Neuropsychopharmacology (2014) 39, 2646–2653
© 2014 American College of Neuropsychopharmacology. All rights reserved 0893-133X/14
www.neuropsychopharmacology.org

Loss of BDNF Signaling in D1R-Expressing NAc Neurons Enhances Morphine Reward by Reducing GABA Inhibition

Ja Wook Koo[1,4], Mary Kay Lobo[2,4], Dipesh Chaudhury[1,3], Benoit Labonté[1], Allyson Friedman[1,3], Elizabeth Heller[1], Catherine Jensen Peña[1], Ming-Hu Han[1,3] and Eric J Nestler*[,1]

[1]Fishberg Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [2]Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD, USA; [3]Departments of Psychiatry and of Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, New York, NY, USA

The nucleus accumbens (NAc) has a central role in the mechanism of action of drugs of abuse. The major neuronal type within the NAc is the GABAergic medium spiny neuron (MSN), with two major subpopulations defined—termed D1-type and D2-type MSNs—based on the predominant dopamine receptor expressed. However, very little is known about the contribution of altered GABAergic function in NAc MSNs to the neural and behavioral plasticity that contributes to the lasting actions of drugs of abuse. In the present study, we show that GABAergic activity is selectively modulated in D1-type MSNs of the NAc by signaling of brain-derived neurotrophic factor (BDNF) and its receptor, tyrosine receptor kinase B (TrkB), and that such adaptations control rewarding responses to morphine. Optical activation of D1-type MSNs, or the knockout of TrkB from D1-type MSNs (D1-TrkB KO), enhances morphine reward, effects not seen for D2-type MSNs. In addition, D1-TrkB KO mice, but not D2-TrkB KO mice, display decreased $GABA_A$ receptor ($GABA_AR$) subunit expression and reduced spontaneous inhibitory postsynaptic currents (sIPSCs) in D1-type, but not D2-type, MSNs in the NAc. Furthermore, we found that $GABA_AR$ antagonism in the NAc enhances morphine reward and that morphine exposure decreases TrkB expression as well as GABAergic activity in D1-type MSNs. Together, these data provide evidence for the enhancement of morphine reward through reduction of inhibitory $GABA_AR$ responses, an adaptation mediated by morphine-induced reduction of BDNF-TrkB signaling in D1-type MSNs.
Neuropsychopharmacology (2014) **39,** 2646–2653; doi:10.1038/npp.2014.118; published online 18 June 2014

INTRODUCTION

Increasing evidence supports the view that persistent alterations in gene expression and excitatory synaptic plasticity in nucleus accumbens (NAc), a main component of the mesolimbic dopamine reward circuit, have an important role in mediating aspects of drug addiction (Kauer and Malenka, 2007; Nestler, 2001). The major neuronal type in the NAc is the GABAergic medium spiny neuron (MSN), which comprises ~95% of all neurons in this brain region (Gerfen, 1988; Graybiel, 2005; Shi and Rayport, 1994). As the activity of these GABAergic MSNs is regulated not only by dopaminergic and glutamatergic inputs (Gerfen and Surmeier, 2011) but also by local GABAergic interneurons and collateral projections between neighboring MSNs (Stuber *et al*, 2012), it is likely that altered GABAergic function in the NAc has an important role in the neural and behavioral plasticity induced by drugs of abuse (Xi and Stein, 2002).

Brain-derived neurotrophic factor-tyrosine receptor kinase B (BDNF-TrkB) signaling in NAc is also important in the actions of drugs of abuse (Graham *et al*, 2007; Graham *et al*, 2009; Horger *et al*, 1999; Pickens *et al*, 2011; Russo *et al*, 2009). Interestingly, BDNF-TrkB signaling modulates the function of $GABA_A$ receptors ($GABA_ARs$) in other brain regions. For example, it increases cell-surface $GABA_AR$ expression in the hippocampus (Jovanovic *et al*, 2004) and promotes construction of inhibitory GABAergic networks in the hippocampus and cerebellum (Huang *et al*, 1999; Rico *et al*, 2002). In addition, BDNF elevates cellular GABA content in cultured striatal neurons and in the striatum of neonatal rats (Mizuno *et al*, 1994). These observations raise the possibility that altered GABA function in NAc might link BDNF-TrkB signaling to its potent effects on behavioral phenotypes associated with drug addiction.

Recent work demonstrates that cell type-specific manipulations of BDNF-TrkB signaling, and downstream effects on neuronal activity, in the two subpopulations of NAc MSNs, those expressing dopamine D1 (*Drd1a*) *vs* D2 (*Drd2*) receptors, produce opposite behavioral responses to cocaine (Lobo *et al*, 2010). Although a growing body of evidence demonstrates some shared and some distinct molecular, cellular, and circuit mechanisms between psychostimulant

*Correspondence: Dr EJ Nestler, Fishberg Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, PO Box 1065, New York, NY 10029, USA, Tel: +1 212 659 5656, Fax: +1 212 659 8510, E-mail: Eric.Nestler@mssm.edu
[4]These authors contributed equally to this work.
Received 3 March 2014; revised 3 May 2014; accepted 5 May 2014; accepted article preview online 23 May 2014

and opiate action (Albertson *et al*, 2006; Badiani *et al*, 2011; Chang *et al*, 1998; Koo *et al*, 2012; Nestler, 2005), the contribution of D1-type *vs* D2-type NAc MSNs to morphine reward remains unknown.

In the present study, we show that cell type-specific optogenetic stimulation of D1-type *vs* D2-type NAc MSNs produces opposite effects on morphine reward, as shown previously for cocaine. In addition, selective deletion of TrkB from D1-type MSNs enhances morphine reward, whereas TrkB deletion from D2-type MSNs is without effect. D1-TrkB KO (knockout of TrkB from D1-type MSNs) mice also display decreased $GABA_AR$ subunit expression and reduced spontaneous inhibitory postsynaptic currents (sIPSCs) in D1-type MSNs. In contrast, morphine reward and sIPSCs are not significantly altered in D2-TrkB KO mice. We demonstrate further that chronic morphine exposure decreases mRNA expression of TrkB and $GABA_AR$ subunits as well as sIPSCs in D1-type MSNs. Together, our findings support a scheme whereby a reduction in inhibitory $GABA_AR$ responses, mediated by morphine-induced reduction in BDNF-TrkB signaling in D1-type MSNs, enhances morphine reward.

MATERIALS AND METHODS

Mice

Drd1a (D1)-Cre (line FK150) and *Drd2* (D2)-Cre (line ER44) bacterial artificial chromosome (BAC) transgenic mice on a C57BL/6J background were obtained from N. Heintz and P. Greengard (Rockefeller), C. Gerfen (NIMH), and NINDS/GENSAT (www.gensat.org) (Gong *et al*, 2007; Gong *et al*, 2003). D1-tdTomato (TMT) mice (line 5) on a C57BL/6J background were obtained from N. Calakos (Duke; Shuen *et al*, 2008). D1-TrkB KO and D2-TrkB KO mice on a C57BL/6J background were generated by crossing D1-Cre or D2-Cre mice to floxed TrkB (flTrkB) mice (Luikart *et al*, 2003). Two- to three-month-old male mice were used in all experiments. Mice were fed *ad libitum* and maintained at 22 ~ 25 °C on a 12-h light/dark cycle. All experiments were performed in accordance with the Animal Care and Use Committee of the Icahn School of Medicine at Mount Sinai.

Stereotaxic Surgery, Virus and Drug Infusion, and Optogenetic Stimulation

Thirty-three gauge needles were used to unilaterally infuse 0.5 μl of adeno-associated virus (AAV) vectors (ie, DIO (double *loxP*-flanked inverted)-AAV-ChR2 (channel rhodopsin)-EYFP (enhanced yellow fluorescent protein) or DIO-AAV-EYFP) into the NAc of the right hemisphere (AP +1.5; ML +1.5; DV −4.4 from bregma; 10° angle) at a rate of 0.1 μl/min. Three weeks later, 20-gauge guide cannulae (4 mm length) were implanted into the right hemisphere (AP +1.5; ML +1.3; DV −3.9; 0° angle). Mice were given 1-week recovery before conditioned place preference (CPP) experiments. For *in vivo* optical stimulation, a modified 200-μm core optic fiber (Thor labs, with ~50 μm of the stripped optic fiber exposed below the cannula) were attached through an FC/PC adaptor to a 473-nm blue laser diode (BCL-473-050-M, Crystal Lasers), and light pulses were generated through a stimulator (33220A, Agilent; Koo *et al*, 2012; Lobo *et al*, 2010). Ten Hertz blue light pulses (100 ms square width) were delivered for four 3-min periods (one period consists of 3 min on and 5 min off) during the 30-min conditioning. Optic fiber light intensity ranged from 15.9 to 27.8 mW/mm^2 at the tip and similarly through experimental groups. For bicuculline (Tocris) infusions, bilateral guide cannulae (26-gauge, 5 mm length) were implanted above the NAc (AP +1.5; ML +0.75; DV −3.9; 0° angle). Bicuculline (5 ng per 0.5 μl/side) was delivered through an injector needle at a rate of 0.1 μl/min. After the 5-min infusion, injector needles remained in place for 4 ~ 5 min until each morphine CPP training session started.

Morphine CPP

An unbiased CPP paradigm was used as previously described (Koo *et al*, 2012; Lobo *et al*, 2010). Mice were evaluated for morphine or morphine/blue light CPP in a three-chamber CPP box using the Med Associates software. The box consisted of a smaller middle chamber (12 (*W*) × 15 (*L*) × 33 (*H*) cm) and two conditioning larger chambers (24.5 (*W*) × 15 (*L*) × 33 (*H*) cm) with different contextual clues including a gray verses stripe chamber, different floor mesh, and different lighting. On day 1, mice were placed in the CPP box for 20 min to ensure no chamber bias. For standard morphine CPP, mice were conditioned to one chamber for 45 min in the morning (saline) and to the opposite chamber in the afternoon (15 mg/kg morphine, sc or ip) over 3 days. The dose was chosen based on a dose–response analysis of morphine CPP in a previous study (Koo *et al*, 2012). On day 5, the CPP test day, mice were allowed to freely explore all three chambers for 20 min. For morphine/light CPP, optic fibers were secured to the cannula before saline or morphine injections. Mice were conditioned to saline/no light and morphine/blue light for 30 min over 2 days. We used a subthreshold morphine dose (5 mg/kg) where our hypothesis was to test for an enhancement of CPP, and higher doses (10 mg/kg) where our hypothesis was to test for an attenuation of CPP. For all experiments, CPP scores represent time spent in the paired − time spent in the unpaired chamber.

Immunohistochemistry

Immunoreactivity of EYFP and Cre was detected as described previously (Koo *et al*, 2012). Thirty-five-micrometer free-floating brain sections were incubated in 1 : 2000 of rabbit polyclonal anti-GFP (A11122, Invitrogen) and/or 1 : 1000 mouse anti-Cre recombinase (MAB3120, Millipore) in block solution overnight at 4 °C. The next day, sections were incubated in 1 : 500 of donkey anti-rabbit Cy2 (Immuno Research) and 1 : 500 of donkey anti-mouse Cy3 in PBS for 1 h. All sections were mounted with antifade solution and subsequently imaged on a LSM 710 confocal microscope.

RNA Extraction and qRT-PCR

For RNA isolation, 14-gauge bilateral NAc punches were processed according to the published protocol (Koo *et al*,

Table 1 Primers for Real-Time PCR

Target gene	Primer sequence (5′—3′)
Gapdh	
F	AACTTTGGCATTGTGGAAGG
R	ACACATTGGGGGTAGGAACA
Gabra1	
F	GCAGATTGGATATTGGGAAGCA
R	GGTCCAGGCCCAAAGATAGTC
Gabra2	
F	CCAGGACTGGGAGACAGTATT
R	CATTGTCATGTTATGGGCCACT
Gabra3	
F	CACTAGAATCTTGGATCGGCTTT
R	CTTTCATCATGCCATGTCTGTCT
Gabra4	
F	ACAATGAGACTCACCATAAGTGC
R	GGCCTTTGGTCCAGGTGTAG
Gabra5	
F	TGACCCAAACCCTCCTTGTCT
R	GTGATGTTGTCATTGGTCTCGT
Gabrb1	
F	TCTATGGACTACGGATCACAACC
R	ATTGACCCCAGTTACTGCTCC
Gabrb2	
F	AAACCGTATGATTCGATTGC
R	ACGATGGAGAACTGAGGAAGC
Gabrb3	
F	AAGACAGCCAAGGCCAAGAA
R	GCCTGCAACCTCATTCATTTC
Gabrg1	
F	ACTCAAGAAAATCGGATGCACA
R	ATGAAGTTGAAGGTAGCACTCTG
Gabrg2	
F	AGAAAAACCCTCTTCTTCGGATG
R	GTGGCATTGTTCATTTGAATGGT
Gabrg3	
F	TAAAACAGCGGAGGCTCACT
R	GCTGGCACTCTGCATTGATA
Gabrd	
F	CTACAGCCTGATGGGGTGAT
R	GTACTTGGCGAGGTCCATGT
Pdyn	
F	AGGTTGCTTTGGAAGAAGGCT
R	GACGCTGGTAAGGAGTCGG
Rn18s	
F	AGGGGAGAGCGGGTAAGAGA
R	GGACAGGACTAGGGGAACA
TrkB	
F	TTGTGTGGCAGAAAACCTTG
R	ACAGTGAATGGAATGCACCA

2012). Primers were designed to amplify regions of 100–250 base pairs located within the gene (Table 1). SYBR Green qRT-PCR was run in triplicate and analyzed using the $\Delta\Delta C_t$ method as previously described (Livak and Schmittgen, 2001) with GAPDH as a normalization control. GAPDH mRNA expression was not altered among the groups in all experiments.

Fluorescence Activated Cell Sorting (FACS)

Twelve-gauge bilateral NAc punches from TMT mice were dissociated to single cells using papain (Worthington). A pure population of D1-tdTomato+ MSNs was obtained via FACS according to a previously published protocol with minimal modifications (Lobo *et al*, 2006). RNA was extracted using a PicoPure RNA Isolation Kit (Life Technologies) including a DNase I digestion according to the manufacturer's protocol. RNA quality and relative concentration were confirmed on the Picochip using Agilent 2100 Bioanalyzer. qRT-PCR was run as described above with 18S rRNA as a normalization control. 18s rRNA mRNA expression was not altered among the groups.

Electrophysiological Recordings

Whole-cell voltage-clamp recordings were obtained from EYFP+ MSNs in the NAc shell of acute brain slices from D1-Cre, D2-Cre, D1-Cre-flTrkB, and D2-Cre-flTrkB mice that had been stereotaxically injected into the NAc with DIO-AAV-EYFP, or from tdTomato+ MSNs in the NAc shell of acute brain slices from TMT mice as previously described (Lobo *et al*, 2010). Briefly, brain slices were maintained in the holding chamber for 1 h at 37 °C. sIPSCs or sEPSCs were recorded in voltage clamp mode (Bessel filtered at 4 kHz) either in the presence of the AMPA/NMDA antagonist (kynurenic acid; 2 mM) or $GABA_AR$ antagonist (picrotoxin; 100 μM), respectively, at 34 °C. Cells were held at −70 mV. Voltage clamp recordings were performed using the amplifier Multiclamp 700B and data acquisition was realized with pClamp 10. Series resistance was monitored during experiments. Responses were obtained from two to six neurons from each animal.

Statistical Analysis

Student's *t*-tests were used for the analysis of experiments with two experimental groups. One-way ANOVAs were used for the analysis of three or more groups, followed by Fisher's PLSD *post hoc* tests, when appropriate. For CPP, electrophysiology, and qRT-PCR data using D1/D2-Cre and/or D1/D2-Cre-flTrkB mice, two-way ANOVAs were used followed by Fisher's PLSD *post hoc* tests, as appropriate. Main and interaction effects were considered significant at $p < 0.05$. All data are expressed as mean ± SEM.

RESULTS

To examine the effect of cell type-specific optical stimulation of NAc neurons on the rewarding effects of morphine, we injected DIO-AAV-ChR2-EYFP into the NAc of D1-Cre or D2-Cre BAC transgenic mice, in which Cre recombinase expression is driven by the D1 or D2 promoter and their regulatory elements (Gong *et al*, 2007) (Figure 1a–c). The activation of D1-type MSNs by use of established stimulating protocols (Chandra *et al*, 2013; Lobo *et al*, 2010) enhanced CPP to a subthreshold dose of morphine (5 mg/kg), with opposite effects induced by activation of D2-type MSNs with a higher morphine dose (10 mg/kg; Figure 1d). The ability of light activation of D1-type MSNs to increase morphine CPP was non-additive with the effect of morphine

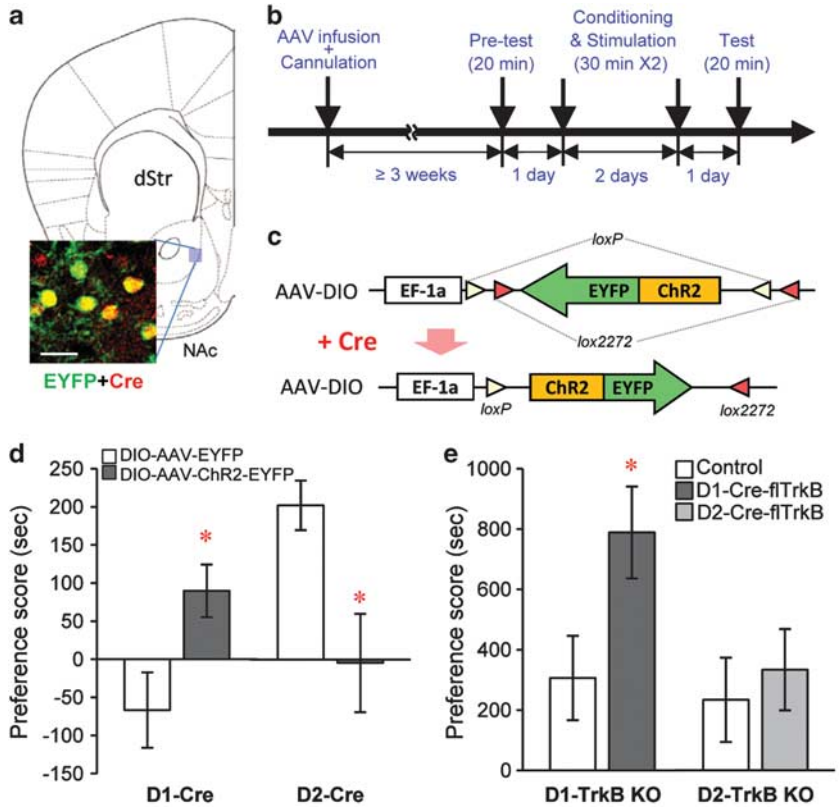

Figure 1 Cell type-specific activation, or selective deletion of tyrosine receptor kinase B (TrkB), from D1- or D2-type medium spiny neurons (MSNs) alters morphine conditioned place preference (CPP). (a) Schematic coronal section from nucleus accumbens (NAc) with an inset depicting localized DIO (double *loxP*-flanked inverted)-AAV (adeno-associated virus)-mediated ChR2-EYFP (channel rhodopsin-enhanced yellow fluorescent protein; green) and Cre (red) in NAc. Scale bar, 20 μm. (b) Schematic diagram depicting the experimental procedures for optical stimulation of D1- or D2-type MSNs in morphine CPP. (c) DIO-AAV-ChR2-EYFP carries an inverted version of ChR2 fused to the fluorescent marker EYFP. In the presence of Cre, ChR2-EYFP is inverted into the sense direction and expressed from the EF-1α (elongation factor 1α) promoter. (d) Activation of D1-type MSNs in D1-Cre DIO-AAV-ChR2-EYFP mice enhances morphine reward compared with D1-Cre DIO-AAV-EYFP controls (5 mg/kg, subcutaneous (sc)). In contrast, activating D2-type MSNs in D2-Cre DIO-AAV-ChR2-EYFP mice decreases morphine reward compared with D2-Cre DIO-AAV-EYFP controls (10 mg/kg, sc; two-way ANOVA, stimulation × cell-type effect: $F_{1,24} = 11.09$, $p < 0.01$; stimulation effect: $F_{1,24} = 0.2051$, $p =$ n.s.; cell-type effect: $F_{1,24} = 2.551$, $p =$ n.s.; $n = 5$–9). *$p < 0.05$, compared with each control group in the Fisher's PLSD *post hoc* test. (e) Loss of TrkB from D1-type MSNs in D1-TrkB KO (knockout of TrkB from D1-type MSNs) mice enhances morphine reward (15 mg/kg, sc), whereas the loss of TrkB from D2-type MSNs in D2-TrkB KO mice has no effect (15 mg/kg, sc; two-way ANOVA, genotype × cell-type effect: $F_{1,29} = 1.896$, $p =$ n.s.; genotype effect: $F_{1,29} = 4.386$, $p < 0.05$; cell-type effect: $F_{1,29} = 3.608$, $p = 0.068$; $n = 7$–10).

itself: such light activation did not enhance morphine CPP to maximal morphine doses (15 mg/kg; data not shown). The opposite effects of optogenetic stimulation of D1-type *vs* D2-type MSNs on morphine reward are similar to those seen for cocaine reward (Lobo *et al*, 2010). We then assessed the functional role of BDNF-TrkB signaling in D1- and D2-type MSNs in morphine reward, using D1-Cre- or D2-Cre-flTrkB mice, where TrkB is selectively deleted from each MSN subtype (Lobo *et al*, 2010). Selective deletion of TrkB from D1-type MSNs (D1-TrkB KO) in NAc and dorsal striatum increased morphine CPP, whereas D2-TrkB KO mice showed no effect (Figure 1e).

To gain insight into a possible functional relationship between deletion of BDNF-TrkB signaling and optogenetic activation in the MSN subtypes, we measured mRNA expression levels of $GABA_AR$ subunits in NAc of D1- and D2-TrkB KO mice. D1-TrkB KO reduces mRNA levels of several $GABA_AR$ subunits including *Gabra4, Gabra5, Gabrb1, Gabrg1,* and *Gabrg3,* but the *Gabra2* subunit was increased (Figure 2a). In contrast, we found a reduction only in *Gabra4*, with an increase in *Gabra2*, *Gabrg2*, and *Gabrg3*, in the NAc of D2-TrkB KO mice (Figure 2a). To determine whether altered $GABA_AR$ subunit expression reflects functional changes in MSNs, we investigated the sIPSCs and the sEPSCs of these MSN subtypes in NAc shell after the cell type-specific deletion of TrkB. Consistent with $GABA_AR$ expression patterns, there was a significant reduction in the amplitude of sIPSCs of D1-TrkB KO mice, but not in D2-TrkB KO mice, compared with control mice (Figure 2b and c). No alterations in the frequency of sIPSCs were found either in D1-TrkB KO or in D2-TrkB KO mice (Figure 2b and d). There were also no alterations in the amplitude or frequency of sEPSCs either in D1-TrkB KO or in D2-TrkB KO mice (Figure 2e and f).

We next investigated the effects of chronic exposure to morphine on $GABA_AR$ subunit expression in whole NAc extracts. We found that repeated intraperitoneal (ip) injections of morphine (20 mg/kg, 7 days) decrease the mRNA expression of several $GABA_AR$ subunits—*Gabra2* and *Gabrd*—in the NAc of C57BL/6J wild-type (WT) mice

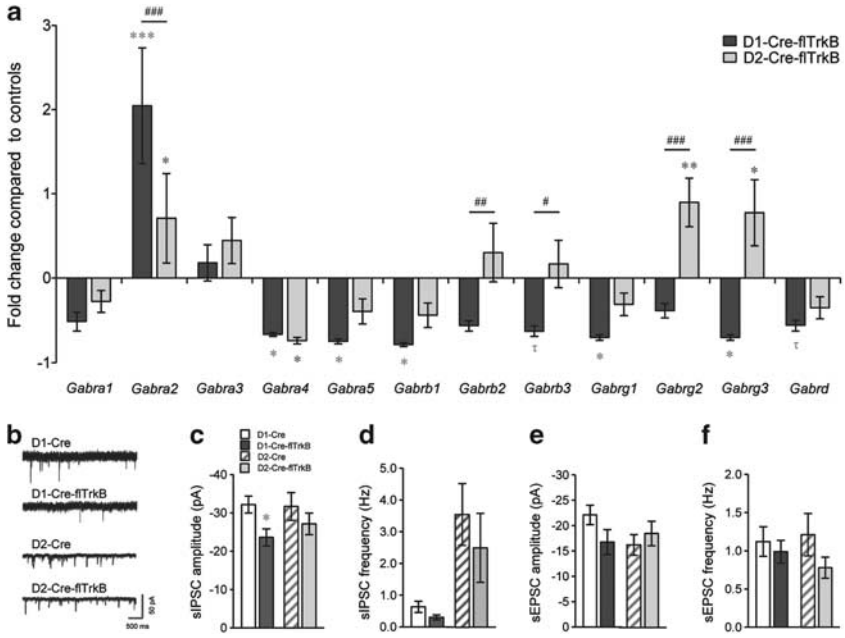

Figure 2 Effects of cell type-specific tyrosine receptor kinase B (TrkB) deletion on $GABA_A$ receptor ($GABA_AR$) subunit gene expression and spontaneous inhibitory postsynaptic currents (sIPSCs) in nucleus accumbens (NAc). (a) Selective deletion of tyrosine receptor kinase B (TrkB) from D1- or D2-type medium spiny neurons (MSNs) alters mRNA expression of $GABA_AR$ subunits. Notably, knockout of TrkB from D1-type MSNs (D1-TrkB KO) reduces mRNA expression of many $GABA_AR$ subunits including *Gabra4, Gabra5, Gabrb1, Gabrg1,* and *Gabrg3* (two-way ANOVA, genotype × cell-type effect: $F_{23,329} = 4.318$, $p < 0.001$; genotype effect: $F_{1,329} = 4.030$, $p < 0.05$; cell-type effect: $F_{23,329} = 4.318$, $p < 0.001$, $n = 6$–9). $^{\tau}p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with each control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared with D1-Cre-flTrkB (floxed TrkB) mice in the Fisher's PLSD *post hoc* test. (b) Raw sIPSC traces recorded from D1- and D2-type MSNs of D1-/D2-TrkB KO and D1-/D2-Cre control mice that were injected with DIO-AAV-EYFP (double *loxP*-flanked inverted-adeno-associated virus-enhanced yellow fluorescent protein) into the NAc for visualization of D1- and D2-type MSNs. (c) D1-TrkB KOs exhibit decreased amplitude of sIPSCs compared with control mice. In contrast, there was no change in the amplitude of sIPSCs in D2-TrkB KO mice (two-way ANOVA, genotype × cell-type effect: $F_{1,28} = 0.4384$, $p =$ n.s.; genotype effect: $F_{1,28} = 4.661$, $p < 0.05$; cell-type effect: $F_{1,28} = 0.2496$, $p =$ n.s.; $n = 4$–13 animals per group). $^{*}p < 0.05$, compared with each control group in the Fisher's PLSD *post hoc* test. (d) There was a significant main effect of cell type, but not a TrkB KO effect on the frequency of sIPSCs (genotype × cell-type effect: $F_{1,28} = 0.6903$, $p =$ n.s.; genotype effect: $F_{1,28} = 2.561$, $p =$ n.s.; cell-type effect: $F_{1,28} = 35.13$, $p < 0.001$). (e and f) There were no changes in the amplitude (e, genotype × cell-type effect: $F_{1,21} = 2.637$, $p =$ n.s.; genotype effect: $F_{1,21} = 0.4618$, $p =$ n.s.; cell-type effect: $F_{1,21} = 1.203$, $p =$ n.s., $n = 5$–8 animals per group) or in the frequency (f, genotype × cell-type effect: $F_{1,21} = 0.5902$, $p =$ n.s.; genotype effect: $F_{1,21} = 2.113$, $p =$ n.s.; cell-type effect: $F_{1,21} = 0.1181$, $p =$ n.s.) of sEPSCs in D1- or D2-TrkB KO mice.

(Figure 3a). We then examined the role of $GABA_A$ signaling in NAc in morphine reward by bilaterally infusing bicuculline (5 ng/side), a competitive $GABA_AR$ antagonist, into NAc before each training session of morphine CPP with a maximal dose of morphine (15 mg/kg). Figure 3b shows that the inhibition of $GABA_A$ signaling selectively in NAc dramatically enhances morphine reward.

Finally, we studied the effects of chronic morphine on inhibitory $GABA_A$ signaling in D1-type MSNs selectively, given the direct connection between the loss of BDNF-TrkB signaling and reduction of inhibitory $GABA_A$ signaling in D1-type MSNs with respect to morphine reward. D1-type MSNs were isolated and purified using FACS from NAc of TMT mice (Figure 3c). The FACS-purified D1-type TMT + cells display enrichment of prodynorphin (*Pdyn*, a marker of D1-type neurons in NAc), compared with TMT-controls (Figure 3d). Consistent with $GABA_AR$ expression patterns in whole NAc extracts from WT mice, chronic morphine reduces mRNA expression levels of several $GABA_AR$ subunits including *Gabra2, Gabra3, Gabra4, Gabra5,* and *Gabrb1* in purified D1-type MSNs (Figure 3e). *Gabra1, Gabrb2,* and *Gabrg2* were not detected in purified D1-type MSNs. In addition, chronic morphine robustly reduced full-length *TrkB* mRNA expression in the D1-type MSNs (Figure 3f). Consistent with $GABA_AR$ expression patterns in D1-type MSNs, the amplitude, but not the frequency, of sIPSCs in D1-type MSNs was significantly reduced by chronic morphine (Figure 3g–i).

DISCUSSION

It has been hypothesized that opioid receptor-mediated inhibition of NAc GABAergic MSNs is critical for opiate reward (Xi and Stein, 2002). Consistent with this hypothesis, self- or local administration of morphine or heroin into the NAc significantly suppresses the spontaneous firing of NAc MSNs (Hakan and Henriksen, 1989; Lee *et al*, 1999). In the present study, we find that chronic morphine administration, or its resulting suppression of BDNF-TrkB signaling in D1-type MSNs, decreases expression of several $GABA_AR$ subunits in this neuronal cell type of the NAc and that the concomitant reduction of inhibitory GABAergic tone in NAc D1-type MSNs promotes morphine reward.

Our observation that chronic morphine suppresses $GABA_AR$ subunit expression in NAc is opposite to prior observations with psychostimulants. For example, we showed recently that chronic cocaine increases the expression of

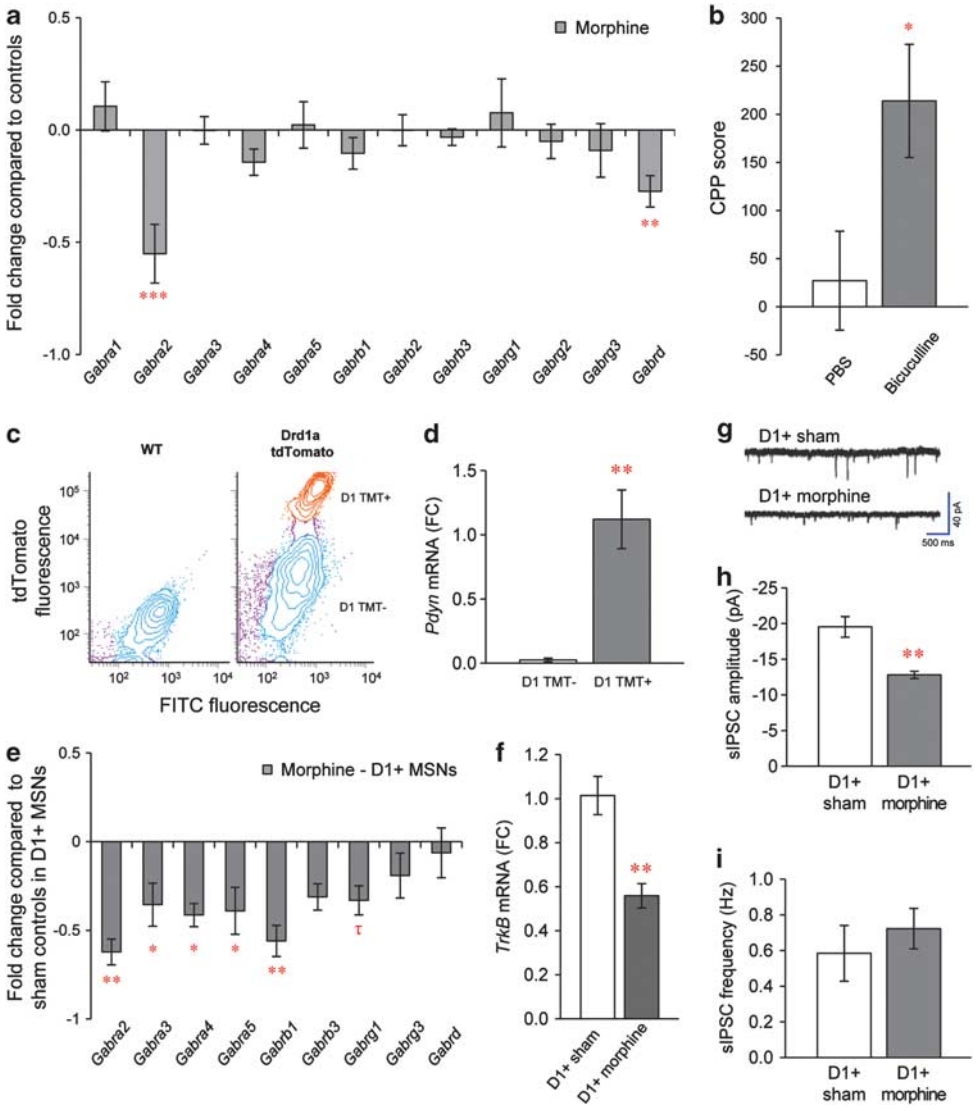

Figure 3 Effects of chronic morphine on $GABA_A$ receptor ($GABA_AR$) subunit gene expression and spontaneous inhibitory postsynaptic currents (sIPSCs) in D1-type medium spiny neurons (MSNs). (a) Chronic morphine (20 mg/kg, ip, 7 days) downregulates mRNA expression of certain $GABA_AR$ subunits in whole nucleus accumbens (NAc) extracts, such as *Gabra2* and *Gabrd* (one-way ANOVA, $F_{23,228} = 3.037$, $p < 0.001$, $n = 8$–12). $^{**}p < 0.01$, $^{***}p < 0.001$, compared with each control in the Fisher's PLSD *post hoc* test. (b) Intra-NAc infusion of bicuculline (10 ng), a competitive $GABA_AR$ antagonist, dramatically blocks morphine reward (15 mg/kg, ip). Student *t*-test, $^{*}p < 0.05$, $n = 9$. (c) Typical scatterplots for fluorescence activated cell sorting (FACS) from wild-type (WT) and D1-tdTomato mice. The data from WT mice were used to gate the tdTomato (TMT+) signals. (d) Purified D1-type TMT+ cells display enrichment of prodynorphin (*Pdyn*) mRNA compared with TMT-negative controls (TMT−). *t*-test, $^{**}p < 0.01$, $n = 4$–5. (e) Chronic morphine (implanted sc with one pellet, 2 days) reduces mRNA expression of several $GABA_AR$ subunits including *Gabra2, Gabra3, Gabra4, Gabra5,* and *Gabrb1* in purified D1-type MSNs, compared with controls (implanted sc with sham pellet; one-way ANOVA, $F_{17,68} = 2.713$, $p < 0.01$, $n = 4$–5). $^{\tau}p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.01$, compared with each control in the Fisher's PLSD *post hoc* test. (f) Chronic morphine also robustly reduces *TrkB* (tyrosine receptor kinase B) mRNA expression in purified D1-type MSNs. *t*-test, $^{**}p < 0.01$, $n = 4$–5. (g) Raw sIPSC traces recorded from TMT+ D1-type MSNs in drug naive *vs* morphine-treated mice. (h and i) Chronic morphine significantly decreases amplitude (h), but not frequency (i), of sIPSCs in D1-type MSNs. *t*-test, $^{**}p < 0.01$, $n = 6$ animals per group.

several $GABA_AR$ subunits, including *Gabra1*, *Gabra2,* and *Gabrb2* in NAc and that $GABA_AR$ antagonism blocks behavioral plasticity to cocaine (Kennedy *et al*, 2013), findings consistent with earlier studies (Dixon *et al*, 2010; Heiman *et al*, 2008). In contrast, we show here that chronic morphine decreases levels of several $GABA_AR$ subunits, including *Gabra2* and *Gabrd*, in whole NAc and that $GABA_AR$ antagonism in this region robustly enhances morphine reward. Such morphine-induced downregulation of $GABA_AR$ subunits is even more apparent when D1-type MSNs are examined selectively (see below). Interestingly, mRNA expression levels of *Gabra2*, which encodes the $GABA_AR$-α2 subunit and is implicated in reward learning and behavioral sensitization as well as human cocaine addiction (Dixon *et al*, 2010), is regulated oppositely in the NAc by morphine *vs* cocaine. A previous study showed that $GABA_AR$-α2 subunit is not directly associated with cocaine reward as evidenced by no alterations in cocaine CPP and conditioned reinforcement in *Gabra2*-deficient mice (Dixon *et al*, 2010). Furthermore, we observed that D1-TrkB KO

enhances *Gabra2* levels, as opposed to reduced expression of many other $GABA_AR$ subunits and to the consequent reduction of sIPSCs, suggesting that the *Gabra2* induction by reduced TrkB in morphine-treated D1-type MSNs might cancel out the effect of *Gabra2* reduction by morphine. Rather, *Gabra4*, another $GABA_AR$ subunit of high abundance in NAc (Schwarzer *et al*, 2001; Wisden *et al*, 1992) that is decreased by morphine in NAc, particularly in D1-type MSNs, as well as by D1-TrkB KO or by itself in the present study, appears to be critical for morphine reward. A very recent report shows that *Gabra4* deletion in D1-type MSNs increases cocaine CPP, but the deletion in D2-type MSNs does not (Maguire *et al*, 2014). These data suggest a $GABA_AR$ subunit-specific and cell type-specific role of $GABA_AR$ subunits for drug reward effects.

A role for BDNF-TrkB signaling in NAc in enhancing the behavioral effects of cocaine is well established (Graham *et al*, 2007; Graham *et al*, 2009; Horger *et al*, 1999). By contrast, less is known about the influence of BDNF-TrkB signaling in NAc on morphine action. Intra-NAc infusion of BDNF or genetic deletion of TrkB from all NAc neurons has no effect on morphine reward (Koo *et al*, 2012). This is consistent with results reported here because TrkB signaling predominates in D2-type MSNs (Lobo *et al*, 2010) and we show enhancement of morphine reward upon TrkB KO from D1-type MSNs, with no effect observed upon TrkB KO from D2-type MSNs. We also showed recently that chronic morphine administration decreases BDNF expression in the VTA, which would be expected to decrease BDNF signaling from the VTA to the NAc (Koo *et al*, 2012). Such reduced signaling, in turn, based on results reported here, would be expected to reduce GABAergic activity in D1-type MSNs only, with a resulting increase in morphine reward. This is in parallel with our recent demonstration that D1-type NAc neurons mediate morphine reward: D1 receptor antagonism, but not D2 receptor antagonism, in NAc blocks the facilitation of morphine reward induced by optogenetically activating dopaminergic inputs to NAc (Koo *et al*, 2012).

In addition, our FACS and electrophysiological studies show that chronic morphine reduces TrkB expression and GABAergic activity selectively in D1-type MSNs in NAc. These findings support the hypothesis that chronic exposure to morphine directly decreases BDNF-TrkB signaling in D1-type MSNs and that the morphine-induced suppression of GABAergic activity in D1-type MSNs occurs through this morphine-induced reduction of BDNF-TrkB signaling, as D1-TrkB KO by itself (ie, without morphine) reduces $GABA_A$ signaling in this NAc neuronal subtype. Nonetheless, it is possible that reduced BDNF signaling in D1-type MSNs of the NAc might enhance the activity of these neurons and morphine reward through $GABA_AR$-independent mechanisms. Likewise, future studies are needed to understand the complex circuit mechanisms by which reduced activity of D1-type MSNs promote morphine reward.

Together, our data indicate that enhanced morphine reward in D1-TrkB KOs is attributed at least in part to the suppression of GABAergic inhibition, and that this accounts for the similar behavioral responses observed upon optogenetic activation of D1-type MSNs. The effect of morphine-induced suppression of BDNF-TrkB signaling in D1-type MSNs on morphine reward suggests a double contrast between morphine and cocaine action, as cocaine-induced increases in BDNF-TrkB signaling in D2-type MSNs promotes cocaine reward (Lobo *et al*, 2010). Whereas chronic exposure to psychostimulants or opiates is known to produce many common molecular and cellular actions in NAc MSNs (Nestler, 2005), there are also clear differences as evidenced by the current findings on BDNF-TrkB signaling. Elucidation of the complicated BDNF-TrkB signaling mechanisms underlying alterations in drug reward by the two main subtypes of NAc MSNs in response to psychostimulants *vs* opiates will help identify and characterize new therapeutic targets for drug addiction.

FUNDING AND DISCLOSURE

This study was supported by grants from the National Institute on Drug Abuse (E.J.N.). The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Ezekiell Mouzon for excellent technical assistance.

REFERENCES

Albertson DN, Schmidt CJ, Kapatos G, Bannon MJ (2006). Distinctive profiles of gene expression in the human nucleus accumbens associated with cocaine and heroin abuse. *Neuropsychopharmacology* **31**: 2304–2312.

Badiani A, Belin D, Epstein D, Calu D, Shaham Y (2011). Opiate versus psychostimulant addiction: the differences do matter. *Nat Rev Neurosci* **12**: 685–700.

Chandra R, Lenz JD, Gancarz AM, Chaudhury D, Schroeder GL, Han MH *et al* (2013). Optogenetic inhibition of D1R containing nucleus accumbens neurons alters cocaine-mediated regulation of Tiam1. *Front Mol Neurosci* **6**: 13.

Chang JY, Janak PH, Woodward DJ (1998). Comparison of mesocorticolimbic neuronal responses during cocaine and heroin self-administration in freely moving rats. *J Neurosci* **18**: 3098–3115.

Dixon CI, Morris HV, Breen G, Desrivieres S, Jugurnauth S, Steiner RC *et al* (2010). Cocaine effects on mouse incentive-learning and human addiction are linked to alpha2 subunit-containing GABAA receptors. *Proc Natl Acad Sci USA* **107**: 2289–2294.

Gerfen CR (1988). Synaptic organization of the striatum. *J Electron Microsc Tech* **10**: 265–281.

Gerfen CR, Surmeier DJ (2011). Modulation of striatal projection systems by dopamine. *Annu Rev Neurosci* **34**: 441–466.

Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N *et al* (2007). Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J Neurosci* **27**: 9817–9823.

Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB *et al* (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* **425**: 917–925.

Graham DL, Edwards S, Bachtell RK, DiLeone RJ, Rios M, Self DW (2007). Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. *Nat Neurosci* **10**: 1029–1037.

Graham DL, Krishnan V, Larson EB, Graham A, Edwards S, Bachtell RK *et al* (2009). Tropomyosin-related kinase B in the mesolimbic dopamine system: region-specific effects on cocaine reward. *Biol Psychiatry* **65**: 696–701.

Graybiel AM (2005). The basal ganglia: learning new tricks and loving it. *Curr Opin Neurobiol* **15**: 638–644.

Hakan RL, Henriksen SJ (1989). Opiate influences on nucleus accumbens neuronal electrophysiology: dopamine and non-dopamine mechanisms. *J Neurosci* **9**: 3538–3546.

Heiman M, Schaefer A, Gong S, Peterson JD, Day M, Ramsey KE *et al* (2008). A translational profiling approach for the molecular characterization of CNS cell types. *Cell* **135**: 738–748.

Horger BA, Iyasere CA, Berhow MT, Messer CJ, Nestler EJ, Taylor JR (1999). Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *J Neurosci* **19**: 4110–4122.

Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF *et al* (1999). BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. *Cell* **98**: 739–755.

Jovanovic JN, Thomas P, Kittler JT, Smart TG, Moss SJ (2004). Brain-derived neurotrophic factor modulates fast synaptic inhibition by regulating GABA(A) receptor phosphorylation, activity, and cell-surface stability. *J Neurosci* **24**: 522–530.

Kauer JA, Malenka RC (2007). Synaptic plasticity and addiction. *Nat Rev Neurosci* **8**: 844–858.

Kennedy PJ, Feng J, Robison AJ, Maze I, Badimon A, Mouzon E *et al* (2013). Class I HDAC inhibition blocks cocaine-induced plasticity by targeted changes in histone methylation. *Nat Neurosci* **16**: 434–440.

Koo JW, Mazei-Robison MS, Chaudhury D, Juarez B, LaPlant Q, Ferguson D *et al* (2012). BDNF is a negative modulator of morphine action. *Science* **338**: 124–128.

Lee RS, Criado JR, Koob GF, Henriksen SJ (1999). Cellular responses of nucleus accumbens neurons to opiate-seeking behavior: I. Sustained responding during heroin self-administration. *Synapse* **33**: 49–58.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**: 402–408.

Lobo MK, Covington HE 3rd, Chaudhury D, Friedman AK, Sun H, Damez-Werno D *et al* (2010). Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science* **330**: 385–390.

Lobo MK, Karsten SL, Gray M, Geschwind DH, Yang XW (2006). FACS-array profiling of striatal projection neuron subtypes in juvenile and adult mouse brains. *Nat Neurosci* **9**: 443–452.

Luikart BW, Nef S, Shipman T, Parada LF (2003). *In vivo* role of truncated trkb receptors during sensory ganglion neurogenesis. *Neuroscience* **117**: 847–858.

Maguire EP, Macpherson T, Swinny JD, Dixon CI, Herd MB, Belelli D *et al* (2014). Tonic inhibition of accumbal spiny neurons by extrasynaptic alpha4betadelta GABAA receptors modulates the actions of psychostimulants. *J Neurosci* **34**: 823–838.

Mizuno K, Carnahan J, Nawa H (1994). Brain-derived neurotrophic factor promotes differentiation of striatal GABAergic neurons. *Dev Biol* **165**: 243–256.

Nestler EJ (2001). Molecular neurobiology of addiction. *Am J Addict* **10**: 201–217.

Nestler EJ (2005). Is there a common molecular pathway for addiction? *Nat Neurosci* **8**: 1445–1449.

Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y (2011). Neurobiology of the incubation of drug craving. *Trends Neurosci* **34**: 411–420.

Rico B, Xu B, Reichardt LF (2002). TrkB receptor signaling is required for establishment of GABAergic synapses in the cerebellum. *Nat Neurosci* **5**: 225–233.

Russo SJ, Mazei-Robison MS, Ables JL, Nestler EJ (2009). Neurotrophic factors and structural plasticity in addiction. *Neuropharmacology* **56**(Suppl 1): 73–82.

Schwarzer C, Berresheim U, Pirker S, Wieselthaler A, Fuchs K, Sieghart W *et al* (2001). Distribution of the major gamma-aminobutyric acid(A) receptor subunits in the basal ganglia and associated limbic brain areas of the adult rat. *J Comp Neurol* **433**: 526–549.

Shi WX, Rayport S (1994). GABA synapses formed *in vitro* by local axon collaterals of nucleus accumbens neurons. *J Neurosci* **14**: 4548–4560.

Shuen JA, Chen M, Gloss B, Calakos N (2008). Drd1a-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. *J Neurosci* **28**: 2681–2685.

Stuber GD, Britt JP, Bonci A (2012). Optogenetic modulation of neural circuits that underlie reward seeking. *Biol Psychiatry* **71**: 1061–1067.

Wisden W, Laurie DJ, Monyer H, Seeburg PH (1992). The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J Neurosci* **12**: 1040–1062.

Xi ZX, Stein EA (2002). GABAergic mechanisms of opiate reinforcement. *Alcohol Alcohol* **37**: 485–494.