slices from sham rats (Fig. 5*B*). Indeed, the reduction in eIPSC amplitude recorded in KML29-treated slices during CCh washout was significantly less pronounced compared with eIPSC amplitudes recorded in vehicle-treated slices (RM two-way ANOVA: KML29 × CCh interaction: $F_{(1,11)} = 11.99$, $p = 0.005$; CCh effect: $F_{(1,11)} = 176.86$, $p < 0.001$; KML29 effect: $F_{(1,11)} = 11.99$, $p = 0.005$; Fig. 5*B*). Moreover, despite a tendency to rescue pharmacologically induced LTD in slices prepared from kindled rats, KML29 was not sufficient to fully restore GABA synaptic depression during CCh washout (Fig. 5*C*). Indeed, no significant effect was observed when we compared the reduction of eIPSC amplitude with respect to the BL during CCh washout in vehicle-treated ($n = 6$ neurons, 4 rats) and KML29-treated ($n = 6$ neurons, 3 rats) slices from kindled rats (RM two-way ANOVA: KML29 × CCh interaction: $F_{(1,10)} = 3.09$, $p = 0.110$; CCh effect: $F_{(1,10)} = 49.68$, $p < 0.001$; KML29 effect: $F_{(1,10)} = 3.09$, $p = 0.110$; Fig. 5*D*).

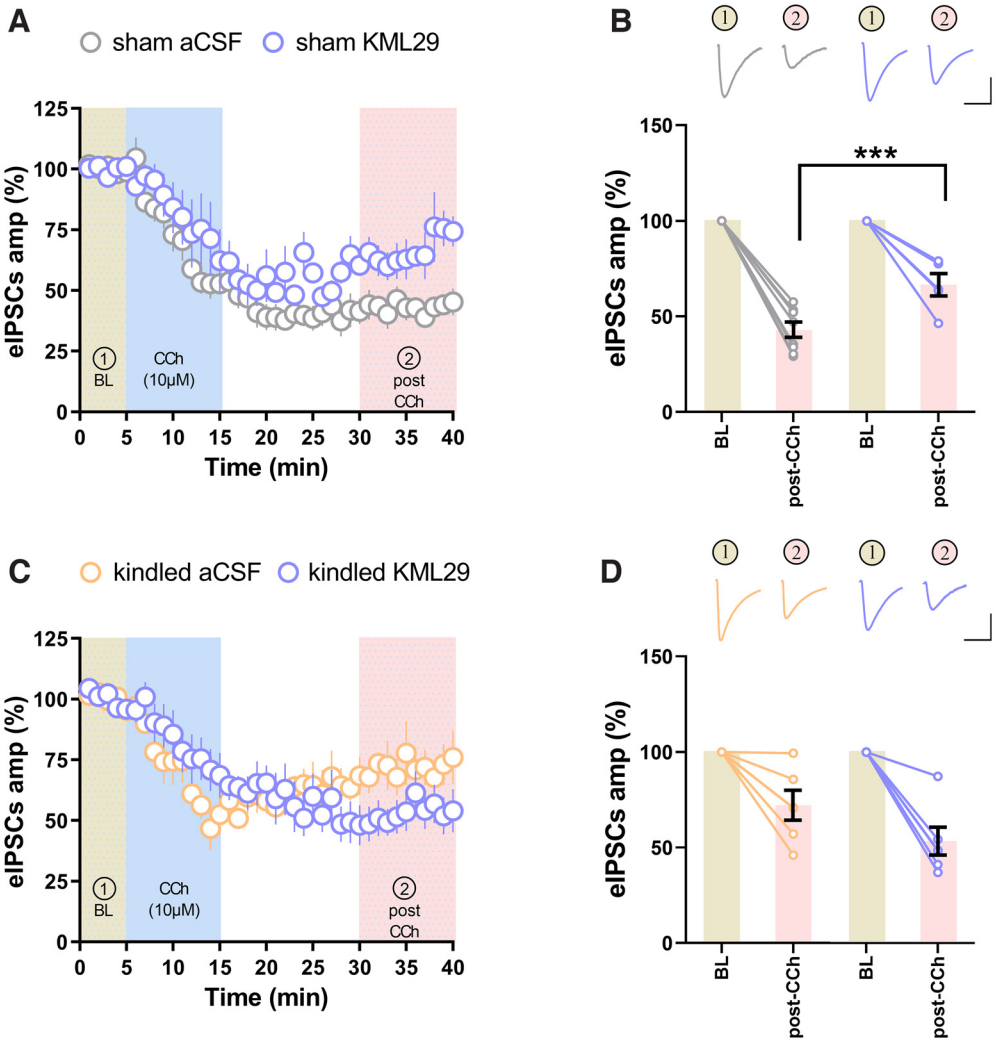

Figure 5. Inhibition of 2-AG degradation with KML29 reduces mAChR-dependent depression on inhibitory transmission in the BLA of sham rats, and it only partially reverts seizure-induced impairment of mAChR-dependent depression on inhibitory transmission in the BLA. ***A***, Time course graph showing the effect of mAChR activation on eIPSC amplitude on slices from sham rats, treated with vehicle or KML29. ***B***, Bar graphs quantifying the percentage inhibition of evoked IPSC amplitude by CCh (10–20 min postapplication) showed that CCh produces a long-term suppression of evoked IPSC amplitude, which is significantly attenuated in the presence of KML29. ***C***, KML29 treatment is not sufficient to fully restore mAChR-LTD in neurons recorded from kindled rats. ***D***, Bar graph quantifying the percentage inhibition of evoked IPSC amplitude by CCh showed no significant difference in mAChR-induced LTD in slices from kindled rats treated with either vehicle or KML29. Calibration: 200 pA, 40 ms. *Post hoc* sham aCSF versus sham KML29 *p*-values: ***$p < 0.001$. Data are shown as the mean ± SEM. Sham aCSF data are repeated in the graphs as they belong to the same experimental design.

Kindling-induced alteration of phasic eCB signaling is not mediated by altered 2-AG tone over GABA transmission

We previously reported that tissue levels of AEA, but not of 2-AG, were reduced in kindled rats 1 week after the last evoked seizure, which mainly impacted the tonic eCB control over glutamate transmission (Colangeli et al., 2020). However, alteration of the 2-AG tone over GABA transmission may occur at the synaptic scale and thereby not be revealed by analyzing the whole amygdala. Since prolonged tonic signaling can cause desensitization and functional antagonism of CB1 receptors, the possibility that the alteration of synaptic plasticity was caused by changes in the 2-AG tone existed. To address this point, we examined basal GABAergic synaptic input onto BLA pyramidal neurons of sham or kindled rats in the presence of either vehicle or DO34 (Fig. 6*A*,*B*). If 2-AG signaling is tonically present under basal conditions to suppress GABAergic transmission, then blockade of its synthesis by DO34 should unmask a facilitation of inhibitory transmission. DO34 treatment did not change sIPSC frequencies and amplitudes in either sham rats (unpaired *t* test for frequency: $t_{(21)} = 0.143$, $p = 0.887$; amplitude: $t_{(21)} = 0.643$, $p = 0.527$; aCSF: $n = 12$ neurons, 9 rats; DO34: $n = 11$ neurons, 9 rats; Fig. 6*A*) or kindled rats (unpaired *t* test for frequency: $t_{(19)} = 0.635$, $p = 0.533$; amplitude: $t_{(19)} = 0.0735$, $p = 0.942$; aCSF: $n = 10$ neurons, 8 rats; DO34: $n = 11$ neurons, 4 rats; Fig. 6*B*). Moreover, to test whether there is a tonic CB1 receptor-mediated control over GABA transmission, presumably mediated by AEA, we performed a time course analysis of sIPSCs before and after bath application of the CB1 receptor inverse agonist AM251. Again, we found no difference in either frequency or amplitude of sIPSCs in both sham (paired *t* test for frequency: $t_{(6)} = 0.77$, $p > 0.05$; amplitude: $t_{(6)} = 1.76$, $p > 0.05$; $n = 7$ neurons, 7 rats; Fig. 6*C*) and kindled rats (paired *t* test for frequency: $t_{(6)} = 1.85$, $p > 0.05$; amplitude: $t_{(6)} = 1.64$, $p > 0.05$; $n = 7$ neurons, 6 rats; Fig. 6*D*). These data confirm previous evidence showing that GABAergic inputs in the BLA are not under a tonic control of eCB signaling (Colangeli et al., 2020; Yasmin et al., 2020) and providing evidence that altered tonic

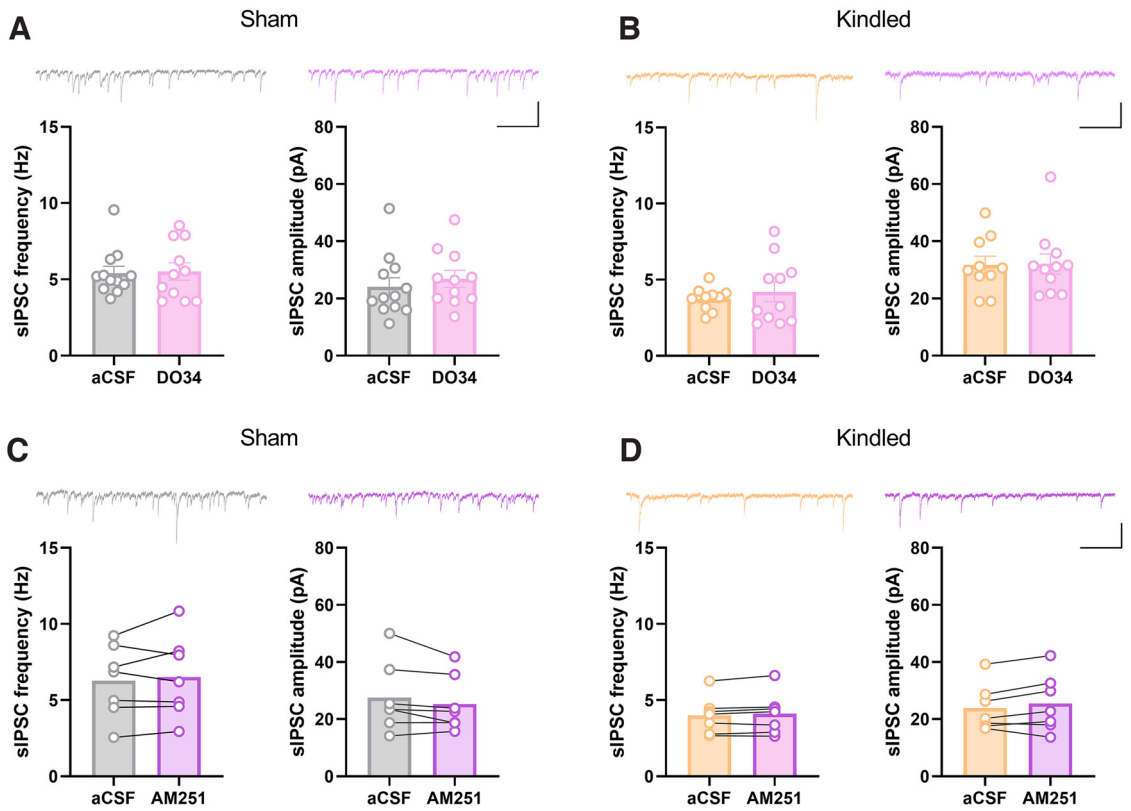

Figure 6. 2-AG synthesis inhibition does not alter GABA transmission in the BLA. ***A***, ***B***, The effect of DO34 on sIPSC frequency and amplitude on slices from sham (***A***) and kindled rats (***B***) is shown. DO34 did not change either the frequency or the amplitude of sIPSCs, ruling out the possibility that 2-AG signaling tonically constrains GABA transmission in the BLA. ***C***, ***D***, Effect of AM251 (2 μM) on sIPSC frequency and amplitude in sham (***C***) and kindled (***D***) rats. AM251 was bath applied after 5 min baseline recording, and sIPSCs were assessed 10–15 min after AM251 application. Calibration: 50 pA, 1 s.

2-AG signaling does not account for the disrupted activity-dependent 2-AG action over GABA transmission.

Kindling-induced alteration of phasic eCB signaling is not mediated by changes in CB1 receptor sensitivity

Repeated seizures could cause functional tolerance of CB1 receptors and thereby lead to an apparent abolition of eCB-mediated plasticity. To test whether repeated seizure activity may directly impair CB1 receptor functionality, we bath applied the cannabinoid agonist CP55,940 (3 μM) and recorded eIPSCs (Fig. 7*A*). In both groups, CP55,940 significantly inhibited synaptic transmission similarly (RM two-way ANOVA; CP55,940 × seizures interaction effect: $F_{(1,9)} = 0.3170$, $p = 0.587$; CP55,940 effect: $F_{(1,9)} = 44.42$, $p < 0.001$; seizure effect: $F_{(1,9)} = 0.3170$, $p = 0.587$; sham: $n = 5$ neurons, 3 rats; kindled: $n = 6$ neurons, 6 rats; Fig. 7*B*). This finding rules out the possibility that altered CB1 receptor sensitivity contributes to the loss of 2-AG signaling in kindled rats.

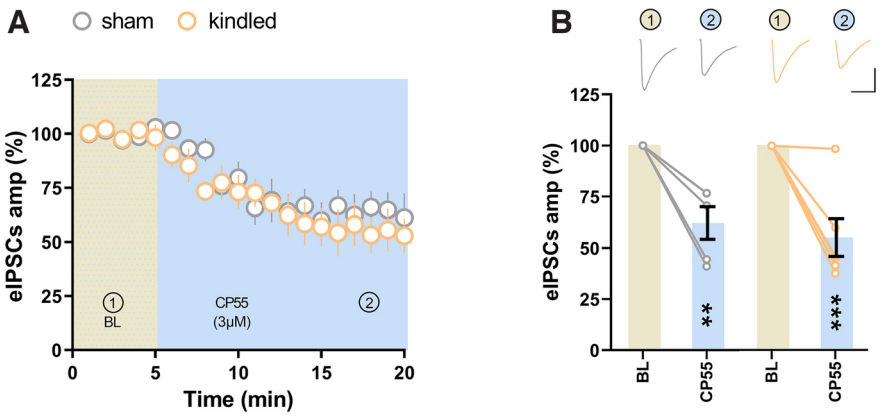

Figure 7. No effects of repeated seizures on CB1 receptor sensitivity. ***A***, CP55,940 (CP55; 3 μM), a specific CB1 receptor agonist, induced a similar depression of eIPSCs in the BLA in both sham and kindled rats. ***B***, The effect of CB1 receptor activation on inhibitory transmission was assessed by measuring the average eIPSC amplitude normalized to the BL obtained 10–15 min after CP55,940 application in both sham and kindled rats. Calibration: 200 pA, 40 ms. *Post hoc* versus BL *p*-values are shown as follows: ***$p < 0.001$, **$p < 0.01$. Data are shown as the mean ± SEM.

Enhanced MAGL enzymatic breakdown activity does not account for the seizure-induced 2-AG-signaling alteration

After having assessed that neither basal 2-AG signaling nor CB1 receptor sensitivity is altered by seizure activity, the loss of activity-dependent 2-AG signaling over GABA transmission after repetitive seizure activity may be attributable to impairments of 2-AG synthesis/release from the postsynaptic cell or enhanced enzymatic breakdown activity. Our data so far indicate that the seizure-induced alteration in 2-AG signaling may be because of a dysfunction in 2-AG synthesis/release machinery since DO34, a synthesis inhibitor, is able to recapitulate these changes; nonetheless, enhanced 2-AG degradation in BLA may lead to a reduction in 2-AG levels, thus contributing to the loss of stimulus-

dependent 2-AG signaling in BLA. Thereby, in both sham and kindled rats, we (1) assessed the effects of KML29, an inhibitor of 2-AG degradation by MAGL, on GABA transmission; and (2) tested the enzymatic activity of MAGL in amygdala tissue. If there is an enhanced enzymatic MAGL activity following repeated seizure activity, KML29 treatment should affect sIPSCs in kindled rats more profoundly than in sham rats. However, in brain slices prepared from sham rats, sIPSC frequency, but not the amplitude, was significantly reduced by KML29 treatment (unpaired t test for frequency: $t_{(23)} = 3.06$, $p = 0.006$; amplitude: $t_{(23)} = 0.886$, $p > 0.05$; aCSF: $n = 11$ neurons, 5 rats; KML29: $n = 14$ neurons, 5 rats; Fig. 8*A*), while this effect was absent in brain slices prepared from kindled rats (unpaired t test for frequency: $t_{(23)} = 0.67$, $p > 0.05$; amplitude: $t_{(23)} = 0.20$, $p > 0.05$; aCSF: $n = 12$ neurons, 5 rats; KML29: $n = 13$ neurons, 6 rats; Fig. 8*B*). Moreover, when MAGL enzymatic activity was assessed in the amygdala, the V_{max} of MAGL was unchanged after kindling (unpaired t test: $t_{(9)} = 0.692$, $p = 0.506$), but K_m for MAGL was found to be increased compared with sham rats (unpaired t test: $t_{(9)} = 2.406$, $p = 0.040$; Fig. 8*C*). This latter finding clearly indicates a decrease in the affinity of MAGL for its substrate, thus a less efficient capability of the enzyme to catabolize 2-AG. Collectively, these results rule out the possibility that enhanced 2-AG degradation accounts for the altered 2-AG-mediated synaptic plasticity alterations over GABA transmission induced by seizure activity.

Discussion

The main findings of the present work are that repeated amygdala seizure activity impairs BLA GABAergic plasticity and that these effects are recapitulated in non-kindled rats by pharmacological disruption of 2-AG synthesis. Moreover, augmentation of 2-AG signaling restores activity-dependent, but not mAChR-induced, synaptic plasticity of GABA transmission. Neither CB1 receptor sensitivity nor 2-AG degradation was altered by seizures, thus, providing evidence that the most likely mechanism underlying aberrant 2-AG signaling over GABA transmission following repeated seizure activity is dysfunctional 2-AG synthesis/release machinery. We previously reported that both tonic AEA control over glutamatergic transmission and phasic AEA signaling over GABA transmission are impaired by repeated seizure activity (Colangeli et al., 2020). We expand these findings by demonstrating that activity-dependent 2-AG signaling dysfunction also contributes to the persistent aberrant GABA plasticity in the BLA following repeated seizures, providing evidence that amygdala kindling causes an enduring alteration of both AEA and 2-AG signaling within the BLA.

Persistent alteration of neuroplasticity is an important hallmark of the pathophysiology of epilepsy and contributes to the negative neurobiological consequences associated with seizure activity (Farrell et al., 2017, 2020). It is well established that the facilitation of eCB signaling acutely dampens seizure expression and provides neuroprotection during seizure activity, whereas eCB signaling blockade often does the opposite (Marsicano et al., 2003; Katona and Freund, 2008; Colangeli et al., 2017; Sugaya and Kano, 2018; Colangeli et al., 2019; Di Maio et al., 2019). Conversely, evidence of pathologic long-term remodeling of the eCB signaling following seizure activity is less consistent across studies, and the relative contributions of AEA and 2-AG are still a matter of debate (Soltesz et al., 2015; Sugaya and Kano, 2018).

DSI is a widespread, 2-AG-mediated phenomenon crucially involved in proper brain plasticity, and alteration of DSI is

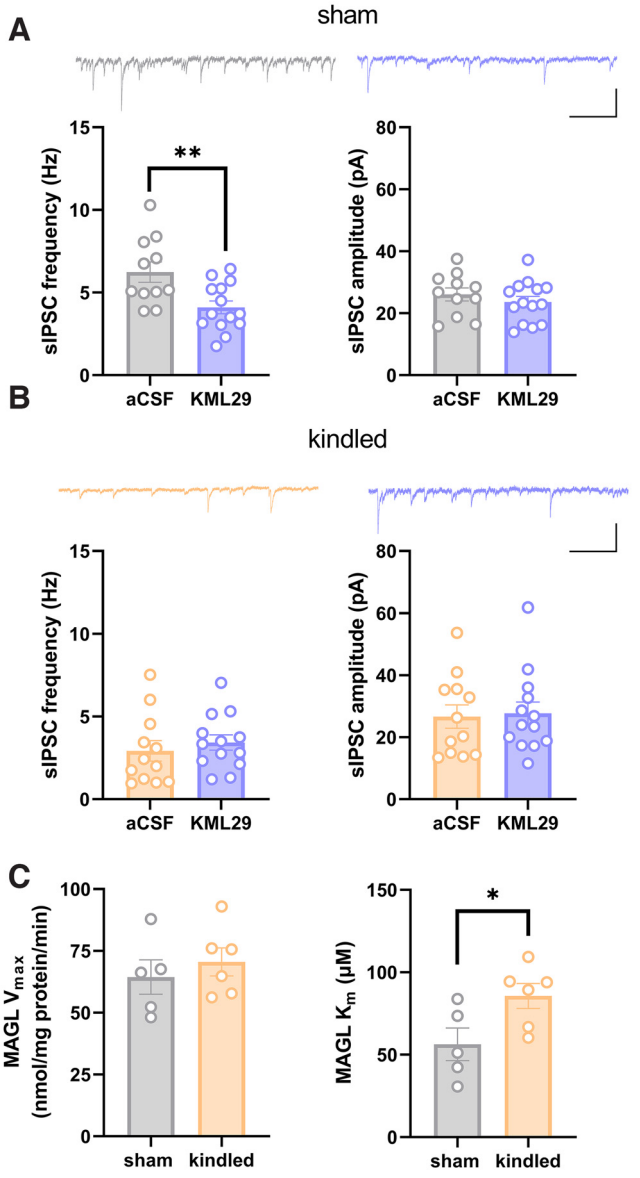

Figure 8. Enhanced 2-AG degradation does not account for eCB signaling alteration in kindled rats. ***A***, ***B***, The effect of KML29, an inhibitor of 2-AG degradation, on sIPSC frequency and amplitude on slices from sham (***A***) and kindled (***B***) rats is shown. KML29 reduced sIPSC frequency but not amplitude in sham rats, while it had no effect on either the frequency or the amplitude of sIPSCs in kindled rats. Calibration: 50 pA, 1 s. Sham aCSF versus sham KML29; $^{**}p < 0.001$. Data are shown as the mean ± SEM. ***C***, Seizures did not change V_{max} but significantly increased K_m of the MAGL enzyme in the amygdala, which is indicative of reduced binding affinity without alteration of hydrolytic activity of the enzyme for its substrate. Sham versus kindled rats: $^{*}p < 0.05$. Data are expressed as the mean ± SEM ($n = 5$–6/group).

associated with a wide range of neurologic diseases (Patel et al., 2009; Wamsteeker et al., 2010; Wang et al., 2010; Jenniches et al., 2016; Folkes et al., 2020). Earlier studies have demonstrated hippocampal and cortical long-term dynamic changes of eCB signaling in animal models of chronic epilepsy, with the most consistent findings being an upregulation of CB1 receptor function at GABAergic terminals following status epilepticus (Falenski et al., 2007; 2009; Maglòczky et al., 2010) and a persistent potentiation of the activity-dependent 2-AG signaling selectively at inhibitory (DSI) synapses following febrile seizures (Chen et al., 2003). As a consequence of the enduring upregulation of the eCB system over GABA transmission,

suppression, rather than enhancement, of eCB signaling seemed to provide protection during epileptogenesis (Chen et al., 2007). In contrast to these findings, here we demonstrate that activity-dependent eCB retrograde signaling at BLA GABA synapses is absent in amygdala-kindled rats, and this alteration is recapitulated in slices of sham rats treated with the 2-AG synthesis inhibitor DO34. Importantly, pharmacological normalization of 2-AG levels restores DSI in kindled rats, thus providing evidence that persistent 2-AG signaling deficiency, rather than enhancement, occurs following amygdala kindling. The apparent controversy between our findings and previous works might be attributable to the model used to induce seizures and an epileptic state. Indeed, while the aforementioned studies used an initial insult to trigger epileptogenesis (i.e., febrile seizure and chemically induced status epilepticus), studies that used kindling consistently indicate that deficiency of the 2-AG signaling occurs, since augmentation of 2-AG signaling retarded epileptogenesis (Griebel et al., 2015; von Rüden et al., 2015) while disruption of 2-AG synthesis increased seizure susceptibility (Sugaya et al., 2016). Thus, the temporal evolution of the pathologic plasticity of the eCB signaling system may differ depending on the epilepsy model used.

It is also important to note that, when pathologic alterations are found at the presynaptic level, the electrophysiological experimental paradigms used to investigate the synaptic activity may account for discrepancies among different findings. For example, extracellular Ca^{2+} concentration used in the recording solution might alter release probability (Mintz et al., 1995), synaptic plasticity (Inglebert et al., 2020), and eCB signaling (Lenz and Alger, 1999; Varma et al., 2002). The extracellular solution in these experiments (2.5 mM Ca^{2+} and 1.5 mM Mg^{2+}) included concentrations of Ca^{2+} in the upper value of physiological levels in rat brain (1–3 mM; Rusakov and Fine, 2003) as it is widely used to test eCB control of synaptic transmission (Báldi et al., 2016).

The eCB system reorganization following seizures may also depend on the brain region involved in seizure activity. In the BLA, eCB signaling controls both glutamatergic and GABAergic transmission; thus, we speculate that repeated electrically evoked seizures may cause a persistent and unselective (glutamate vs GABA) dysregulation of eCB signaling in the BLA when it is required for retrograde suppression of synaptic transmission. The consequences of eCB signaling breakdown are a lack of control over seizure generation when impacting glutamatergic terminals (mainly AEA) and aberrant synaptic plasticity when impacting GABAergic transmission (mainly 2-AG). In this context, augmentation of eCB signaling provides neuroprotection against epileptiform discharges and restores proper GABA plasticity, which is crucially involved in amygdala-mediated emotional behavior (Ehrlich et al., 2009).

In addition to the 2-AG-mediated short-term plasticity, the production of eCBs is also tied to the activity of the metabotropic receptors such as postsynaptic mGluR and mAChR (Kano et al., 2009), which are critical for eCB-mediated LTD at central synapses (Chevaleyre and Castillo, 2003; Katona and Freund, 2012). Several studies have demonstrated that, in the BLA, low-frequency stimulation can mobilize eCBs, and particularly AEA, via the activation of postsynaptic mGluR1 (Marsicano et al., 2002; Azad et al., 2004; Chevaleyre et al., 2007), and consistently, via seizure-induced alteration of LTD of inhibitory transmission is completely rescued by AEA signaling augmentation (Colangeli et al., 2020). On the other hand, "on demand" eCB synthesis is not linked to special metabotropic receptors (Heifets and Castillo, 2009) and, independently of the mGluR system, mAChR activation mediates LTD through 2-AG signaling in several brain regions (Tanimura et al., 2010). Since BLA pyramidal neurons richly express mAChR, which are closely positioned to DAGL clusters (Yoshida et al., 2011), we tested whether, in addition to AEA–mGluR-driven LTD, mAChR activation also triggers eCB-LTD at BLA GABAergic synapses and whether it requires 2-AG/CB1 signaling. Our results further confirmed our previous report that seizures severely impact LTD at GABA transmission within the BLA (Colangeli et al., 2020), as mAChR-driven LTD was consistently induced in sham rats while significantly attenuated in kindled rats. However, our results do not fully elucidate whether this seizure-induced alteration of LTD might be ascribed to differences in 2-AG signaling. Indeed, while DSI was completely abolished with DO34, we were unable to completely abolish mAChR-induced synaptic depression by inhibiting the DAGL enzyme in the sham group. It is important to note that different sensitivities to DAGL inhibitors between metabotropic receptor-driven 2-AG mobilization and stimulus-dependent 2-AG mobilization have been previously reported. For example, pharmacological DAGL inhibition fully blocked DSI but only partially prevented mGluR-driven 2-AG mobilization in the CA1 region of the hippocampus (Zhang et al., 2011). This could explain why mAChR-induced LTD might be less sensitive to DO34 than DSI.

Another possible explanation is that, unlike other brain regions, 2-AG activity plays only a partial role in the expression of mAChR-driven LTD at GABA transmission in the BLA. In support of this hypothesis, we found that AM251 treatment completely blocked mAChR-induced LTD in naive rats; thus, the residual synaptic depression observed after CCh washout in DO34-treated rats is likely to be ascribed to AEA signaling released by the activation of mAChR receptors (van der Stelt et al., 2005). This latter hypothesis, together with our previous findings showing an altered AEA signaling in the amygdala of kindled rats, could explain why boosting the 2-AG signal, which fully restored DSI in kindled rats, did not completely rescue kindling-induced LTD impairments, as AEA signaling dysfunction may contribute to LTD dysfunction. The possible contribution of AEA signaling on CCh-induced LTD may also explain why KML29 treatment impaired, rather than enhanced, CCh-induced LTD in sham rats. Indeed, if both AEA and 2-AG are involved in this form of plasticity, boosting 2-AG signaling might occlude LTD in sham rats by interfering with AEA signaling.

A further possibility is that other neuromodulators might influence eCB-mediated LTD and may account for the attenuated plasticity seen in kindled rats. For example, in some conditions, nitric oxide appears to be necessary for the induction of eCB-mediated LTD (Crosby et al., 2011) and eCB-mediated short-term regulation of GABAergic synaptic transmission (Makara et al., 2007). Intriguingly, in this latter finding, nitric oxide is involved in DSI expression only in the presence of cholinergic receptor agonists (Makara et al., 2007). Given the contribution of nitric oxide signaling in seizure expression (Gupta and Dettbarn, 2003; Kato et al., 2005; Kovács et al., 2009), a potential involvement of nitric oxide signaling of seizure-induced disruption of eCB-mediated plasticity is an interesting mechanism that it is worth further investigation.

We have previously reported (Colangeli et al., 2020) and confirmed in the current work (data not shown) that sIPSC frequency is significantly reduced in the kindled group. Since the eCB signaling subserves tonic constraint of basal synaptic activity

in many brain regions, it is possible that enhanced 2-AG tone over GABA transmission in kindled rats occurred. This would lead to a reduction of the basal GABA synaptic activity and may also result in the occlusion of activity-dependent 2-AG depression of inhibitory transmission. However, we found that neither CB1 receptor blockade nor interference with either 2-AG synthesis or degradation altered the sIPSC frequency reduction found in kindled rats. Together with the lack of effect of AEA degradation inhibitor on sIPSC reduction found in kindled rats in our previous article (Colangeli et al., 2020), we ruled out any possible occlusion of phasic 2-AG signaling after repeated seizure activity performed by an enhanced tonic eCB signaling over GABA transmission. Conversely, we found that inhibiting the degradation of 2-AG in sham rats did reduce sIPSC frequency, suggesting that CCh-induced LTD impairment obtained in sham rats treated with MAGL inhibitor might be ascribed to an occlusion effect.

A further possible explanation for the seizure-induced loss of 2-AG signaling in kindled rats is an altered CB1 receptor functionality. It has been recently shown that seizure activity induces intense and prolonged activation of CB1 receptors by 2-AG during ictal events, which quickly returns to baseline soon after seizure cessation (Farrell et al., 2021). Thus, it is possible that seizure-evoked transient but repeated 2-AG signaling activation of CB1 receptors can cause a persistent compensatory CB1 receptor desensitization and, in turn, an enduring deficiency in the 2-AG-mediated responses over GABA transmission. However, we excluded this possibility since our results provide evidence that CB1 receptor sensitivity is not altered after 20 evoked amygdala seizures in BLA.

We finally asked whether the most likely mechanism subserving phasic 2-AG signaling collapse following repeated seizures is a reduction in the stimulus-dependent 2-AG mobilization or an enhanced 2-AG degradation. 2-AG is generated postsynaptically, but its synaptic effect is regulated strictly by presynaptic MAGL activity. Here we found that, while MAGL activity in the amygdala is not altered following seizure activity, the binding affinity of 2-AG for MAGL enzyme was found to be reduced. This reduction in binding affinity indicates a less efficient capability of the enzyme to catabolize 2-AG, which would actually lead to increased 2-AG signaling. This counterintuitive finding is likely explained by the reduction of MAGL binding affinity for 2-AG as a compensatory mechanism to counteract the impaired capability of postsynaptic neurons to mobilize 2-AG when needed.

In conclusion, we show that persistent 2-AG signaling maladaptation occurs in the BLA following repeated seizures, leading to aberrant plasticity in a brain region crucially involved in emotional behavior. Thereby, enhancing 2-AG-CB1 signaling via MAGL inhibition could represent an attractive therapeutic approach for the treatment of epilepsy-related emotional disorders.

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