Selectively Impaired Endocannabinoid-Dependent Long-Term Depression in the Lateral Habenula in an Animal Model of Depression

Highlights

- Different types of long-term depression (LTD) occur in the lateral habenula (LHb)

- Some, but not all, types of LTD require endocannabinoid (eCB) signaling

- Stress exposure selectively blocks eCB-dependent LTD in the LHb

- CaMKII blockade or PKA inhibition restores LFS-LTD even after stress exposure

In Brief

Park et al. find that long-term synaptic depression occurs in the lateral habenula after low- or moderate-frequency stimulation and DHPG application. In a depression model, CB1R-dependent LFS- and DHPG-LTD, but not MFS-LTD, were completely abolished. Stress exposure impairs CB1R signaling, leading to the abnormal activation of CaMKII and PKA.

Authors

Hoyong Park, Jeehae Rhee, Seongju Lee, ChiHye Chung

Correspondence

cchung@konkuk.ac.kr

Park et al., 2017, Cell Reports 20, 289–296

July 11, 2017 © 2017 The Author(s).

http://dx.doi.org/10.1016/j.celrep.2017.06.049
Selective Impaired Endocannabinoid-Dependent Long-Term Depression in the Lateral Habenula in an Animal Model of Depression

Hoyong Park,1 Jeehae Rhee,1 Seongju Lee,1 and ChiHye Chung1,2,*
1Department of Biological Sciences, Konkuk University, Seoul 05029, South Korea
2Lead Contact
*Correspondence: cchung@konkuk.ac.kr
http://dx.doi.org/10.1016/j.celrep.2017.06.049

SUMMARY
Abnormal potentiation in the lateral habenula (LHb) has been suggested to mediate depression-like behaviors. However, the underlying mechanisms of the synaptic efficacy regulation of LHb synapses and the potential for their modulation are only poorly understood. Here, we report that long-term synaptic depression (LTD) occurs in the LHb upon both low-frequency stimulation (LFS) and moderate-frequency stimulation (MFS). LFS-induced LTD (LFS-LTD) is accompanied by a reduction in presynaptic release probability, which is endocannabinoid (eCB) signaling dependent. Surprisingly, exposure to an acute stressor completely masks the induction of LFS-LTD in the LHb while leaving the MFS-induced LTD intact. Pharmacological activation of cannabinoid receptor 1 (CB1R) or blockade of αCaMKII successfully restored LTD in the LHb in an animal model of depression. Thus, our findings reveal a form of synaptic strength regulation and a stress-induced shift of synaptic plasticity in the LHb.

INTRODUCTION
The lateral habenula (LHb) has recently attracted much attention regarding its prominent role in reward prediction, drug addiction, and helplessness behaviors (Matsumoto and Hikosaka, 2007; Proulx et al., 2014). In animal models of depression, the LHb is shown to be abnormally potentiated, and inhibition of the LHb activity by using lesion, deep brain stimulation, or Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) showed a successful reversal of depressive behaviors (Li et al., 2011; Meng et al., 2011; Nair et al., 2013; Yang et al., 2008). Therefore, reducing LHb activity, which may contribute to the alleviation of depression-like behaviors, is of considerable clinical relevance.

Acute and sustained exposure to stressors are known to alter synaptic plasticity in vulnerable brain areas, including the hippocampus and prefrontal cortex (Bohacek et al., 2015; Krishnan and Nestler, 2008; Segev et al., 2014); however, synaptic plasticity in the LHb and its possible modulation upon stress exposure have not been fully investigated. We have recently shown that mild long-term potentiation (LTP) can be induced in a subpopulation of LHb neurons. Intriguingly, single exposure to acute stressors significantly enhanced the propensity to induce LTP (Park et al., 2017). However, it remains to be investigated whether the LHb exhibits long-term depression (LTD) and, if so, how stressful experiences may modulate LTD induction in the LHb. Here, we demonstrated how low-frequency stimulation (LFS), moderate-frequency stimulation (MFS), and DHPG application induce LTD in the LHb. An endocannabinoid (eCB)-dependent decrease in presynaptic release probability mediated the LFS-induced LTD (LFS-LTD) and metabotropic glutamate receptor (mGluR)-LTD in LHb synapses. Surprisingly, a single exposure to restraint plus tail shock (RTS) selectively prevented LFS-LTD and mGluR-LTD induction while leaving a 13-Hz-induced LTD (MFS-LTD) intact. Together with our previous report (Park et al., 2017), our results show that the LHb exhibits bidirectional synaptic plasticity and that acute stress can greatly disrupt the balance between LTP and LTD in the LHb, shifting to further potentiation of LHb activity.

RESULTS
LFS Induces Long-Lasting Synaptic Depression, or LFS-LTD, in the LHb
To study whether LTD occurs in the LHb, evoked excitatory postsynaptic currents (eEPSCs) were recorded from LHb neurons while stimulating the stria medullaris. LFS (1 Hz, for 10 min) significantly depressed the eEPSC amplitudes selectively in the LFS pathway (Figures 1A–1C; p < 0.001). Two slices out of 25 slices exhibited no depression after LFS. The magnitude of LTD was independent of eEPSC size; there was no correlation between initial eEPSC amplitudes and the magnitude of LTD (Figure 1J; R^2 < 0.001). MFS (13 Hz for 10 min) that was known to induce LTD in the nucleus accumbens (NAc) (Huang and Hsu, 2012) also decreased the eEPSC amplitudes in a pathway-specific manner (Figures 1G–1I; p < 0.01). These observations suggest that LTD occurs in the LHb upon LFS and MFS. LTD in the LHb was accompanied with an increase in the paired-pulse ratio (PPR) (Figures 1D–1F; p < 0.05). The increase in PPR after LFS suggests that presynaptic release probability was decreased upon LFS; therefore, LTD in the LHb is presynaptically expressed (Figure 1F).
Exposure to RTS Paradigm Blocks LFS-LTD

To determine whether a single exposure to extreme stress alters the propensity to LTD in the LHb, we used the RTS stress paradigm (Chen et al., 2010; Kim et al., 1996; Park et al., 2017; Yang et al., 2004) and obtained acute brain slices 24 hr after the RTS exposure. Surprisingly, LHb neurons in the RTS group exhibited little depression upon LFS, if any (Figures 2A–2C; p > 0.1, compared to the baseline; p < 0.001, compared to the naive wild-type [WT] group). In addition, the PPR was largely unchanged after LFS in the RTS group (Figures 2D–2F; p > 0.4). Interestingly, MFS-LTD remained intact even after the exposure to RTS (Figures 2G–2I; p < 0.01), resulting in a comparable magnitude of LTD with the WT group (p > 0.7). These data indicate that LTD occurs in the LHb and that a single exposure to a stressor selectively impairs presynaptic LFS-LTD in the LHb, but not MFS-LTD.

LFS-LTD in the LHb Requires the eCB Signaling

Given the presynaptic properties of LFS-LTD, we examined whether eCB signaling, a well-known, activity-dependent retrograde signaling pathway, is involved in this particular type of LTD in the LHb. To delineate the principles of LFS-LTD in the WT LHb, we gave LFS after the incubation of 5 μM AM251, a CB1R antagonist, for 10 min. AM251 successfully blocked LFS-LTD from WT animals (Figure 3A; p > 0.7, compared to the control pathway; p < 0.05, compared to LTD in the WT group). The blockade of CB1R signaling did not have any additional effect on LHb obtained from the RTS group (Figure 3A; p > 0.2), suggesting that LFS-LTD in the LHb requires intact CB1R signaling.

Interestingly, a brief activation of CB1R by bath application of a CB1R agonist (WIN55,212-2, 1 μM) in the absence of LFS successfully induced LTD in the LHb neurons in both the WT and RTS groups (Figure 3B; p > 0.2 between the WT and RTS groups). Given that the pharmacological activation of CB1R successfully induces LTD in the RTS group, acute stressor exposure
Figure 2. A Single Exposure to a Stressor Selectively Impairs LFS-LTD in the LHb

(A–C) LFS-LTD was abolished in the LHb obtained after exposure to RTS. (A) Example experiment showing LFS-LTD in the LHb (scale bars, 20 ms and 100 pA). (B) Normalized amplitudes in both the Ctrl. pathway (p > 0.2) and the LFS pathway (n = 16; p > 0.1) did not change compared to baseline. (C) The magnitude of LTD in the Ctrl. and LFS pathways. The LFS pathway exhibited a slightly decreased magnitude of LTD compared to that of the Ctrl. pathway (p < 0.01). However, there was no difference in the magnitude of LTD in both pathways compared to the baseline. Shaded area represents the averaged magnitude of LTD observed in the WT. (D–F) No changes in presynaptic release probability upon LFS in the LHb of RTS animals. (D) Example experiment showing changes in PPR during recording of the LHb after RTS exposure. (E) Normalized PPR upon LFS in the RTS group. Changes in PPR were no longer observed after LFS in the LHb obtained from the RTS group (n = 7; LFS pathway, p > 0.4; Ctrl. pathway, p > 0.1). (F) Fold changes in PPR after LFS in the RTS group. LFS did not change PPR after RTS exposure (p > 0.4).

(G–I) MFS-LTD was intact in the LHb obtained from stressed animals. (G) Example experiment showing MFS-LTD in the LHb after RTS exposure. (H) Normalized eEPSC amplitudes decreased after MFS compared to the baseline (n = 6; p < 0.01), while those of the Ctrl. pathway remained unchanged (p > 0.5). (I) The magnitude of depression after MFS in the RTS group. MFS induced a selective decrease in eEPSC amplitudes compared to the Ctrl. pathway (p < 0.05).

Amp., amplitude; data are indicated as mean ± SEM. **p < 0.01; Student’s t test for within-group comparisons; p < 0.05; †p < 0.01, Student’s t test for between-group comparisons. n.s, not significant.

is not likely to impair the functional receptors for eCBs. Instead, RTS may interfere with the de novo synthesis of eCBs in the LHb. To test this possibility, we pre-incubated slices in tetrahydrolipstatin (THL, 10 μM), an inhibitor of diacylglycerol lipases (DGLs) for more than 45 min prior to recording. It is well reported that the DGLs synthesize 2-arachidonylglycerol (2-AG), one of the most abundant endogenous CB1R agonists, from DAG (Tanimura et al., 2010) and are present in the LHb (Suarez et al., 2011). The inhibition of 2-AG synthesis via pre-incubation with THL prevented LTD in WT LHb slices (Figure 3C; p > 0.6), suggesting that the removal of CB1R ligands blocked the LFS-LTD in the LHb. Previous studies have suggested that cAMP-dependent protein kinase A (PKA) inactivation is necessary for eCB-mediated LTD in the hippocampus. Furthermore, CB1R is known to inactivate adenyl cyclase, thereby turning off PKA (Chevaleyre et al., 2007; Lafourcade, 2009; Mato et al., 2008; Yasuda et al., 2008). Brief pre-incubation with a PKA inhibitor (H89, 10 μM for 10 min) lacked the ability to induce LFS-LTD in the control group (Figure 3D; p > 0.4). To examine whether the PKA inhibition only during the LFS is sufficient to restore LTD in the LHb of the RTS group, H89 was applied only during 10 min of LFS, while the CB1R antagonist (AM251, 5 μM) was present throughout the entire recording session. PKA inhibition during the LFS successfully restored the LTD, even in the blockade of CB1R (Figure 3E; n = 6, p < 0.05), suggesting that the inactivation of PKA is necessary and sufficient to induce LFS-LTD from stressed animals.

We found that application of 50 μM DHPG, indeed, induced LTD in the LHb of unstressed WT animals, as very recently reported (Figures S1A and S1B; p < 0.001) (Valentinova and Mamel, 2016). Interestingly, we found that mGluR-LTD was also impaired in the RTS group (Figures S1A and S1B; p > 0.7), allowing us to observe a significant difference in the magnitude of LTD between groups (Figure S1B; p < 0.05). The changes in PPRs after LTD induction were observed in the WT group (p < 0.05) but not in the RTS group (p > 0.2; Figure S1C). Given that mGluR-LTD in the LHb was reported to be mediated by protein kinase C (PKC) as well as the eCB signaling pathway (Valentinova and Mamel, 2016), we tested whether PKC is involved in the LFS-LTD. A PKC inhibitor, G06983 (5 μM), was added in the recording chamber for at least 10 min, and LTD was given. Interestingly, we found that the LFS-LTD was successfully induced in the presence of G06983 (Figure S1D; n = 9, p < 0.05), suggesting a selective requirement of PKA during the LFS-LTD. Our observations implicate that distinct kinases are activated during LFS-LTD and mGluR-LTD. These observations...
suggest that CB1R-initiated PKA activation mediates LFS-LTD in the LHb and that the activation of CB1R rescues presynaptic LTD in an animal model of depression.

**Blockade of CaMKII Restores LFS-LTD in the LHb, even after the Exposure to RTS**

αCaMKII is proposed to inhibit DGL₂, a synthesizing enzyme for eCB via direct interactions in the striatum (Shonesy et al., 2013), and increased CaMKII levels were reported in the LHb of animals with congenitally learned helplessness (Li et al., 2013). We examined whether acute stress exposure alters αCaMKII expression in the LHb. We found that exposure to RTS significantly increased protein levels of αCaMKII as well as βCaMKII in the LHb (n = 4; Figure 4A), while mRNA levels remained unchanged (Figure 4B). These observations suggest that acute stress exposure post-transcriptionally increased both αCaMKII and βCaMKII expression in the LHb and that increased αCaMKII may interfere with the synthesis of eCB in the LHb.

Therefore, to examine whether the increased CaMKII after RTS exposure may have prevented the induction of LTD upon LFS, we recorded eEPSCs of LHb neurons in the presence of a CaMKII antagonist, autacamide-2-related inhibitory peptide (AIP; 10 μM) in the internal solution. The blockade of postsynaptic CaMKII by AIP successfully uncovered LFS-LTD, even in the RTS group (Figures 4C and 4D; p < 0.05 compared to the baseline; p < 0.05 compared to the LTD in the RTS group presented in Figure 2B). There was no additional depression by postsynaptic treatment of AIP in the WT group (Figures 4C and 4D; p < 0.001 compared to the baseline; p > 0.5 compared to the LTD in the WT group presented in Figure 1B). These data support the idea that LFS-LTD in an eCB-dependent manner, and that a single exposure to an extreme stressor, acutely impairs eCB-dependent LTD in the LHb, presumably due to an increase of basal CaMKII levels.

**DISCUSSION**

Here, we showed that different types of LTD occur in the LHb and in alterations in synaptic plasticity of the LHb after stress exposure. First, we observed that LHB neurons exhibited LFS-LTD by decreasing presynaptic release probability, which was eCB dependent. Prolonged MFS also induced LTD in the LHb. We recently showed that mild LTP also can occur in the LHb upon theta burst stimulation, although the magnitude of potentiation was smaller compared to that observed in other structures, including the hippocampus (Park et al., 2017). These findings implicate that the LHb exhibits bidirectional synaptic plasticity, although the efficacy for potentiation and that for depression do not seem to be equally balanced in the LHb.

---

**Figure 3. LFS-LTD in the LHb Depends on eCB Signaling**

(A) AM251 (5 μM) abolished the LFS-LTD in the LHb of both groups. The normalized amplitudes in the LFS pathway were comparable to the baseline (WT: n = 11, p > 0.1; RTS: n = 10, p > 0.2) and to those in the Ctrl. pathway (WT: p > 0.7; RTS: p > 0.4) in the presence of AM251 (scale bars, 20 ms and 100 pA). The magnitudes of LTD in the presence of AM251 in the WT and RTS groups were not different (p > 0.6). The averaged magnitude of LTD observed in the WT is shown as a shaded area in the background of all bar graphs for comparison.

(B) Bath application of WIN55,212-2 (1 μM, 10 min) was sufficient to induce LTD in the WT control group (n = 8; p < 0.001) and in the RTS group (n = 9; p < 0.01). The magnitude of LTD was comparable between the WT and RTS groups (p > 0.2).

(C) Pre-incubation with DGL inhibitor (THL, 10 μM) for 45 min masked the LFS-LTD in the LHb (n = 7; LFS pathway, p > 0.6). Control pathway, p > 0.5). Fold change in the magnitude of LTD in the LFS pathway was not different from that in the Ctrl. pathway (p > 0.8).

(D) PKA inhibitor (H89, 10 μM, for 10 min) impairs LFS-LTD in the LHb of WT animals. Fold changes in magnitude of LTD. There is no significant difference in both pathways (n = 8; LFS pathway, p > 0.4; Ctrl. pathway, p > 0.3) and between two pathways (p > 0.7).

(E) PKA inhibition only during LFS successfully restored LTD in the LHb obtained from RTS-exposed animals, even in the absence of CB1R activation. 5 μM AM251 was present throughout the recording to block the CB1R activation.

Amp., amplitude; data are indicated as mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001. Student’s t test for within-group comparisons. n.s, not significant.
Strikingly, a single exposure to a strong stressor completely blocked LTD in the LHb. However, the stress-mediated LTD impairment was selective for LFS-LTD; MFS-LTD remained comparable to that in the WT group, even after the stress exposure. Stress exposure greatly facilitated the induction of LTP in the LHb (Park et al., 2017). Therefore, stress exposure may facilitate LTP while masking LTD in the LHb, leading to the abnormal enhancement of LHb synapses overall.

The requirement for eCB signaling may dissociate LFS- and MFS-LTD in the LHb. Several previous studies suggest that group 1 mGluRs regulate eCB signaling (Chevaleyre and Castillo, 2003; Lafourcade et al., 2007). MFS-LTD is known to depend on mGluRs in the NAc (Huang and Hsu, 2012), yet MFS successfully induces LTD in the LHb of both the WT and RTS groups (Figures 2 and S1). How does DHPG application also induce LTD in the LHb obtained after exposure to RTS (Figures 2 and S1). How does LFS turn on eCB signaling in the LHb? Gq-coupled receptor activation or intracellular [Ca2+] increase is known to trigger eCB signaling (Heifets and Castillo, 2009; Piomelli, 2003). Several studies in the hippocampus and striatum have demonstrated that LFS can activate CB1Rs via both activation of Gq-coupled receptors and a rise in intracellular [Ca2+]. It is suggested that LFS (1 Hz for 5 min) induces persistent eCB release in the hippocampal slices (Zhu and Lovinger, 2007). In addition, patterns of stimulation such as 3 Hz (5 min) and 5 Hz (10 min), which induce eCB-mediated LTD in the hippocampus or the striatum, require the activation of group 1 mGluRs and TRV1 (Hoffman et al., 2003; Xu et al., 2010). A number of Gq-coupled receptors, including group 1 mGluRs, serotonin receptors, muscarinic acetylcholine receptors, and transient receptor potentials (TRPs) are also expressed in the LHb (Valentinova and Mameli, 2016; Zapata et al., 2017; Zuo et al., 2016). Therefore, LFS in the LHb is likely to share cellular mechanisms for turning on eCB signaling reported in other brain areas.

Notably, signaling pathways activated by LFS or DHPG application seem to be distinct. A computational study showed that DAG, a metabolite synthesized by the activation of Gq-coupled receptors or elevated intracellular Ca2+, competitively activates PKC or DGL in a striatal model network. In this setting, LTD occurs when DAG activates DGL, while LTP is expected when DAG turns on the PKC pathway, although the exact determinants of which pathway DAG activates are unknown (Kim et al., 2013). A recent study suggested that PKC is involved in DHPG-mediated mGluR-LTD in the LHb (Valentinova and Mameli, 2016).
However, we found that the LFS-LTD activates CB1R independent of PKC (Figure S1D). Therefore, the LFS is likely to induce LTD through distinct mechanisms to mGluR-dependent LTD, suggesting that the cellular milieu may determine the direction of predominant signaling cascades.

Other candidates to regulate stress-induced disruption of the eCB system in the LHb are receptors responsive to stress hormones. Recent studies suggest that chronic stress exposure reduces eCB signaling in the amygdala (Gray et al., 2015; Qin et al., 2015). In these studies, eCB production was interrupted by glucocorticoid receptor activation, while eCB hydrolysis was enhanced by the activation of corticotropin-releasing hormone receptor. Therefore, it is possible that the LHb may share stress-induced alterations in the eCB system via the activation of stress hormone receptors as observed in the amygdala.

Besides eCB receptor activation, we found that αCaMKII inhibition successfully restored LTD in the LHb in an animal model of depression. In a recent study, overexpression of βCaMKII, but not αCaMKII, in the LHb was shown to induce depressive behaviors in rodents (Li et al., 2013). In this study, αCaMKII protein levels showed a trend of increase, while βCaMKII expression was significantly increased in the LHb obtained from a congenitally learned helpless (cLH) animal model of depression. In our acute stress model, we found a significant increase in both isoforms of CaMKII (Figure 4A), suggesting that slightly distinct molecular and cellular responses may be activated in different models of depression. mRNA levels of CaMKII isoforms obtained from the RTS group remain comparable to those of WT animals (Figure 4B), as shown in a previous study comparing WT and cLH animals (Li et al., 2013). Data from previous and current observations suggest that eCB signaling was impaired in the LHb of the LTS group, presumably due to the increased αCaMKII level, shifting the balance of bidirectional synaptic plasticity toward further potentiation (Park et al., 2017). No behavioral animal models of depression cause depressive behaviors in all animals tested. Therefore, even though the overexpression of αCaMKII, per se, may not be sufficient to induce helpless behaviors, as shown in a previous study (Li et al., 2013), αCaMKII levels in the LHb may serve as a determinant for susceptibility to stress exposure by setting the neuronal output upon given stimulations.

Stress-induced imbalance in bidirectional synaptic plasticity is not limited to the LHb: acute stress appears to impair synaptic plasticity in several brain areas that mediate helpless behaviors. Previous studies showed that both LTP and LTD were altered in the hippocampus of animals that underwent RTS (Kim et al., 1996; Yang et al., 2004). Increased mitogen-activated protein kinase (MAPK) activity or enhanced NMDA receptor activity upon acute stress was suggested to mediate impaired hippocampal synaptic plasticity. Chronic restraint stress for a week impaired LTD in the dorsal raphe nucleus and LTD in layer 1 of the medial prefrontal cortex (mPFC). Impaired LTD after chronic restraint stress in the mPFC was accompanied with a decrease in AMPA-receptor-mediated synaptic transmission (Haj-Dahmane and Shen, 2014; Negrón-Oyarzo et al., 2015). Chronic social defeat stress was shown to impair the induction of NMDA-receptor-dependent LTP in dopaminergic neurons in the ventral tegmental area (Stelly et al., 2016). LFS-LTD was impaired in the cortico-accumbal pathway of animals susceptible to chronic social defeat stress (Jiang et al., 2013).

Together, our study revealed a signaling pathway underlying LFS-LTD in the epithalamic area and selective impairment in eCB-mediated LTD in an animal model of depression. With our recent investigation on LTP in the LHb, we expect that stress exposure imbalances bidirectional synaptic plasticity occurring in the LHb, which may contribute to determining the threshold for the onset of depression.

**EXPERIMENTAL PROCEDURES**

**Animals and RTS Paradigm**

All procedures were performed in accordance with the guidelines of the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Konkuk University. Five- to 7-week-old male Sprague-Dawley rats were purchased from Orient Bio (a branch of Charles River Laboratories), and at least a week of acclimation was allowed upon arrival. Animals were group housed (four rats per cage), maintained on a 12-hr/12-hr light/dark cycle (lights on at 07:00 and off at 19:00) in a controlled vivarium (45% humidity, 23°C ± 1°C), and provided food and water ad libitum. For exposure to RTS, animals were given unpredictable electric shocks on their tails for 1 hr (1 mA, 1 s each; total, 60 shocks) while restrained, as described previously (Park et al., 2017). Animals were housed individually after stress exposure, and brain slices were prepared 24 hr after RTS.

**Acute Brain Slice Preparation**

Animals were anesthetized using isoflurane, and brains were removed into ice-cold oxygenated choline dissection buffer (containing in millimolar: 110 choline chloride, 11.6 Na-ascorbate, 3.1 pyruvate, 25 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, 7 MgCl2, 0.5 CaCl2, 25 glucose). Coronal brain slices containing the LHb (350 μm thick) were prepared using the Leica VT1000 S, stored for 1 hr at 35°C in oxygenated artificial cerebrospinal fluid (aCSF, containing in millimolar: 1 NaH2PO4, 26.2 NaHCO3, 118 NaCl, 2.5 KCl, 11 glucose, 2 CaCl2, and 1 MgCl2), and stored at room temperature thereafter. Slices were transferred to a recording chamber and constantly perfused with warmed aCSF (30°C ± 2°C).

**Slice Electrophysiology**

To record the eEPSCs, LHb neurons were voltage clamped at ~60 mV using the Axopatch 200B amplifier and Clampex 10.2 software (Molecular Devices), filtered at 5 kHz, and sampled at 10 kHz. Recording pipettes (2- to 6-MΩ resistance) were filled with (in millimolar): 115 Cs methanesulphonate, 20 CsCl, 10 HEPEs, 2.5 MgCl2, 0.6 EGTA, 5 QX-314, 4 Na2-ATP, 0.4 Na2-GTP, and 10 Na-phosphocreatine. eEPSCs were elicited by a bipolar electric stimulator (FHC) placed on the striata medullaris in the presence of picrotoxin (PTX, 50 μM in DMSO) at 0.1 Hz. In experiments using H89 and Gö6983, to rule out the effect of kinase inhibition in baseline eEPSCs, series resistance was monitored and shown instead of the control pathway. The series resistance was measured by a test pulse (5 mV, 25 ms) in each stimulation sweep. The PPR was measured by giving two pulses 50 ms apart and 20 times at 0.05 Hz and was calculated as the peak amplitude of the second eEPSC divided by that of the first eEPSC. For pharmacological experiments, drugs were bath applied for at least 20 min before the LTD induction, unless described otherwise. Salts and drugs were obtained from Sigma-Aldrich, except for WIN55,212-2 (Tocris Bioscience) and for AIP (Calbiochem).

**Lysate Preparation and Western Blot Analysis**

The LHb dissected from the WT group or the RTS group was quickly immersed in ice-cold artificial cerebrospinal fluid (aCSF, containing in millimolar: 25 NaHCO3, 118 NaCl, 2.5 KCl, 11 glucose, 2 CaCl2, and 1 MgCl2) at 0°C for 10 min and was stored at −80°C. Tissue was thawed on ice, rinsed in aCSF, and was homogenized in a glass Teflon homogenizer. Homogenates (150 mM sodium chloride, 1% NP-40, 0.1% sodium deoxycholate, 0.1% SDS, and 50 mM Tris [pH 8.0] with protease inhibitor cocktail and phosphatase inhibitor cocktail (Roche) and homogenized with a glass Teflon homogenizer. After constant agitation for 1 hr at 4°C, the lysates were clarified by
centrifugation (14,000 × g) for 20 min at 4 °C. The supernatants were transferred to new tubes, and the protein amount was measured with the Bradford protein assay. Thirty micrograms of proteins were loaded on each lane of SDS-PAGE gels and subjected to western blot analysis, as described previously (Lee et al., 2016). The antibodies used were anti-cαCaMKII (1:1,000, Millipore), anti-βCaMKII (1:1,000, Abcam), and anti-β-tubulin (1:1,000, Sigma-Aldrich). Amersham ECL Prime (GE Healthcare) was used to detect immunoreactive proteins using a ChemiDoc XRS+ System with Image Lab software (Bio-Rad).

**Real-Time qPCR**

RNA was extracted from LHB using the WelPrep Total RNA Prep Kit (Welgene). Real-time qPCR was performed with SYBR Green PCR Master Mix (Euro-technologies) using the Applied Biosystems StepOne System. Experiments were performed in triplicate using three independent animals. The real-time qPCR results were normalized to GAPDH and calculated following the 2−ΔΔCT method. The following sequences of primers were used: GAPDH-F: 5′-GG TCGGAGTCACGGATTTGTC-3′; GAPDH-R: 5′-CCTCCGACGGCCTGCTTA -CA-3′; cαCaMKII-F: 5′-C CAAATATCGTCCGACTCA-3′; cαCaMKII-R: 5′-CCAGCACAAGATTCTCAGGC-3′; βCaMKII-F: 5′-CAAGGAGGAAGCTCAC GGGAGA-3′; βCaMKII-R: 5′-A TG TGTGTTGTTGCTGTC-3′.

**Data Analysis and Statistics**

All values in the text and figures are given as mean ± SEM. The peak eEPSC amplitudes were measured by using Clampfit 10.3 (Molecular Devices) and averaged for 5 min to calculate the mean amplitude. Student’s t test was used for within-group comparisons (*p < 0.05, **p < 0.01, and ***p < 0.001) and for between-group comparisons (*p < 0.05, **p < 0.01, and ***p < 0.001), with p < 0.05 deemed significant.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes one figure and can be found with this article online at http://dx.doi.org/10.1016/j.cerep.2017.06.049.

**AUTHOR CONTRIBUTIONS**

C.C. conceived this work and designed the experiments. H.P. and C.C. performed all the electrophysiological experiments and acquired the data presented in this paper. J.R. participated in obtaining preliminary electrophysiological experiments. S.L. performed western blot analysis. H.P. and C.C. analyzed and interpreted the data and prepared the manuscript and the figures. All authors have approved the final version of the manuscript.

**ACKNOWLEDGMENTS**

We would like to thank members of the Chung laboratory for helpful discussion on our manuscript. This work was supported by grants from the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI14C2136 and HI14C2137).

Received: December 2, 2016
Revised: May 22, 2017
Accepted: June 19, 2017
Published: July 11, 2017

**REFERENCES**


Huang, C.C., and Hsu, K.S. (2012). Activation of NMDA receptors reduces metabotropic glutamate receptor-induced long-term depression in the nucleus accumbens via a CaMKII-dependent mechanism. Neuropharmacology 63, 1298–1307.


