Development/Plasticity/Repair

Brain-Derived Neurotrophic Factor Controls Cannabinoid CB_1 Receptor Function in the Striatum

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The role of brain-derived neurotrophic factor (BDNF) in emotional processes suggests an interaction with the endocannabinoid system. Here, we addressed the functional interplay between BDNF and cannabinoid CB_1 receptors (CB_1Rs) in the striatum, a brain area in which both BDNF and CB_1s play a role in the emotional consequences of stress and of rewarding experiences.

BDNF potently inhibited CB_1R function in the striatum, through a mechanism mediated by altered cholesterol metabolism and membrane lipid raft function. The effect of BDNF was restricted to CB_1Rs controlling GABA-mediated IPSCs ($CB_1R_{(GABA)}$), whereas CB_1Rs modulating glutamate transmission and $GABA_B$ receptors were not affected. The action of BDNF on $CB_1R_{(GABA)}$ function was tyrosine kinase dependent and was complete even after receptor sensitization with cocaine or environmental manipulations activating the dopamine (DA)-dependent reward system. In mice lacking one copy of the BDNF gene ($BDNF^{+/-}$), $CB_1R_{(GABA)}$ responses were potentiated and were preserved from the action of haloperidol, a DA D_2 receptor (D_2R) antagonist able to fully abolish $CB_1R_{(GABA)}$ function in rewarded animals. Haloperidol also enhanced BDNF levels in the striatum, suggesting that this neurotrophin may act as a downstream effector of D_2Rs in the modulation of cannabinoid signaling. Accordingly, 5 d cocaine exposure both reduced striatal BDNF levels and increased $CB_1R_{(GABA)}$ activity, through a mechanism dependent on D_2Rs.

The present study identifies a novel mechanism of CB_1R regulation mediated by BDNF and cholesterol metabolism and provides some evidence that DA D_2R-dependent modulation of striatal CB_1R activity is mediated by this neurotrophin.

Introduction

Brain-derived neurotrophic factor (BDNF) is a widely expressed, activity-regulated secretory protein with pleiotropic actions within the CNS. Besides its role in promoting neuronal proliferation, differentiation, migration, and survival, BDNF is a key regulator of synaptic transmission and plasticity in the adult brain (Carvalho et al., 2008; Waterhouse and Xu, 2009). These composite actions are likely to mediate the neuroprotective effects of BDNF (Castrén, 2004; Lu et al., 2005; Hennigan et al., 2007), as well as its complex effects on cognition and mood (Lu et al., 2005; Martinowich et al., 2007). Signaling through cannabinoid CB_1 receptors (CB_1Rs) is also emerging as a critical determinant in neuroprotection (Martínez-Orgado et al., 2007; Galve-Roperh et al., 2008), learning and memory (Horder et al., 2009; Puighermanal et al., 2009), and emotional control (Aso et al., 2008; Juhasz et al., 2009; Moreira et al., 2009), raising the possibility that BDNF and CB_1Rs interact to regulate multiple functions in the brain.

Indirect evidence suggests a BDNF–CB_1R interaction. BDNF levels, in fact, are decreased in the brain of mice lacking CB_1Rs (Aso et al., 2008), whereas activation of these receptors increases BDNF in rodents (Butovsky et al., 2005) and in humans (D'Souza et al., 2009). Furthermore, BDNF release triggered by CB_1R stimulation mediates the neuroprotective effects of cannabinoids (Khaspekov et al., 2004). Although these data indicate that stimulation of CB_1Rs promotes BDNF release and activity, the effects of BDNF activity on CB_1R function are poorly characterized. BDNF, in fact, has been found to inhibit CB_1R responses in the visual cortex (Huang et al., 2008) and to increase the expression of CB_1R transcripts in cultured cerebellar granule neurons (Maison et al., 2009).

In the striatum, BDNF and CB_1Rs seem to act in opposite ways to regulate emotionality. Intrastriatal infusion of BDNF, in fact, elicits a depressive behavior (Eisch et al., 2003), as also does a stress protocol causing the downregulation of striatal CB_1Rs (Berton et al., 2006; Rossi et al., 2008). Furthermore, the anxious-depressive behavior induced by social stress is abolished by either blockade of BDNF signaling in this brain area (Berton et al., 2006) or sensitization of CB_1Rs regulating striatal GABA synapses (De Chiara et al., 2010). These data argue against a synergistic action

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of CB_1Rs and BDNF and rather suggest that BDNF contrasts the activity of CB_1Rs.

Here, we addressed the effects of BDNF on CB_1R function in the striatum. We found that *in vitro* and *in vivo* BDNF treatments potently inhibited the activity of a subset of CB_1Rs controlling GABA synapses ($CB_1Rs_{(GABA)}$) in the striatum. This effect was mediated by an action on cholesterol metabolism in lipid rafts and was prevented by tyrosine kinase inhibition. BDNF also blocked $CB_1R_{(GABA)}$ function after receptor sensitization with chronic cocaine or with environmental manipulations activating the dopamine (DA)-dependent reward system. $CB_1R_{(GABA)}$ responses were potentiated in mice with reduced expression of BDNF. In these mice, $CB_1R_{(GABA)}$ sensitivity was also preserved from the action of haloperidol, a DA receptor antagonist able to abolish $CB_1R_{(GABA)}$ function in rewarded animals.

Materials and Methods

Mice. Male C57BL/6 mice (8–10 weeks old) were used for all the experiments. All animals were housed, four per cage, on a 12 h light/dark cycle with lights on at 6:00 A.M.

A group of control mice (reared in standard cages and never exposed to sucrose) received a single intracerebroventricular injection of BDNF ($n = 6$) or vehicle ($n = 6$) and was used for the electrophysiological experiments 1 d later. Another group of mice lacking one copy of the BDNF gene ($BDNF^{+/-}$) and expressing reduced activity of BDNF (Jeanblanc et al., 2006; Saylor and McGinty, 2008) received intramuscular haloperidol ($n = 6$) or the appropriate vehicle ($n = 6$) at days 1 and 4 before the electrophysiological experiments (day 7). Control mice treated with intramuscular haloperidol or vehicle were killed at day 7 to measure BDNF levels in the striatum and the hippocampus ($n = 8$ for both groups) or for electrophysiological recordings ($n = 6$ for both groups). Another group of control mice received five daily intraperitoneal injections of cocaine (Centonze et al., 2007). Then, they received a single intracerebroventricular injection of BDNF ($n = 7$) or vehicle ($n = 6$) and killed 24 h later for the electrophysiological experiments. Another sample of mice treated with cocaine received haloperidol or vehicle intramuscular injections (days 1 and 4) and were killed 24 after the last injection of cocaine for electrophysiological ($n = 5$ per group) or BDNF measures ($n = 6$ per group).

To study the effects of natural rewards, a group of mice ($n = 30$) were housed in a cage equipped with a running wheel for 15 d. Another group of mice ($n = 30$) was reared in control cages, but they were allowed to consume *ad libitum* a drinking fluid containing sucrose (3% in tap water) for 7 d. Both 15 d running wheel and 7 d sucrose consumption have already been reported to enhance the sensitivity of $CB_1Rs_{(GABA)}$ to the CB_1 receptor agonist HU210 [(6a*R*,10a*R*)-3-(1,1′-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[b,d]pyran-9-methanol] (De Chiara et al., 2010). At the end of these environmental manipulations, mice from both rewarded groups ($n = 8$ per group) were killed along with control animals of the same age ($n = 8$) to measure BDNF levels in the striatum and in the hippocampus. Twelve mice per group were randomly allocated to receive a single intracerebroventricular injection of BDNF ($n = 6$ for both groups) or vehicle ($n = 6$ for both groups) and killed 24 h later for the electrophysiological experiments. The remaining 10 mice per group received intramuscular injections of haloperidol ($n = 5$) or vehicle ($n = 5$) during the behavioral manipulations (days 1 and 4 in mice exposed to sucrose for 1 week; days 1, 4, 8, and 11 in mice exposed to running wheel for 2 weeks). These mice were killed for the electrophysiology at the end of the running wheel (15 d) or sucrose (7 d) procedure.

All efforts were made to minimize animal suffering and to reduce the number of mice used, in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Electrophysiology. Whole-cell patch-clamp recordings from single striatal neurons in corticostriatal coronal slices (200 μm) were performed as described previously (Rossi et al., 2008; De Chiara et al., 2010). To detect spontaneous (sIPSCs) or miniature (mIPSCs) $GABA_A$-mediated IPSCs, intraelectrode solution had the following composition (in mM): 110 CsCl, 30 K^+-gluconate, 1.1 EGTA, 10 HEPES, 0.1 $CaCl_2$, 4 Mg-ATP, and 0.3 Na-GTP. MK-801 [(+)-5-methyl-10,11-dihydro-5*H*-dibenzo [a,d] cyclohepten-5,10-imine maleate] (30 μM) and CNQX (10 μM) were added to the external solution to block, respectively, NMDA and non-NMDA glutamate receptors. Tetrodotoxin (TTX) (1 μM) was also continuously applied to study mIPSCs (Centonze et al., 2007; De Chiara et al., 2010). For kinetic analysis, events with peak amplitude between 10 and 50 pA were grouped, aligned by half-rise time, normalized by peak amplitude, and averaged to obtain rise times, decay times, and half-widths.

Conversely, to study spontaneous glutamate-mediated EPSCs (sEPSCs), the recording pipettes were filled with internal solution of the following composition (in mM): 125 K^+-gluconate, 10 NaCl, 1.0 $CaCl_2$, 2.0 $MgCl_2$, 0.5 BAPTA, 19 HEPES, 0.3 GTP, and 1.0 Mg-ATP 1.0, adjusted to pH 7.3 with KOH. Bicuculline (10 μM) was added to the perfusing solution to block $GABA_A$-mediated transmission.

BDNF measurements. Mice were decapitated, the brains were quickly removed, and the striatum was dissected on ice together with the hippocampus and immediately used for additional processing. The brain regions were freehand and roughly dissected. Brain tissue samples were homogenized in ice-cold lysis buffer, containing 137 mM NaCl, 20 mM Tris-HCl, pH 8.0, 1% NP-40, 10% glycerol, 1 mM PMSF, 10 μg/ml aprotinin, 1 μg/ml leupetin, and 0.5 mM sodium vanadate. The tissue homogenate solutions were centrifuged at 14,000 × *g* for 5 min at 4°C. The supernatants were collected and used for quantification of BDNF by using a two-site enzyme immunoassay kit (Promega). BDNF concentrations were determined from the regression line for the neurotrophin standard (ranging from 7.8 to 500 pg/ml purified mouse BDNF) incubated under similar conditions in each assay. Cross-reactivity with other related neurotrophic factors, e.g., NGF and neurotrophins 3 and 4, was <3%. BDNF concentration was expressed as picograms per gram wet weight, and all assays were performed in triplicate.

Concentrations of BDNF proteins were assessed. Briefly, 96-well immunoplates (Nalge Nunc International) were coated with 50 μl/well with the corresponding captured antibody, which binds the neurotrophin of interest, overnight at 4°C. The next day, serial dilutions of known amounts of BDNF ranging from 0 to 500 pg/ml were performed in duplicate for generating the standard curve. Then, the plates were washed three times with wash buffer, and the standard curves and supernatants of brain tissue homogenates were incubated in the coated wells (100 μl each) for 2 h at room temperature (RT) with shaking. After additional washes, the antigen was incubated with second specific antibody for 2 h at RT, as specified in the protocol. The plates were washed again with wash buffer and then incubated with an anti-IgY HRP for 1 h at RT. After another wash, the plates were incubated with a TMB/peroxidase substrate solution for 15 min and phosphoric acid at 1 M (100 μl/well) was added to the wells. The colorimetric reaction product was measured at 450 nm using a microplate reader (MR 5000; Dynatech).

Drugs. Single corticostriatal slices were prepared from tissue blocks of the brain with the use of a vibratome. Unless otherwise specified, they were incubated for 60 min in the presence of BDNF (10 ng/ml, first dissolved in 10 μg/ml water; Tocris Cookson), lavendustin A (10 μM, first dissolved in 10 mM DMSO; Calbiochem, Merck), BDNF plus lavendustin A, K252a [methyl-9-(*S*)-12(*R*)-epoxy-1*H*-diindolo[1,2,3-fg: 3′2′1′-kl]pyrrolo[3,4-i][1,6]benzodiazocine-2,3,9,10,11,12-hexahydro-10-(*R*)-hydroxy-9-methyl-1-oxo-10-carboxilate] (10 μM, first dissolved in DMSO; Tocris Cookson), or BDNF plus K252a. In some experiments, methyl-β-cyclodextrin (MCD) (5 mM, first dissolved in water; Sigma/RBI) was applied for 30 min in slices from control or BDNF intracerebroventricularly treated mice or added to the BDNF-containing bathing solution after 30 min. In other experiments, striatal slices were incubated with mevestatin (10 μM, first dissolved in ethanol; Tocris Cookson) or with BDNF plus mevestatin for 1 h. Slices were then transferred to the recording chamber and submerged in a continuously flowing artificial CSF (ACSF) (32°C, 2–3 ml/min) gassed with 95% O_2–5% CO_2. The composition of the ACSF included the following (in mM): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 11 glucose, and 25 $NaHCO_3$.

Drugs used during the electrophysiological recordings were applied for 10 min, with the exception of BDNF, whose application was contin-

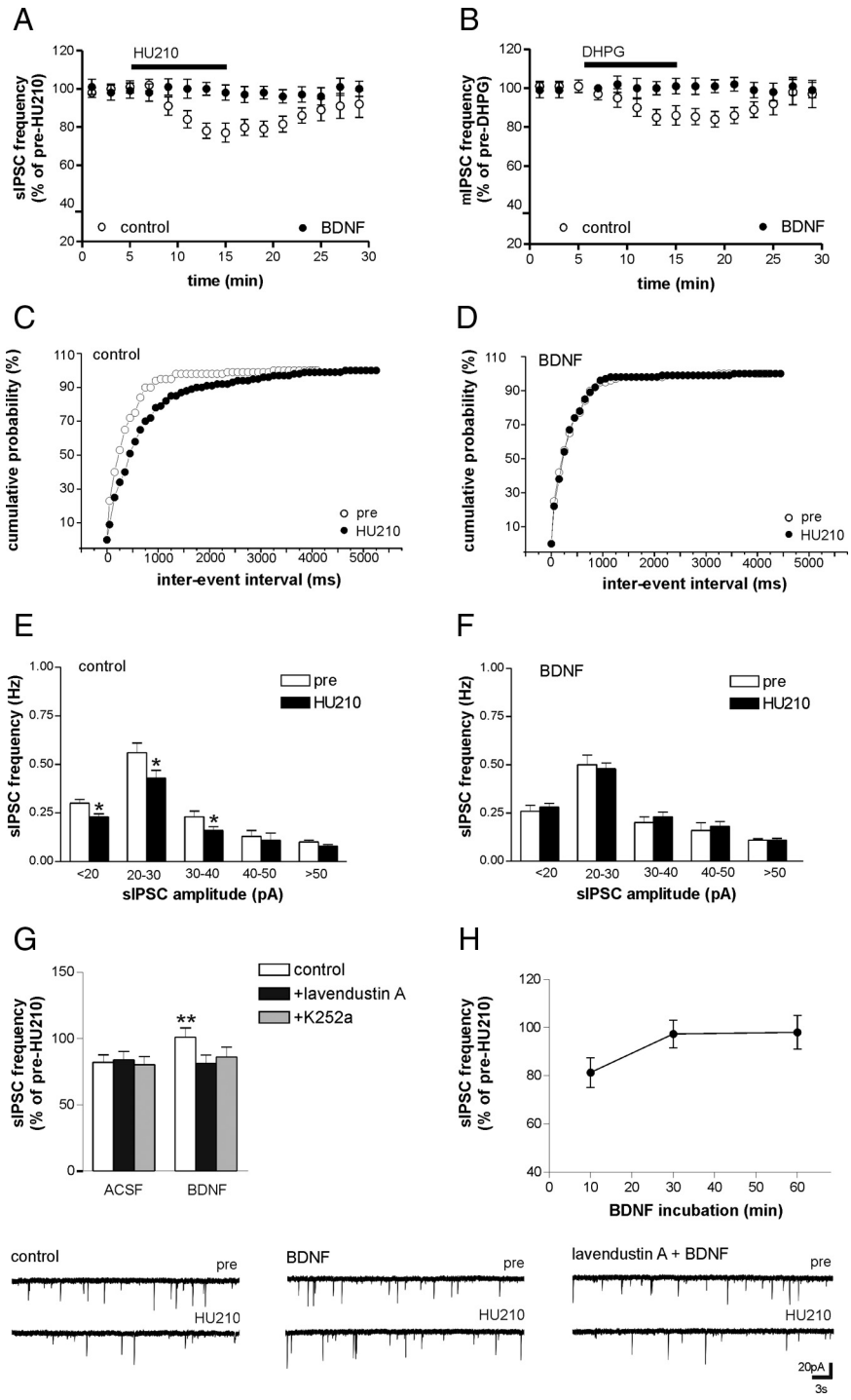

Figure 1. BDNF blocks $CB_1R_{(GABA)}$ function via tyrosine kinase activation. ***A***, The graph shows that the depressant effect of the CB_1 receptor agonist HU210 on sIPSC was completely abolished by incubation of striatal slices with BDNF (control, $n = 9$ cells; BDNF, $n = 11$ cells). ***B***, DHPG was ineffective in reducing mIPSC frequency in BDNF-treated slices (control, $n = 7$ cells; BDNF, $n = 9$ cells). ***C***, ***D***, Cumulative distribution of sIPSC interevent interval recorded from control slices (***C***) and from BDNF-treated slices (***D***) before and during the application of HU210. The effect of HU210 on cumulative probability distribution was blocked in BDNF-treated slices, as reveled by Kolmogorov–Smirnov test. ***E***, ***F***, Amplitude–frequency histograms of sIPSCs before and during the application of HU210 in control (***E***) and BDNF-treated (***F***) slices. ***G***, Lavendustin A and K252a, inhibitors of TrkB tyrosine kinase, did not affect HU210 responses per se on sIPSC frequency ($n = 7$ cells for each group) but were able to rescue the effect of HU210 in BDNF-treated slices (lavendustin A, $n = 10$ cells; K252a, $n = 8$ cells). ***H***, The graph shows that the HU210-induced reduction on sIPSC frequency was normal after 10 min of BDNF application ($n = 9$ cells). The effect of HU210 was fully blocked after 30 and 60 min of BDNF application ($n = 11$ cells for each group). The electrophysiological traces below are examples of voltage-clamp recordings showing the loss of sIPSC frequency reduction induced by HU210 in a neuron from BDNF-treated slices and the recovery of the effects of HU210 induced by lavendustin A. $^*p < 0.05$; $^{**}p < 0.01$.

ued, in half of the recordings (6 of 13) and after the initial 60 min incubation, for 20–40 min in the recording chamber. In both experimental conditions, the electrophysiological effects were comparable, and the results were therefore pooled together. Drugs were first dissolved in DMSO (AM251 [*N*-1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-1-piperidinyl-1*H*-pyrazole-3-carboxamide] and HU210) or in water and then in the bathing ACSF to the desired final concentration. The concentrations of the various drugs were chosen according to previous *in vitro* studies on corticostriatal brain slices (Rossi et al., 2008; De Chiara et al., 2010) and were as follows: 10 μM AM251, 3 μM baclofen, 10 μM CNQX, 50 μM (*RS*)-3,5-dihydroxyphenylglycine (DHPG), 1 μM HU210, 30 μM MK-801, and 1 μM TTX (from Tocris Cookson). Bicuculline was used at 10 μM (from Sigma/RBI). In the experiments with drugs dissolved in DMSO, the control samplings were obtained during DMSO and ACSF applications.

A single dose of BDNF (1 μl) was also administered *in vivo* by an intracerebroventricular injection (0.2 μg/μl saline) under stereotaxic coordinates (anterior, +0 mm; lateral, +0.8 mm; dorsal, −2.4 mm) and general anesthesia with 2,2,2-tribromoethanol (10 mg/ml; 1/27 of body weight).

Cocaine (15 mg/kg, in 200 μl saline) was injected intraperitoneally for 5 consecutive days in control mice. At the end of the treatment period, cocaine-exposed mice received a single intracerebroventricular injection of vehicle or BDNF and were killed for the electrophysiological experiments 24 h after. Haloperidol (Haldol Decanoas at 1 mg/kg; Jansenn-Cirag) was dissolved in 200 μl of saline (0.9% NaCl) and administrated by intramuscular injections for 1 or 2 weeks.

Mice receiving the same number of intracerebroventricular, intraperitoneal, or intramuscular injections of the appropriate volume of vehicle were used as controls.

Statistical analysis. The analyses were performed on a per animal basis, and, throughout text, *n* refers to the number of mice used. Five to eight mice were used for a single electrophysiological experiment. Electrophysiological results from neurons recorded from the same animal were treated as a separate sample and averaged before calculating statistics. One to six neurons per animal were recorded. For data presented as the mean ± SEM, statistical analysis between two groups was performed using a paired or unpaired Student's *t* test or Wilcoxon's test. Multiple comparisons were analyzed by one-way ANOVA, followed by Tukey's honestly significant difference test. One or two animals per day were used for the electrophysiology. The significance level of the results was established at $p < 0.05$. To determine differences between two cumulative distributions, the Kolmogorov–Smirnov test was used.

Results

BDNF blocks $CB_1R_{(GABA)}$ function via tyrosine kinase activation

We first tested the effects of *in vitro* BDNF on $CB_1R_{(GABA)}$ function. Incubation of

striatal slices with BDNF did not alter frequency and amplitude of sIPSCs ($n = 8$ and $p > 0.05$ for both experimental groups and electrophysiological parameters) or frequency ($p > 0.05$, $n = 6$ for both groups) and amplitude ($n = 6$ and $p > 0.05$ for both groups and electrophysiological parameters) of mIPSCs. BDNF also failed to affect rise time, decay time, and half-width of sIPSCs ($n = 6$ and $p > 0.05$ for each parameter in both groups) (supplemental table, available at www.jneurosci.org as supplemental material).

BDNF, however, fully blocked the effects of the CB_1R agonist HU210 on sIPSC and mIPSC frequency. In control slices, in fact, HU210 caused a significant inhibition of sIPSC ($n = 8$, $p < 0.01$) (Fig. 1*A*,*C*,*E*) and mIPSC [82.3 ± 4% in HU210 (10 min), $n = 6$, $p < 0.01$] frequency, whereas sIPSC ($n = 8$, $p > 0.05$) and mIPSC ($n = 6$, $p > 0.05$) amplitude were unaffected. The effects of HU210 on sIPSC and mIPSC frequency were abolished in the presence of AM251, a selective antagonist of CB_1Rs [sIPSC frequency in HU210 plus AM251 (10 min), 99 ± 2%; mIPSC frequency in HU210 plus AM251 (10 min), 100 ± 3%; $n = 6$ for both sIPSCs and mIPSCs, $p > 0.05$]. These data are consistent with a large body of previous results on sIPSCs, mIPSCs, and evoked IPSCs in slices, concluding that the effects of HU210 on striatal GABA-mediated transmission are entirely mediated by a presynaptic mechanism (Centonze et al., 2007; Maccarrone et al., 2008; Rossi et al., 2008). In BDNF-treated slices, HU210 effects were negligible on both sIPSC ($n = 12$, $p > 0.05$) and mIPSC (101.3 ± 3%; $n = 13$, $p > 0.05$) frequency (Fig. 1*A*,*D*,*F*).

Activation of metabotropic glutamate receptor 5 by DHPG enhances the synthesis of the endocannabinoid 2-arachidonoyl, which in turn stimulates $CB_1Rs_{(GABA)}$ and depresses mIPSC frequency in the striatum (Maccarrone et al., 2008, 2009). Thus, to see whether BDNF also interfered with the effect of this endocannabinoid on its receptors, we measured the action of DHPG in slices pretreated with BDNF. As already reported, DHPG reduced the frequency of mIPSCs in striatal slices [mIPSC frequency in control slices (pre-DHPG), 1.15 ± 0.39 Hz; $n = 5$, $p < 0.01$], an effect that was fully blocked by AM251 [mIPSC frequency in DHPG plus AM251 (10 min), 101 ± 4%; $n = 5$, $p > 0.05$] (Maccarrone et al., 2008, 2009). In BDNF-treated slices, the effect of DHPG was absent [mIPSC frequency in BDNF-bathed slices (pre-DHPG), 1.17 ± 0.38 Hz; $n = 6$, $p > 0.05$] (Fig. 1*B*).

The effects of BDNF are primarily mediated by the TrkB tyrosine kinase receptor, and previous studies in slices reported that they were prevented by inhibiting tyrosine kinases with lavendustin A (Frerking et al., 1998). Lavendustin A was unable per se in altering sIPSC frequency (sIPSC frequency in lavendustin A-bathed slices, 1.39 ± 0.43 Hz) or amplitude (sIPSC amplitude in lavendustin A-bathed slices, 35.00 ± 3.23 pA) ($n = 6$, $p > 0.05$ for both parameters), and it did not affect HU210 responses ($n = 6$, $p > 0.05$ compared with HU210 alone). This pharmacological compound, however, fully prevented the effects of BDNF on HU210-mediated inhibition of sIPSC frequency (pre-HU210 sIPSC frequency in slices bathed with lavendustin A plus BDNF, 1.40 ± 0.45 Hz; $n = 7$, $p < 0.01$). Similar effects were obtained with K252a, another tyrosine kinase inhibitor. K252a, in fact, did not change sIPSC frequency (sIPSC frequency in K252a-bathed slices, 1.48 ± 0.40 Hz) or amplitude (sIPSC amplitude in K252a-bathed slices, 31.20 ± 3.20 pA) ($n = 6$, $p > 0.05$ for both parameters). K252a also failed to change the sensitivity to HU210 of $CB_1Rs_{(GABA)}$ but blocked the effect of BDNF on sIPSCs [frequency of sIPSCs in slices bathed with K252a plus BDNF (pre-HU210), 1.41 ± 0.39; $n = 5$, $p > 0.05$ compared with HU210 alone] (Fig. 1*G*).

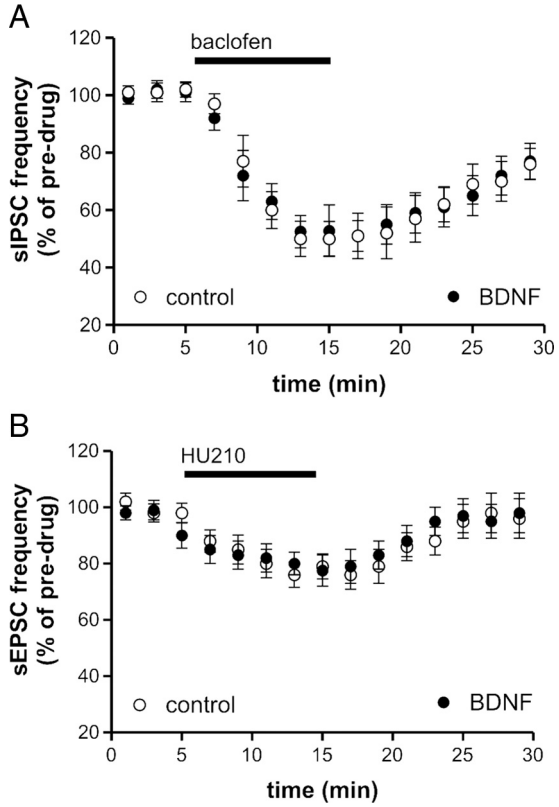

Figure 2. $GABA_B$ receptors and $CB_1Rs_{(Glu)}$ are insensitive to BDNF. ***A***, The depressant effect of the $GABA_B$ receptor agonist baclofen on sIPSC frequency was similar in control and BDNF-treated slices (control, $n = 8$ cells; BDNF, $n = 10$ cells). ***B***, The graph shows that the depressant action of HU210 on sEPSC frequency was similar in control and BDNF-treated slices (control, $n = 9$ cells; BDNF, $n = 9$ cells). $^{**}p < 0.01$.

Finally, we also determined the time course of BDNF effects on $CB_1Rs_{(GABA)}$. BDNF application in slices for 10 min did not alter HU210 effects on sIPSCs ($n = 5$, $p < 0.01$), whereas 30 min application fully blocked the sensitivity of $CB_1Rs_{(GABA)}$ to this agonist ($n = 5$, $p > 0.05$) (Fig. 1*H*).

$GABA_B$ receptors and $CB_1Rs_{(Glu)}$ are insensitive to BDNF

Striatal GABA synapses are modulated by many receptor subtypes inhibiting transmitter release. Thus, we investigated whether the effect of BDNF on presynaptic control of GABA transmission was specific for $CB_1Rs_{(GABA)}$ or also involved other receptors. Application of the $GABA_B$ receptor agonist baclofen ($n = 7$) significantly ($p < 0.01$) reduced striatal sIPSC frequency in control slices (pre-baclofen sIPSC frequency in control slices, 1.43 ± 0.45 Hz) (Calabresi et al., 1991; Rossi et al., 2008; De Chiara et al., 2010). In BDNF-treated slices, the inhibitory effect of baclofen was similar to that seen in control condition (pre-baclofen sIPSC frequency in BDNF-bathed slices, 1.43 ± 0.37 Hz) ($n = 8$, $p > 0.05$ compared with baclofen in control slices) (Fig. 2*A*).

CB_1Rs also control glutamate transmission in the striatum ($CB_1Rs_{(Glu)}$) by a presynaptic mechanism. According to previous findings (Maccarrone et al., 2008; Rossi et al., 2008; De Chiara et al., 2010), HU210 inhibited glutamate-mediated sEPSC frequency in control slices (pre-HU210 sEPSC frequency in control slices, 2.63 ± 0.25 Hz) ($n = 6$, $p < 0.01$). In BDNF-treated slices, the effect of HU210 on sEPSCs was indistinguishable from that of

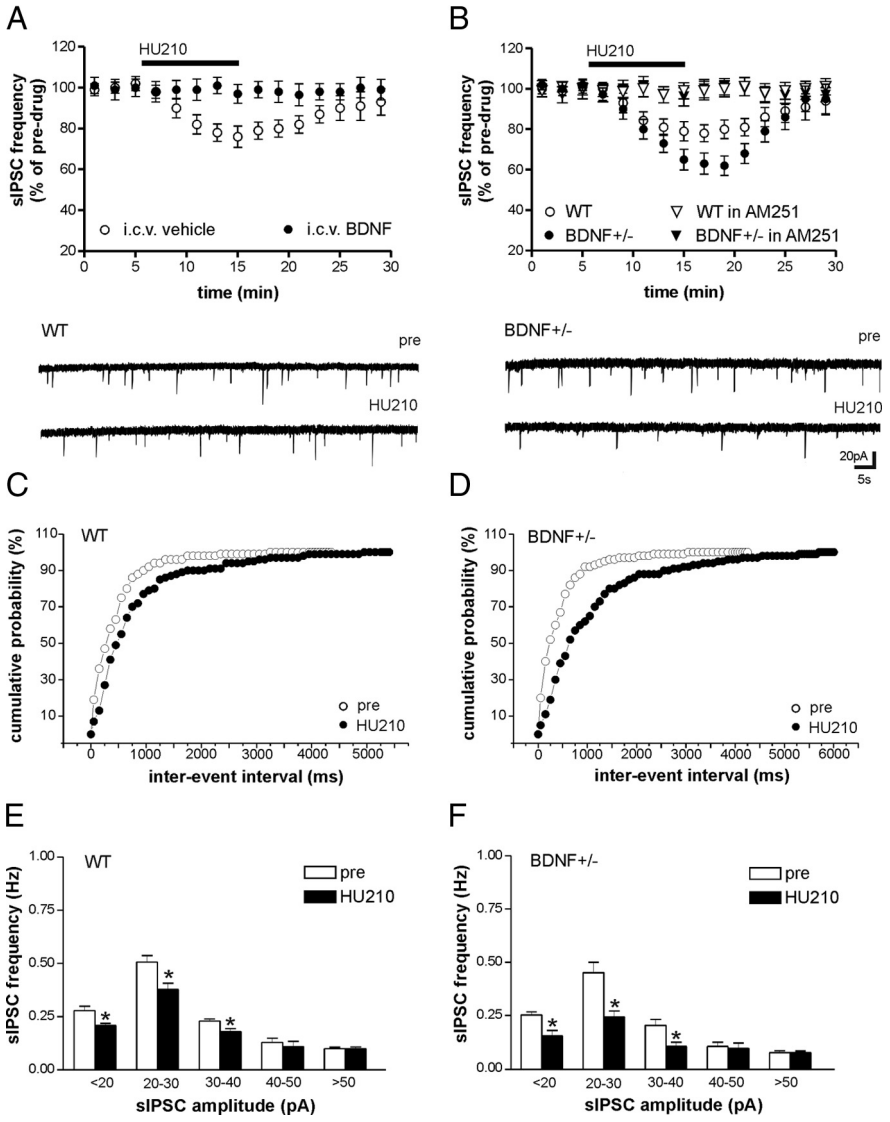

Figure 3. *In vivo* manipulations of BDNF levels alters the sensitivity of $CB_1Rs_{(GABA)}$. ***A***, HU210 was ineffective in reducing sIPSC frequency in mice receiving BDNF via a single intracerebroventricular injection (i.c.v. vehicle, $n = 10$ cells; i.c.v. BDNF, $n = 11$ cells). ***B***, HU210-mediated inhibition of sIPSC frequency was selectively potentiated in striatal neurons from mice with partial genetic BDNF depletion [wild type (WT), $n = 8$ cells; $BDNF^{+/-}$, $n = 9$ cells]. This effect was completely prevented by preincubation with the CB_1 receptor antagonist AM251 ($n = 8$ cells for each group). The electrophysiological traces below are examples of voltage-clamp recordings before and during the application of HU210 in control and $BDNF^{+/-}$ mice. ***C***, ***D***, Cumulative distribution of sIPSC interevent interval recorded from wild-type and $BDNF^{+/-}$ mice before and during the application of HU210. The effect of HU210 on cumulative probability distribution was enhanced in $BDNF^{+/-}$ mice, as reveled by Kolmogorov–Smirnov test. ***E***, ***F***, Amplitude–frequency histograms of sIPSCs before and during the application of HU210 in slices from wild-type and $BDNF^{+/-}$ mice. $^*p < 0.05$; $^{**}p < 0.01$.

controls (pre-HU210 sEPSC frequency in BDNF-treated slices, 2.58 ± 0.22 Hz; 10.2 ± 0.90 pA) ($n = 7$, $p > 0.05$ compared with HU210 in control slices), indicating that BDNF selectively modulates CB_1Rs regulating GABA synapses (Fig. 2*B*).

In vivo manipulations of BDNF levels alter the sensitivity of $CB_1Rs_{(GABA)}$

In an additional set of experiments, we wanted to explore whether alterations of BDNF levels in living animals resulted in concomitant changes of $CB_1R_{(GABA)}$ sensitivity in the striatum. BDNF administered via a single intracerebroventricular injection resulted in the complete suppression of HU210 effect on sIPSC frequency ($n = 6$ for both intracerebroventricular BDNF and intracerebroventricular vehicle, $p > 0.05$ for BDNF and $p < 0.01$ for vehicle) (Fig. 3*A*). Both intracerebroventricular BDNF and intracerebroventricular vehicle failed to affect sIPSC frequency and amplitude [intracerebroventricular vehicle: 1.43 ± 0.45 Hz (frequency), 31.00 ± 3.34 pA (amplitude); intracerebroventricular BDNF: 1.44 ± 0.37 Hz (frequency), 32.45 ± 3.78 pA (amplitude)].

We also measured the effects of HU210 on sIPSCs in mice with partial genetic BDNF depletion (Jeanblanc et al., 2006; Saylor and McGinty, 2008). In $BDNF^{+/-}$ mice, sIPSC frequency (1.14 ± 0.25 Hz) and amplitude (32.81 ± 4.10 pA) were similar to those of the respective control group (sIPSC frequency, 1.27 ± 0.32 Hz; sIPSC amplitude, 35.17 ± 3.82 pA) ($n = 6$, $p > 0.05$ for both parameters). Conversely, HU210-mediated inhibition of sIPSC frequency was selectively potentiated in striatal neurons from $BDNF^{+/-}$ mice ($n = 6$, $p < 0.05$ compared with HU210 in control mice). Even in these mice, AM251 prevented the effect of HU210 on sIPSCs, confirming that BDNF plays a crucial role in the control of striatal $CB_1Rs_{(GABA)}$ (Fig. 3*B–F*).

Involvement of cholesterol metabolism in the BDNF-mediated suppression of $CB_1R_{(GABA)}$ sensitivity

Striatal $CB_1Rs_{(GABA)}$ function within lipid rafts, which are subdomains of the plasma membranes that contain high concentrations of cholesterol (Maccarrone et al., 2009). BDNF has been found recently to increase cholesterol content in lipid rafts but not in nonrafts (Suzuki et al., 2007), raising the possibility that altered lipid composition in rafts might mediate the effects of BDNF on $CB_1Rs_{(GABA)}$. Thus, we first investigated whether MCD, which causes a significant depletion of cholesterol in striatal slices (Maccarrone et al., 2009), was able to reverse the effects of BDNF on $CB_1R_{(GABA)}$ sensitivity. As reported previously (Maccarrone et al., 2009), preincubation of striatal slices with MCD did not increase per se the sensitivity of $CB_1Rs_{(GABA)}$ to HU210 (pre-HU210 frequency of sIPSCs in MCD-bathed slices, 1.36 ± 0.34 Hz) ($n = 6$, $p > 0.05$ compared with HU210 in control slices). MCD, however, was able to rescue the effect of HU210 on sIPSC frequency after BDNF administration *in vitro* (pre-HU210 frequency of sIPSCs in slices bathed in BDNF plus MCD, 1.37 ± 0.44 Hz) ($n = 5$ of 7, $p > 0.05$ compared with pre-HU210) or *in vivo* [pre-HU210 frequency of sIPSCs in MCD-bathed slices prepared from intracerebroventricular BDNF-treated mice, 1.41 ± 0.43 Hz; $81 \pm 4\%$ in HU210 (10 min)] ($n = 4$ of 6, $p > 0.05$ compared with pre-HU210).

We also explored the effect of mevastatin, an inhibitor of cholesterol synthesis able to block BDNF-induced cholesterol

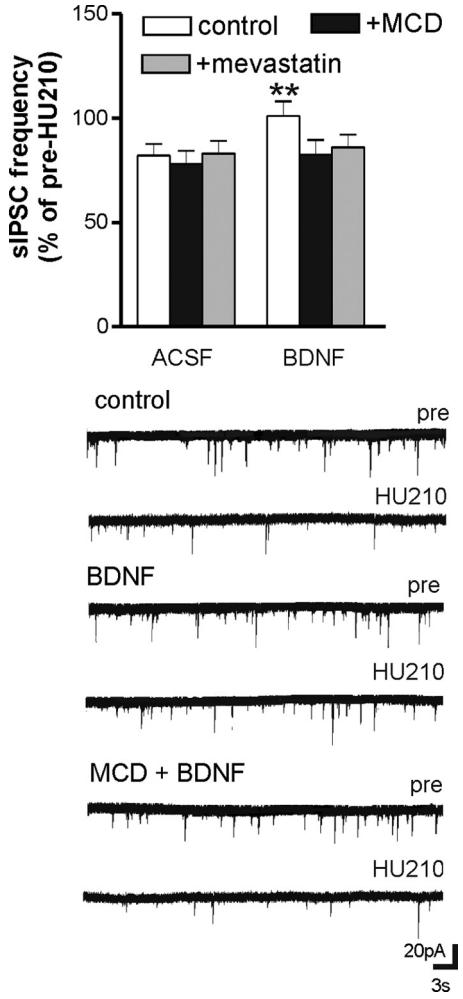

Figure 4. Involvement of cholesterol metabolism in BDNF-mediated suppression of $CB_1R_{(GABA)}$ sensitivity. Reduction of cholesterol contents with MCD (30 min incubation, $n = 10$ cells) or mevastatin (60 min incubation, $n = 9$ cells) did not affect per se HU210 responses on sIPSC frequency. Both treatments were, however, able to rescue the effect of HU210 in BDNF (60 min)-treated slices (BDNF plus MCD, $n = 9$ cells; BDNF plus mevastatin, $n = 9$ cells). The electrophysiological traces are examples of voltage-clamp recordings showing the loss of sIPSC frequency reduction induced by HU210 in a neuron from BDNF-treated slices and the recovery of the effects of HU210 induced by MCD. $^{**}p < 0.01$.

increase in lipid rafts (Suzuki et al., 2007). As with MCD, mevastatin did not affect the sensitivity of $CB_1Rs_{(GABA)}$ to HU210 (pre-HU210 frequency of sIPSCs in mevastatin-bathed slices, 1.37 ± 0.44 Hz) ($n = 5$, $p > 0.05$ compared with HU210 in control slices) but significantly interfered with BDNF-induced suppression of HU210 effects (pre-HU210 frequency of sIPSCs in mevastatin plus BDNF-bathed slices, 1.38 ± 0.47 Hz) ($n = 5$ of 8, $p > 0.05$ compared with pre-HU210) (Fig. 4).

BDNF is able to fully block sensitized $CB_1Rs_{(GABA)}$

The sensitivity of striatal $CB_1Rs_{(GABA)}$ can be enhanced by pharmacological or environmental manipulations activating the brain reward system (Centonze et al., 2007; De Chiara et al., 2010). To see whether BDNF was able to contrast the activity of striatal $CB_1Rs_{(GABA)}$ not only in normal conditions but also after their sensitization, we measured the effects of BDNF in cocaine-treated mice. Cocaine, in fact, has strong rewarding properties and sensitizes striatal $CB_1Rs_{(GABA)}$ (Centonze et al., 2004, 2007). In cocaine-treated mice receiving intracerebroventricular vehicle, HU210 effects on sIPSC frequency were remarkably potentiated (pre-HU210 sIPSC frequency in cocaine-treated mice receiving a single intracerebroventricular injection of vehicle, 1.44 ± 0.39 Hz) ($n = 6$, $p < 0.01$ compared with pre-HU210 and with HU210 effects in control mice), whereas they were fully blocked in mice treated with a single intracerebroventricular injection of BDNF at the end of the cocaine treatment period (pre-HU210 sIPSC frequency in cocaine-treated mice receiving a single intracerebroventricular injection of BDNF, 1.31 ± 0.38 Hz; $n = 7$, $p > 0.05$) (Fig. 5*A*).

We also reared mice in cages equipped with a running wheel for 15 d or with sucrose in their drinking solution for 7 d. Both protocols have been found to cause a dramatic sensitization of $CB_1Rs_{(GABA)}$ but not of $CB_1Rs_{(Glu)}$ to HU210, thus mimicking the effect of cocaine treatment (De Chiara et al., 2010). BDNF, administered intracerebroventricularly at the end of the $CB_1R_{(GABA)}$ sensitizing protocols, fully blocked the HU210-mediated inhibition of sIPSCs (pre-HU210 sIPSC frequency in running mice receiving a single intracerebroventricular injection of BDNF, 1.13 ± 0.22 Hz; pre-HU210 sIPSC frequency in sucrose-drinking mice receiving a single intracerebroventricular injection of BDNF, 1.34 ± 0.39 Hz) ($n = 6$, $p > 0.05$ for both running wheel and sucrose), whereas intracerebroventricular vehicle did not prevent the enhancing effects of both running wheel ($n = 6$) and sucrose drinking ($n = 6$) on HU210-mediated inhibition of sIPSC frequency ($n = 6$) ($p < 0.01$ compared with intracerebroventricular vehicle administered in nonrewarded animals) (Fig. 5*A*).

Haloperidol blocks $CB_1Rs_{(GABA)}$ in rewarded animals

Activation of the DA signaling in the striatum mediates the reinforcing properties of cocaine and natural rewards, including running wheel (El Rawas et al., 2009) and sucrose consumption (Mark et al., 1991; Hajnal et al., 2004). Because pharmacological stimulation of DA D_2 receptors (D_2Rs) has been shown to upregulate the striatal cannabinoid system (Giuffrida et al., 1999; Beltramo et al., 2000; Centonze et al., 2004), we wondered whether the effects of cocaine, running wheel, or sucrose on $CB_1R_{(GABA)}$ function were mediated by endogenous DA acting on D_2Rs. We found that neither cocaine ($n = 5$), running wheel ($n = 5$), nor sucrose administration ($n = 5$) increased the sensitivity of sIPSCs to HU210 in mice treated with the D_2R antagonist haloperidol. Haloperidol, in fact, fully blocked the HU210-mediated inhibition of striatal sIPSC frequency in rewarded mice (pre-HU210 sIPSC frequency in cocaine plus haloperidol-treated mice, 1.14 ± 0.19 Hz; pre-HU210 sIPSC frequency in running mice treated with haloperidol, 1.35 ± 0.36 Hz; pre-HU210 sIPSC frequency in sucrose-drinking mice treated with haloperidol, 1.42 ± 0.41 Hz) ($p > 0.05$ compared with pre-HU210 values for the three experimental groups), whereas intramuscular saline treatment of mice exposed to cocaine ($n = 5$), running wheel ($n = 5$), or sucrose ($n = 5$) caused the expected potentiation of HU210 responses (pre-HU210 sIPSC frequency in cocaine plus vehicle-treated mice, 1.22 ± 0.21 Hz; pre-HU210 sIPSC frequency in running mice treated with vehicle, 1.42 ± 0.41 Hz; pre-HU210 sIPSC frequency in sucrose-drinking mice treated with vehicle, 1.44 ± 0.26 Hz) (Fig. 5*B–D*).

Effects of cocaine and natural rewards on striatal and hippocampal BDNF levels

BDNF has been found to both enhance (McGinty et al., 2010) or inhibit (Berglind et al., 2009) cocaine effects in the brain, and

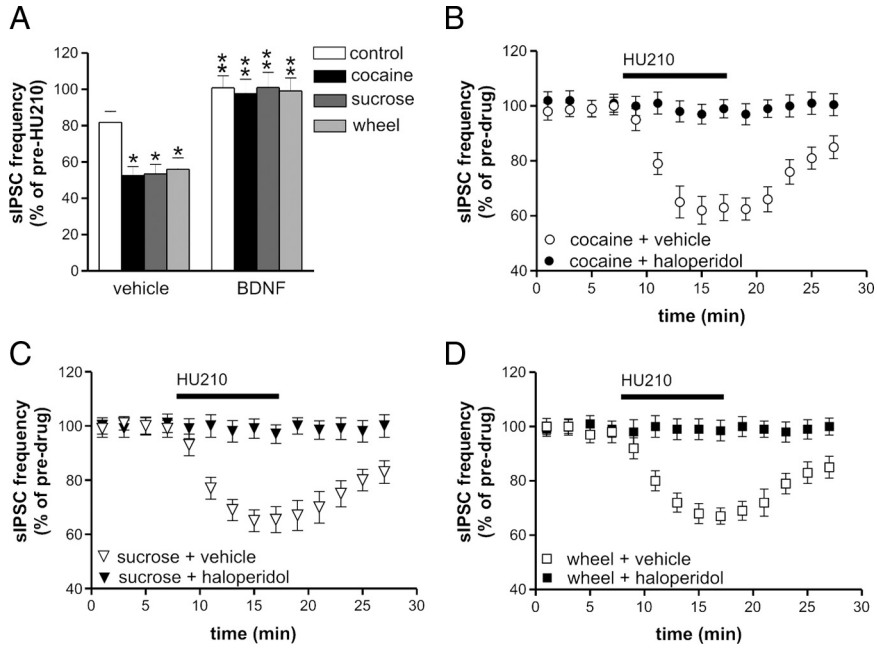

Figure 5. BDNF and haloperidol block $CB_1Rs_{(GABA)}$ in rewarded mice. ***A***, The graph shows that HU210-induced reduction of sIPSC were potentiated after 5 d of cocaine treatment ($n = 11$ cells), 7 d of sucrose exposure ($n = 12$ cells), and after 15 d of exposure to running wheel ($n = 12$ cells). Intracerebroventricular injection of BDNF, at the end of cocaine ($n = 10$ cells), sucrose ($n = 8$ cells), and wheel ($n = 8$ cells) exposure protocols, fully blocked the HU210-mediated inhibition of sIPSC. ***B–D***, The DA D_2R antagonist haloperidol fully blocked the HU210-mediated inhibition of striatal sIPSC frequency in cocaine (5 d)-exposed mice (***B***), in sucrose (7 d)-exposed mice (***C***), and in wheel (15 d)-exposed mice (***D***) ($n = 9$ cells for each group). $^*p < 0.05$; $^{**}p < 0.01$.

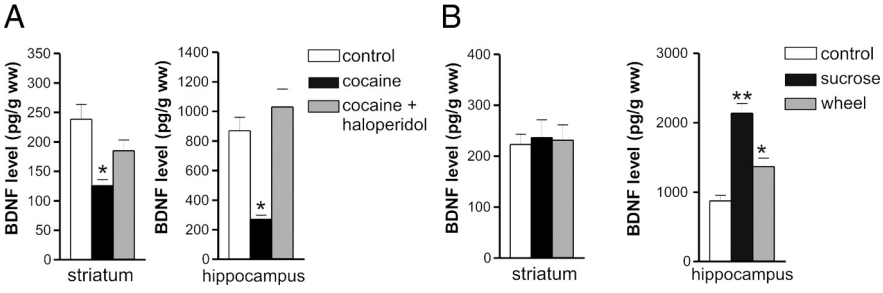

Figure 6. Effects of cocaine and natural rewards on striatal and hippocampal BDNF levels. ***A***, The graph shows BDNF levels in the striatum and the hippocampus in control mice, in cocaine-treated mice, and in cocaine plus haloperidol-treated mice. BDNF levels were reduced in the hippocampus and the striatum of cocaine-treated mice. Treatment with haloperidol blocked cocaine-induced BDNF reduction ($n = 6$ mice for each group and brain area). ***B***, The graph shows BDNF levels in the striatum and the hippocampus in control mice, in sucrose (7 d)-exposed mice, and in wheel (15 d)-exposed mice. BDNF levels were increased in the hippocampus of both groups of rewarded mice, whereas striatal contents of BDNF were similar to control mice ($n = 8$ mice for each group and brain area). $^*p < 005$; $^{**}p < 001$.

cocaine exposure can both increase (Williams and Undieh, 2010) or decrease (Angelucci et al., 2007) BDNF levels. Our neurophysiological data show that BDNF contrasts the effects of cocaine on $CB_1R_{(GABA)}$ sensitivity in the striatum, thus suggesting that DA-mediated $CB_1R_{(GABA)}$ upregulation can be mediated by a negative effect on striatal BDNF levels. We addressed this possibility by measuring BDNF levels in the striatum and the hippocampus of cocaine-treated mice ($n = 6$). Our results showed that cocaine significantly reduced BDNF contents in the striatum and the hippocampus ($p < 0.05$), an effect that was primarily mediated by D_2Rs in both brain areas, because treatment with haloperidol blocked cocaine-induced BDNF reduction ($n = 6$, $p > 0.05$) (Fig. 6*A*).

Voluntary wheel-running exercise and palatable foods have been reported to increase BDNF levels in the hippocampus (Oliff et al., 1998; Johnson et al., 2003; Berchtold et al., 2005; Teegarden et al., 2008). Whether a similar BDNF mobilization also occurs in the striatum in response to natural rewards is less clear, and it would be hardly compatible with the finding that BDNF-sensitive $CB_1R_{(GABA)}$ function is enhanced, and not reduced, by running wheel or sucrose exposure. To address this issue, we measured BDNF levels in the striatum and the hippocampus of control mice ($n = 8$) and mice exposed to running wheel for 15 d ($n = 8$) or to sucrose for 7 d ($n = 8$). Compared with control animals, BDNF levels were increased in the hippocampus of both groups of rewarded mice (running wheel, $p < 0.05$ and sucrose, $p < 0.01$ compared with control mice), whereas striatal contents of BDNF were similar in the three experimental groups ($p > 0.05$) (Fig. 6*B*).

Haloperidol does not affect $CB_1R_{(GABA)}$ function in *BDNF*$^{+/-}$ mice

The above results indicate that BDNF and D_2Rs play opposite roles in the regulation of striatal $CB_1Rs_{(GABA)}$. Here, we tested whether haloperidol was able to interfere with the enhancing effects of partial BDNF gene deletion on $CB_1R_{(GABA)}$ function, as it did in mice receiving treatments with cocaine, running wheel, or sucrose. The effects of HU210 on sIPSC frequency were remarkably similar in *BDNF*$^{+/-}$ mice treated *in vivo* with haloperidol (pre-HU210 sIPSC frequency, 1.38 ± 0.40 Hz; $n = 6$) or with vehicle (pre-HU210 sIPSC frequency, 1.27 ± 0.48 Hz; $n = 6$) ($p < 0.01$ compared with pre-HU210 values; $p > 0.05$ by comparing HU210 effects in the two groups) and were potentiated compared with untreated control animals ($p < 0.05$). These findings indicate that D_2R blockade does not overcome the effects of reduced BDNF signaling on $CB_1R_{(GABA)}$ function (Fig. 7*A*).

Haloperidol increases BDNF levels in the striatum

The lack of effect of haloperidol on HU210 action in *BDNF*$^{+/-}$ mice further suggests that BDNF acts as a downstream effector of the DA signaling in the striatum and that D_2R blockade interferes with $CB_1R_{(GABA)}$ function by enhancing striatal contents of BDNF in control mice but not in mice with depleted contents of the neurotrophin (Jeanblanc et al., 2006; Saylor and McGinty, 2008). We explored this possibility by a direct measurement of BDNF levels in the striatum and hippocampus of mice treated with intraperitoneal haloperidol ($n = 8$) or control vehicle ($n = 8$). We found that BDNF levels were increased in mice treated with haloperidol in both the hippocampus ($p < 0.01$) and the striatum ($p < 0.05$) compared with untreated control animals (Fig. 7*B*).

The same regimen of haloperidol treatment in control mice fully blocked HU210 effects on sIPSC frequency (pre-HU210

sIPSC frequency, 1.21 ± 0.24 Hz; $n = 6$, $p > 0.05$ for both haloperidol and vehicle) (Fig. 7*C*).

Discussion

BDNF exerts its complex effects in the adult brain by modulating synaptic transmission and plasticity. At glutamatergic synapses, BDNF increases transmitter release (Li et al., 1998; Numakawa et al., 2001) and controls AMPA and NMDA receptor phosphorylation, expression, and trafficking (Carvalho et al., 2008; Waterhouse and Xu, 2009). It also regulates GABA synapses through presynaptic and postsynaptic actions (Frerking et al., 1998; Wardle and Poo, 2003; Baldelli et al., 2005). Although the effects of BDNF on transmitter release and receptor function explain its role in the control of the early stages of synaptic plasticity, its effects on mRNA trafficking and translation in dendrites account for its critical role in the late phase of long-term potentiation, which is dependent on local dendritic *de novo* protein synthesis (Carvalho et al., 2008; Waterhouse and Xu, 2009). Of note, the majority of the BDNF synaptic effects described to date are mediated by the stimulation of the receptor tyrosine kinase system TrkB, whereas activation of the other receptor system $p75^{NTR}$ by pro-BDNF has only been associated with NMDA receptor-dependent long-term depression in the hippocampus (Woo et al., 2005).

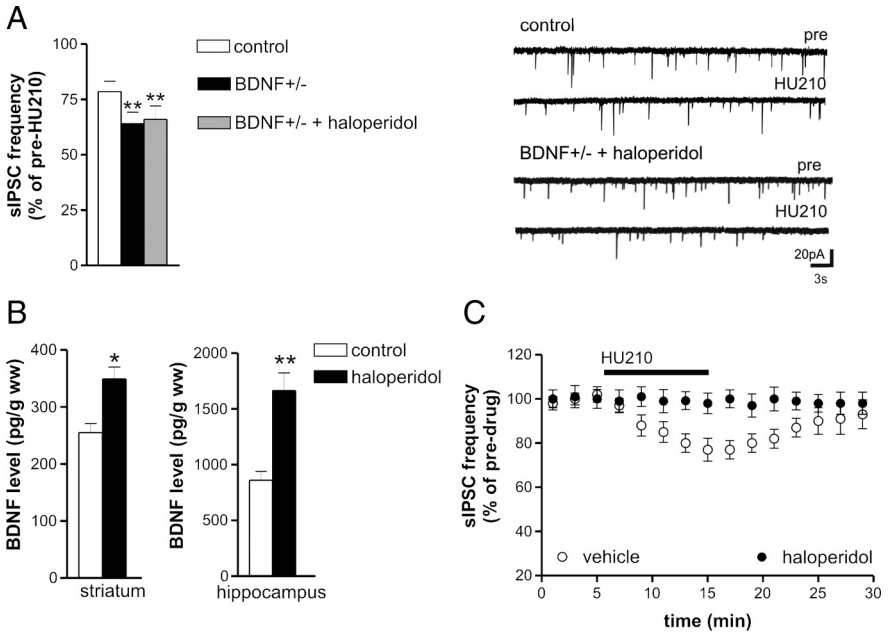

Figure 7. Haloperidol does not block $CB_1Rs_{(GABA)}$ in *BDNF* $^{+/-}$ mice and increases BDNF levels in the striatum. ***A***, Haloperidol failed to block the effects of HU210 on sIPSC frequency in *BDNF* $^{+/-}$ mice ($n = 11$ cells). The electrophysiological traces on the right are examples of voltage-clamp recordings before and during the application of HU210 in control and haloperidol-treated *BDNF* $^{+/-}$ mice. ***B***, BDNF levels were enhanced in both the striatum and the hippocampus of mice treated with intraperitoneal haloperidol compared with mice treated with intraperitoneal control vehicle ($n = 8$ mice for each group and brain area). ***C***, The graph shows that HU210-induced reduction of sIPSC was fully blocked in haloperidol-treated mice (vehicle, $n = 10$ cells; haloperidol, $n = 9$ cells). *$p < 0.05$; **$p < 0.01$.

The results of the present study show that BDNF is a strong regulator of CB_1R function in the striatum, providing evidence for a novel action of this neurotrophin in this brain area. The inhibition of CB_1R activity by BDNF was restricted to GABA synapses, indicating that striatal $CB_1Rs_{(GABA)}$ and $CB_1Rs_{(Glu)}$ have distinct regulatory mechanisms. In this respect, we have demonstrated that BDNF controls striatal $CB_1R_{(GABA)}$ activity mainly by regulating cholesterol metabolism. Several G-protein-coupled receptors (GPCRs), including $CB_1R_{(GABA)}$ (Maccarrone et al., 2009), segregate within lipid rafts, and cholesterol is essential for their function by controlling the fluidity of membranes, thus affecting the freedom of movements of lipid raft-associated proteins, as well as the ability of vesicles to deform and fuse with plasma membranes (Lucero and Robbins, 2004; Hanzal-Bayer and Hancock, 2007; Suzuki et al., 2007). In addition, cholesterol can modulate the activity of GPCRs by promoting receptor dimerization and hence reducing agonist binding (Cherezov et al., 2007; Rosenbaum et al., 2007).

Our data also show that BDNF does not result in a widespread dysregulation of the presynaptic control of striatal GABA synapses, because it did not alter the sensitivity of sIPSCs to the stimulation of other $G\alpha_{i/o}$-coupled receptors, such as $GABA_B$ receptors. Finally, pharmacological inhibition of tyrosine kinase activity prevented the action of BDNF on $CB_1R_{(GABA)}$, confirming the crucial role of TrkB in the synaptic effects of this neurotrophin.

Previous findings have emphasized the involvement of striatal neuron activity in the control of anxiety-related behavior in humans (Reiman et al., 1989; Yoo et al., 2005; Mathew and Ho, 2006) and rodents (Favilla et al., 2008). In addition, it has been shown that BDNF itself may participate in these events (Hashimoto, 2007). Thus, the regulation of striatal CB_1R function might be important for the effect of BDNF on mood control. Accordingly, we have already found that stress-induced anxious-depressive behavior in mice is associated with the selective loss of $CB_1R_{(GABA)}$ function in the striatum and with preserved activity of $GABA_B$ receptors and $CB_1Rs_{(Glu)}$ (Rossi et al., 2008). The BDNF synaptic effects here described mirror these observations and are also in good agreement with the evidence that intrastriatal infusion of BDNF induces a depressive effect (Eisch et al., 2003), whereas inhibition of BDNF–TrkB signaling in this brain area elicits antidepressive actions (Eisch et al., 2003) and contrasts the behavioral consequences of the same stress protocol causing striatal $CB_1R_{(GABA)}$ downregulation in mice (Berton et al., 2006; Rossi et al., 2008).

The important role of extrastriatal BDNF in mood control, and especially the evidence that hippocampal BDNF infusion causes antidepressant-like effects (Siuciak et al., 1997; Shirayama et al., 2002), prevented us from analyzing the emotional consequences of intracerebroventricular BDNF administration, to address the behavioral counterpart of our synaptic findings. Additional lines of evidence, however, support the idea that BDNF-mediated inhibition of $CB_1R_{(GABA)}$ function represent a synaptic correlate of the anxious-depressive behavior induced by the activation of BDNF–TrkB signaling in the striatum (Eisch et al., 2003; Berton et al., 2006).

Enhancement of cAMP signaling in the striatum has been associated with increased anxious-depressive behavior in mice (Favilla et al., 2008; Kim et al., 2008; Zhang et al., 2008) and confirms the relevance of our findings for the pathophysiology of emotional disorders. CB_1Rs, in fact, reduce transmitter release by

inhibiting cAMP levels in presynaptic nerve terminals (Howlett et al., 2004), implying that BDNF-mediated loss of CB_1R sensitivity results in enhanced cAMP signaling in striatal nerve terminals. GABA-mediated inhibition of striatal neuron activity possibly disrupts a circuitry normally limiting fearful or anxiety-related behaviors (Rogan et al., 2005). Furthermore, the effects of *in vivo* manipulations contrasting anxiety in stressed animals (De Chiara et al., 2010) are associated with increased sensitivity of striatal $CB_1Rs_{(GABA)}$ and are fully blocked by treatment with BDNF (present study).

Another finding of the present study is that blockade of D_2Rs with haloperidol inhibits the activity of $CB_1Rs_{(GABA)}$ in control mice and in mice exposed to rewarding experiences but not in mice with reduced brain levels of BDNF. Together, these results highlight the importance of the DA system in the control of cannabinoid signaling in the striatum (Centonze et al., 2004, 2007) and further confirm the crucial role of BDNF in the modulation of $CB_1R_{(GABA)}$ activity and its sensitivity to other regulatory receptor systems.

Complex interactions between DA and the (endo)cannabinoid system, as well as between DA and BDNF, have been described. Stimulation of D_2Rs, in fact, increases endocannabinoid levels (Giuffrida et al., 1999; Beltramo et al., 2000; Centonze et al., 2004) and upregulates the expression of CB_1 receptors in the striatum (Centonze et al., 2004). Furthermore, endocannabinoids act as downstream effectors of D_2R signaling in the inhibition of striatal synaptic transmission (Yin and Lovinger, 2006) and in the generation of corticostriatal long-term depression (Gerdeman et al., 2002, 2003). With respect to the interaction between DA and BDNF, it has been reported that acute administration of levodopa (Okazawa et al., 1992), dopamine, or direct D_1R agonists (Küppers and Beyer, 2001; Williams and Undieh, 2009) upregulates BDNF signaling in the striatum. In contrast, other studies reported that both activation and inhibition of the DA system with, respectively, chronic cocaine (Fumagalli et al., 2007) and 6-OHDA treatment (Zhang et al., 2006) failed to affect striatal levels of BDNF. Furthermore, blockade of D_2Rs with haloperidol treatment for 3 d (Dawson et al., 2001) or 90 d (Pillai et al., 2006) significantly reduced striatal BDNF, whereas intermediate time windows of haloperidol exposure (21 d) caused a partial rebound of striatal BDNF immunoreactivity (Pillai et al., 2006). Together, these data indicate that DA-dependent regulation of striatal BDNF is not unidirectional and that stimulatory effects are evident only in response to acute treatments. Our schedule of haloperidol treatment resulted in a significant enhancement of BDNF levels in the striatum, suggesting that, at least under the present experimental conditions, BDNF might act as a downstream effector of D_2R-mediated enhancement of CB_1R sensitivity. Of note, this model is highly speculative and needs to be validated in future studies (supplemental Fig. 1, available at www.jneurosci.org as supplemental material).

In conclusion, the present study identified a mechanism of striatal CB_1R regulation mediated by BDNF, with potentially relevant implications for important cognitive and behavioral functions.

References

Angelucci F, Ricci V, Pomponi M, Conte G, Mathé AA, Attilio Tonali P, Bria P (2007) Chronic heroin and cocaine abuse is associated with decreased serum concentrations of the nerve growth factor and brain-derived neurotrophic factor. J Psychopharmacol 21:820–825.

Aso E, Ozaita A, Valdizán EM, Ledent C, Pazos A, Maldonado R, Valverde O (2008) BDNF impairment in the hippocampus is related to enhanced despair behavior in CB1 knockout mice. J Neurochem 105:565–572.

Baldelli P, Hernandez-Guijo JM, Carabelli V, Carbone E (2005) Brain-derived neurotrophic factor enhances GABA release probability and nonuniform distribution of N- and P/Q-type channels on release sites of hippocampal inhibitory synapses. J Neurosci 25:3358–3368.

Beltramo M, de Fonseca FR, Navarro M, Calignano A, Gorriti MA, Grammatikopoulos G, Sadile AG, Giuffrida A, Piomelli D (2000) Reversal of dopamine D_2 receptor responses by an anandamide transport inhibitor. J Neurosci 20:3401–3407.

Berchtold NC, Chinn G, Chou M, Kesslak JP, Cotman CW (2005) Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. Neuroscience 133:853–861.

Berglind WJ, Whitfield TW Jr, LaLumiere RT, Kalivas PW, McGinty JF (2009) A single intra-PFC infusion of BDNF prevents cocaine-induced alterations in extracellular glutamate within the nucleus accumbens. J Neurosci 29:3715–3719.

Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ (2006) Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311:864–868.

Butovsky E, Juknat A, Goncharov I, Elbaz J, Eilam R, Zangen A, Vogel Z (2005) In vivo up-regulation of brain-derived neurotrophic factor in specific brain areas by chronic exposure to Delta-tetrahydrocannabinol. J Neurochem 93:802–811.

Calabresi P, Mercuri NB, De Murtas M, Bernardi G (1991) Involvement of GABA systems in feedback regulation of glutamate-and GABA-mediated synaptic potentials in rat neostriatum. J Physiol 440:581–599.

Carvalho AL, Caldeira MV, Santos SD, Duarte CB (2008) Role of the brain-derived neurotrophic factor at glutamatergic synapses. Br J Pharmacol 153 [Suppl 1]:S310–S324.

Castrén E (2004) Neurotrophins as mediators of drug effects on mood, addiction, and neuroprotection. Mol Neurobiol 29:289–302.

Centonze D, Battista N, Rossi S, Mercuri NB, Finazzi-Agrò A, Bernardi G, Calabresi P, Maccarrone M (2004) A critical interaction between dopamine D2 receptors and endocannabinoids mediates the effects of cocaine on striatal GABAergic transmission. Neuropsychopharmacology 29:1488–1497.

Centonze D, Rossi S, De Chiara V, Prosperetti C, Battista N, Bernardi G, Mercuri NB, Usiello A, Maccarrone M (2007) Chronic cocaine sensitizes striatal GABAergic synapses to the stimulation of cannabinoid CB1 receptors. Eur J Neurosci 25:1631–1640.

Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK, Stevens RC (2007) High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science 318:1258–1265.

Dawson NM, Hamid EH, Egan MF, Meredith GE (2001) Changes in the pattern of brain-derived neurotrophic factor immunoreactivity in the rat brain after acute and subchronic haloperidol treatment. Synapse 39:70–81.

De Chiara V, Errico F, Musella A, Rossi S, Mataluni G, Sacchetti L, Siracusano A, Castelli M, Cavasinni F, Bernardi G, Usiello A, Centonze D (2010) Voluntary exercise and sucrose consumption enhance cannabinoid CB1 receptor sensitivity in the striatum. Neuropsychopharmacology 35:374–387.

D'Souza DC, Pittman B, Perry E, Simen A (2009) Preliminary evidence of cannabinoid effects on brain-derived neurotrophic factor (BDNF) levels in humans. Psychopharmacology 202:569–578.

Eisch AJ, Bolaños CA, de Wit J, Simonak RD, Pudiak CM, Barrot M, Verhaagen J, Nestler EJ (2003) Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. Biol Psychiatry 54:994–1005.

El Rawas R, Thiriet N, Lardeux V, Jaber M, Solinas M (2009) Environmental enrichment decreases the rewarding but not the activating effects of heroin. Psychopharmacology 203:561–570.

Favilla C, Abel T, Kelly MP (2008) Chronic Galphas signaling in the striatum increases anxiety-related behaviors independent of developmental effects. J Neurosci 28:13952–13956.

Frerking M, Malenka RC, Nicoll RA (1998) Brain-derived neurotrophic fac-

tor (BDNF) modulates inhibitory, but not excitatory, transmission in the CA1 region of the hippocampus. J Neurophysiol 80:3383–3386.
Fumagalli F, Di Pasquale L, Caffino L, Racagni G, Riva MA (2007) Repeated exposure to cocaine differently modulates BDNF mRNA and protein levels in rat striatum and prefrontal cortex. Eur J Neurosci 26:2756–2763.
Galve-Roperh I, Aguado T, Palazuelos J, Guzmán M (2008) Mechanisms of control of neuron survival by the endocannabinoid system. Curr Pharm Des 14:2279–2288.
Gerdeman GL, Ronesi J, Lovinger DM (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nat Neurosci 5:446–451.
Gerdeman GL, Partridge JG, Lupica CR, Lovinger DM (2003) It could be habit forming: drugs of abuse and striatal synaptic plasticity. Trends Neurosci 26:184–192.
Giuffrida A, Parsons LH, Kerr TM, Rodríguez de Fonseca F, Navarro M, Piomelli D (1999) Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat Neurosci 2:358–363.
Hajnal A, Smith GP, Norgren R (2004) Oral sucrose stimulation increases accumbens dopamine in the rat. Am J Physiol Regul Integr Comp Physiol 286:R31–R37.
Hanzal-Bayer MF, Hancock JF (2007) Lipid rafts and membrane traffic. FEBS Lett 581:2098–2104.
Hashimoto K (2007) BDNF variant linked to anxiety-related behaviors. Bioessays 29:116–119.
Hennigan A, O'Callaghan RM, Kelly AM (2007) Neurotrophins and their receptors: roles in plasticity, neurodegeneration and neuroprotection. Biochem Soc Trans 35:424–427.
Horder J, Cowen PJ, Di Simplicio M, Browning M, Harmer CJ (2009) Acute administration of the cannabinoid CB1 antagonist rimonabant impairs positive affective memory in healthy volunteers. Psychopharmacology 205:85–91.
Howlett AC, Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Porrino LJ (2004) Cannabinoid physiology and pharmacology: 30 years of progress. Neuropharmacology 47 [Suppl 1]:345–358.
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.
Jeanblanc J, He DY, McGough NN, Logrip ML, Phamluong K, Janak PH, Ron D (2006) The dopamine D_3 receptor is part of a homeostatic pathway regulating ethanol consumption. J Neurosci 26:1457–1464.
Johnson RA, Rhodes JS, Jeffrey SL, Garland T Jr, Mitchell GS (2003) Hippocampal brain-derived neurotrophic factor but not neurotrophin-3 increases more in mice selected for increased voluntary wheel running. Neuroscience 121:1–7.
Juhasz G, Chase D, Pegg E, Downey D, Toth ZG, Stones K, Platt H, Mekli K, Payton A, Elliott R, Anderson IM, Deakin JF (2009) CNR1 gene is associated with high neuroticism and low agreeableness and interacts with recent negative life events to predict current depressive symptoms. Neuropsychopharmacology 34:2019–2027.
Khaspekov LG, Brenz Verca MS, Frumkina LE, Hermann H, Marsicano G, Lutz B (2004) Involvement of brain-derived neurotrophic factor in cannabinoid receptor-dependent protection against excitotoxicity. Eur J Neurosci 19:1691–1698.
Kim KS, Lee KW, Baek IS, Lim CM, Krishnan V, Lee JK, Nestler EJ, Han PL (2008) Adenylyl cyclase-5 activity in the nucleus accumbens regulates anxiety-related behavior. J Neurochem 107:105–115.
Küppers E, Beyer C (2001) Dopamine regulates brain-derived neurotrophic factor (BDNF) expression in cultured embryonic mouse striatal cells. Neuroreport 12:1175–1179.
Li YX, Xu Y, Ju D, Lester HA, Davidson N, Schuman EM (1998) Expression of a dominant negative TrkB receptor, T1, reveals a requirement for presynaptic signaling in BDNF-induced synaptic potentiation in cultured hippocampal neurons. Proc Natl Acad Sci U S A 95:10884–10889.
Lu B, Pang PT, Woo NH (2005) The yin and yang of neurotrophin action. Nat Rev Neurosci 6:603–614.
Lucero HA, Robbins PW (2004) Lipid rafts-protein association and the regulation of protein activity. Arch Biochem Biophys 426:208–224.
Maccarrone M, Rossi S, Bari M, De Chiara V, Fezza F, Musella A, Gasperi V, Prosperetti C, Bernardi G, Finazzi-Agrò A, Cravatt BF, Centonze D (2008) Anandamide inhibits metabolism and physiological actions of 2-arachidonoylglycerol in the striatum. Nat Neurosci 11:152–159.
Maccarrone M, De Chiara V, Gasperi V, Viscomi MT, Rossi S, Oddi S, Molinari M, Musella A, Finazzi-Agrò A, Centonze D (2009) Lipid rafts regulate 2-arachidonoylglycerol metabolism and physiological activity in the striatum. J Neurochem 109:371–381.
Maison P, Walker DJ, Walsh FS, Williams G, Doherty P (2009) BDNF regulates neuronal sensitivity to endocannabinoids. Neurosci Lett 467:90–94.
Mark GP, Blander DS, Hoebel BG (1991) A conditioned stimulus decreases extracellular dopamine in the nucleus accumbens after the development of a learned taste aversion. Brain Res 551:308–310.
Martínez-Orgado J, Fernández-López D, Lizasoain I, Romero J (2007) The seek of neuroprotection: introducing cannabinoids. Recent Pat CNS Drug Discov 2:131–139.
Martinowich K, Manji H, Lu B (2007) New insights into BDNF function in depression and anxiety. Nat Neurosci 10:1089–1093.
Mathew SJ, Ho S (2006) Etiology and neurobiology of social anxiety disorder. J Clin Psychiatry 67 [Suppl 12]:9–13.
McGinty JF, Whitfield TW Jr, Berglind WJ (2010) Brain-derived neurotrophic factor and cocaine addiction. Brain Res 1314:183–193.
Moreira FA, Grieb M, Lutz B (2009) Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. Best Pract Res Clin Endocrinol Metab 23:133–144.
Numakawa T, Matsumoto T, Adachi N, Yokomaku D, Kojima M, Takei N, Hatanaka H (2001) Brain-derived neurotrophic factor triggers a rapid glutamate release through increase of intracellular Ca^{2+} and Na^{+} in cultured cerebellar neurons. J Neurosci Res 66:96–108.
Okazawa H, Murata M, Watanabe M, Kamei M, Kanazawa I (1992) Dopaminergic stimulation up-regulates the in vivo expression of brain-derived neurotrophic factor (BDNF) in the striatum. FEBS Lett 313:138–142.
Oliff HS, Berchtold NC, Isackson P, Cotman CW (1998) Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus. Brain Res Mol Brain Res 61:147–153.
Pillai A, Terry AV Jr, Mahadik SP (2006) Differential effects of long-term treatment with typical and atypical antipsychotics on NGF and BDNF levels in rat striatum and hippocampus. Schizophr Res 82:95–106.
Puighermanal E, Marsicano G, Busquets-Garcia A, Lutz B, Maldonado R, Ozaita A (2009) Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. Nat Neurosci 12:1152–1158.
Reiman EM, Raichle ME, Robins E, Mintun MA, Fusselman MJ, Fox PT, Price JL, Hackman KA (1989) Neuroanatomical correlates of a lactate-induced anxiety attack. Arch Gen Psychiatry 46:493–500.
Rogan MT, Leon KS, Perez DL, Kandel ER (2005) Distinct neural signatures for safety and danger in the amygdala and striatum of the mouse. Neuron 46:309–320.
Rosenbaum DM, Cherezov V, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Yao XJ, Weis WI, Stevens RC, Kobilka BK (2007) GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. Science 318:1266–1273.
Rossi S, De Chiara V, Musella A, Kusayanagi H, Mataluni G, Bernardi G, Usiello A, Centonze D (2008) Chronic psychoemotional stress impairs cannabinoid-receptor-mediated control of GABA transmission in the striatum. J Neurosci 28:7284–7292.
Saylor AJ, McGinty JF (2008) Amphetamine-induced locomotion and gene expression are altered in BDNF heterozygous mice. Genes Brain Behav 7:906–914.
Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioural models of depression. J Neurosci 22:3251–3261.
Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM (1997) Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacol Biochem Behav 56:131–137.
Suzuki S, Kiyosue K, Hazama S, Ogura A, Kashihara M, Hara T, Koshimizu H, Kojima M (2007) Brain-derived neurotrophic factor regulates cholesterol metabolism for synapse development. J Neurosci 27:6417–6427.
Teegarden SL, Nestler EJ, Bale TL (2008) Delta FosB-mediated alterations in dopamine signaling are normalized by a palatable high-fat diet. Biol Psychiatry 64:941–950.
Wardle RA, Poo MM (2003) Brain-derived neurotrophic factor modulation of GABAergic synapses by postsynaptic regulation of chloride transport. J Neurosci 23:8722–8732.

Waterhouse EG, Xu B (2009) New insights into the role of brain-derived neurotrophic factor in synaptic plasticity. Mol Cell Neurosci 42:81–89.

Williams SN, Undieh AS (2009) Dopamine D1-like receptor activation induces brain-derived neurotrophic factor protein expression. Neuroreport 20:606–610.

Williams SN, Undieh AS (2010) Brain-derived neurotrophic factor signaling modulates cocaine induction of reward-associated ultrasonic vocalization in rats. J Pharmacol Exp Ther 332:463–468.

Woo NH, Teng HK, Siao CJ, Chiaruttini C, Pang PT, Milner TA, Hempstead BL, Lu B (2005) Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. Nat Neurosci 8:1069–1077.

Yin HH, Lovinger DM (2006) Frequency-specific and D2 receptor-mediated inhibition of glutamate release by retrograde endocannabinoid signaling. Proc Natl Acad Sci U S A 103:8251–8256.

Yoo HK, Kim MJ, Kim SJ, Sung YH, Sim ME, Lee YS, Song SY, Kee BS, Lyoo IK (2005) Putaminal gray matter volume decrease in panic disorder: an optimized voxel-based morphometry study. Eur J Neurosci 22:2089–2094.

Zhang HT, Huang Y, Masood A, Stolinski LR, Li Y, Zhang L, Dlaboga D, Jin SL, Conti M, O'Donnell JM (2008) Anxiogenic-like behavioral phenotype of mice deficient in phosphodiesterase 4B PDE4B. Neuropsychopharmacology 33:1611–1623.

Zhang X, Andren PE, Svenningsson P (2006) Repeated l-DOPA treatment increases c-fos and BDNF mRNAs in the subthalamic nucleus in the 6-OHDA rat model of Parkinson's disease. Brain Res 1095:207–210.