Neuropsychopharmacology (2011) 36, 993–1002

www.neuropsychopharmacology.org

In Vivo Ethanol Experience Increases D_2 Autoinhibition in the Ventral Tegmental Area

Simona Perra[1], Michael A Clements[1], Brian E Bernier[1] and Hitoshi Morikawa*[,1]

[1]*Section of Neurobiology, Waggoner Center for Alcohol and Addiction Research, University of Texas at Austin, Austin, TX, USA*

Alcoholism is characterized by compulsive alcohol intake after a history of chronic consumption. A reduction in mesolimbic dopaminergic transmission observed during abstinence may contribute to the negative affective state that drives compulsive intake. Although previous *in vivo* recording studies in rodents have demonstrated profound decreases in the firing activity of ventral tegmental area (VTA) dopamine neurons after withdrawal from long-term ethanol exposure, the cellular mechanisms underlying this reduced activity are not well understood. Somatodendritic dopamine release within the VTA exerts powerful feedback inhibition of dopamine neuron activity via stimulation of D_2 autoreceptors and subsequent activation of G protein-gated inwardly rectifying K^+ (GIRK) channels. Here, by performing patch-clamp recordings from putative dopamine neurons in the VTA of mouse brain slices, we show that D_2 receptor/GIRK-mediated inhibition becomes more potent and exhibits less desensitization after withdrawal from repeated *in vivo* ethanol exposure (2 g/kg, i.p., three times daily for 7 days). In contrast, $GABA_B$ receptor/GIRK-mediated inhibition and its desensitization are not affected. Chelating cytosolic Ca^{2+} with BAPTA augments D_2 inhibition and suppresses its desensitization in control mice, while these effects of BAPTA are occluded in ethanol-treated mice. Furthermore, inositol 1,4,5-trisphosphate (IP_3)-induced intracellular Ca^{2+} release and Ca^{2+}/calmodulin-dependent protein kinase II are selectively involved in the desensitization of D_2, but not $GABA_B$, receptor signaling. Consistent with this, activation of metabotropic glutamate receptors that are coupled to IP_3 generation leads to cross-desensitization of D_2/GIRK-mediated responses. We propose that enhancement of D_2 receptor-mediated autoinhibition via attenuation of a Ca^{2+}-dependent desensitization mechanism may contribute to the hypodopaminergic state during ethanol withdrawal.
Neuropsychopharmacology (2011) **36,** 993–1002; doi:10.1038/npp.2010.237; published online 19 January 2011

Keywords: alcoholism; ethanol; dopamine neuron; calcium; D_2 receptor; desensitization

INTRODUCTION

The mesolimbic dopamine system, which originates in the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc) and other limbic structures, is critically involved in reward processing, behavioral reinforcement, and addictive behaviors. Acute exposure to ethanol, the principal psychoactive constituent of all alcoholic beverages, stimulates mesolimbic dopaminergic transmission (Boileau *et al*, 2003; Imperato and Di Chiara, 1986; Weiss *et al*, 1993), primarily via direct excitation of VTA dopamine neuron firing activity (Brodie *et al*, 1990; Gessa *et al*, 1985). This effect is believed to mediate the rewarding and reinforcing actions of ethanol. In contrast, profound decreases in NAc dopamine release have been observed in animals withdrawn from long-term ethanol exposure (Rossetti *et al*, 1992; Weiss *et al*, 1996) and also in detoxified alcoholics (Martinez *et al*, 2005; Volkow *et al*, 2007). In line with these observations, a marked reduction in VTA dopamine neuron activity has been reported in ethanol-withdrawn animals (Diana *et al*, 1993, 1996). This 'hypodopaminergic state', which can be reversed by administration of ethanol itself (Diana *et al*, 1993; Weiss *et al*, 1996), may, at least partially, contribute to the emotional/motivational component of ethanol withdrawal symptoms, such as dysphoria and anhedonia (Koob and Volkow, 2010; Melis *et al*, 2005; Trevisan *et al*, 1998). However, the cellular mechanisms underlying the hypoactivity of dopamine neurons after ethanol withdrawal remain poorly understood.

It is well known that activation of dopamine D_2 receptors and $GABA_B$ receptors can mediate powerful inhibition of dopamine neurons both *in vitro* (Beckstead *et al*, 2004; Labouebe *et al*, 2007; Lacey *et al*, 1987) and *in vivo* (Erhardt *et al*, 2002; White and Wang, 1984). These receptors are coupled to the activation of G protein-gated inwardly rectifying K^+ (GIRK) channels via $G_{i/o}$ G proteins, resulting

*Correspondence: Dr H Morikawa, Section of Neurobiology, Waggoner Center for Alcohol and Addiction Research, University of Texas at Austin, 2400 Speedway, PAT 402, Austin, TX 78712, USA, Tel: +1 512 232 9299, Fax: +1 512 471 3878,
E-mail: morikawa@mail.utexas.edu
Received 1 November 2010; revised 2 December 2010; accepted 5 December 2010

in membrane hyperpolarization and suppression of neuronal excitability (Luscher and Slesinger, 2010). *In vivo* recording studies have shown that VTA DA neuron firing activity is tonically inhibited by these receptors (Erhardt *et al*, 2002; White and Wang, 1984), raising the possibility that enhancement of D_2 and/or $GABA_B$ inhibition might contribute to the tonic hypoactivity of dopamine neurons observed in ethanol-withdrawn animals.

Previous studies have shown that D_2 autoreceptor-mediated inhibition of VTA dopamine neurons is reduced after repeated psychostimulant exposure (Henry *et al*, 1989; Marinelli *et al*, 2003; Wolf *et al*, 1993), whereas the $GABA_B$ receptor-GIRK channel coupling efficiency is increased after repeated exposure to morphine or the $GABA_B$ agonist γ-hydroxybutyrate (Labouebe *et al*, 2007). In this study, by performing patch-clamp recordings from putative dopamine neurons in the VTA of mouse brain slices, we show that repeated *in vivo* exposure to ethanol induces sensitization of D_2-mediated inhibition without affecting $GABA_B$-mediated inhibition. Consistent with this differential modulation, the D_2 receptor signaling is uniquely regulated by a Ca^{2+}-dependent desensitization mechanism involving inositol 1,4,5-trisphosphate (IP_3)-induced Ca^{2+} release from intracellular stores and subsequent activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). Furthermore, *in vivo* ethanol treatment occludes the enhancement of D_2 inhibition and suppression of its desensitization produced by the Ca^{2+} chelator BAPTA observed in control mice, suggesting that the Ca^{2+}-dependent desensitization machinery may be suppressed by repeated ethanol exposure.

SUBJECTS AND METHODS

Subjects

Male C57BL/6J mice (3–4 weeks old; Jackson Laboratory) were housed under a 12-h light–dark cycle (lights on at 0700 hours). Food and water were available *ad libitum*. All animal procedures were approved by the University of Texas Institutional Animal Care and Use Committee.

In Vivo Ethanol Treatment

Mice received three times daily i.p. injections of saline or ethanol (2 g/kg, 20% v/v in saline) for 7 days. It should be noted that previous studies reporting reduced dopamine neuron firing *in vivo* after ethanol withdrawal used comparable ethanol administration protocol (2–5 g/kg, intragastric, four times daily for 6 days) (Diana *et al*, 1993, 1995). Midbrain slices were prepared 1 day after the final injection. This ethanol treatment protocol produced an increase in the amount of ethanol consumption measured using a 24-h continuous access two-bottle choice paradigm (Supplementary Figure 1).

Slices and Solutions

Mice were killed by cervical dislocation under halothane or isoflurane anesthesia, and horizontal midbrain slices (200–210 μm) were prepared using a vibratome (VT1000S; Leica Microsystems). Slices were routinely cut in ice-cold physiological saline containing (in mM): 126 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 11 glucose, 21.4 $NaHCO_3$, saturated with 95% O_2, and 5% CO_2 (pH 7.4, ~295–300 mOsm/kg). MK-801 was added to this solution to prevent NMDA receptor-mediated excitotoxicity. For experiments performing loose-patch recordings of action potential firing, slices were cut in high-sucrose cutting solution containing (in mM): 205 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 7.5 $MgCl_2$, 0.5 $CaCl_2$, 10 glucose, 25 $NaHCO_3$, saturated with 95% O_2, and 5% CO_2 (~305 mOsm/kg). Slices were incubated at 35 °C for >1 h in the physiological saline described above. Recordings were made at 34–35 °C in the same physiological saline perfused at 2–3 ml/min. Unless noted otherwise, the pipette solution contained (in mM): 115 K-gluconate or K-methylsulfate, 20 KCl, 1.5 $MgCl_2$, 10 HEPES, 0.025 EGTA, 2 Mg-ATP, 0.2 Na_2-GTP, and 10 Na_2-phosphocreatine (pH 7.25, ~280 mOsm/kg).

Electrophysiological Recordings

Cells were visualized using a 40× objective on an upright microscope (BX51WI; Olympus) with infrared/oblique illumination optics. Putative dopamine neurons were identified by low-frequency (<4 Hz) pacemaker firing with broad action potentials (>1.2 ms) in cell-attached configuration before break-in and the presence of large whole-cell I_h currents (>200 pA in response to a 1.5-s voltage step from −62 to −112 mV) after break-in. Recordings were restricted to the lateral part of the VTA within ~150 μm from the medial border of the medial terminal nucleus of the accessory optic tract (MT) in horizontal midbrain slices, where dopamine neurons projecting to the core and lateral shell regions of the NAc are present (Ikemoto, 2007). It should be noted that reduced dopamine release after ethanol withdrawal has been detected in both the core and shell of the NAc (Weiss *et al*, 1996). Although electrophysiological identification of dopamine neurons has been challenged (Margolis *et al*, 2006), the criteria described above have been used in recent studies performing recordings from the lateral part of the VTA in horizontal slices (Ahn *et al*, 2010; Beckstead *et al*, 2009; Ford *et al*, 2006; Riegel and Williams, 2008; Wanat *et al*, 2008; Zhang *et al*, 2010).

Whole-cell voltage-clamp recordings were routinely made at a holding potential of −62 mV, corrected for a liquid junction potential of −7 mV. Pipettes had an open tip resistance of 1.7–2.3 MΩ when filled with the pipette solution. Series resistance after break-in was typically ~5–10 MΩ and was periodically monitored during the recording. Recordings were discarded if the series resistance increased beyond 20 MΩ. A Multiclamp 700A or 700B amplifier (Molecular Devices) was used to record the data, which were filtered at 1 kHz, digitized at 2 kHz, and collected using AxoGraph X.

Loose-patch recordings (~10–20 MΩ seal) of action potential firing were made using glass pipettes filled with 150 mM NaCl (2.0–2.5 MΩ tip resistance). Firing data were filtered at 5 kHz and digitized at 10 kHz. In these recordings, whole-cell break-in was not made afterward to measure I_h currents, and thus low-frequency pacemaker firing and action potential width (>1.2 ms) were the criteria to

identify putative dopamine neurons, in accordance with (Ford *et al*, 2006).

Pressure Ejection of Dopamine

Patch pipettes (~2–3 μm tip diameter), filled with dopamine (100 μM) and ascorbate (1.3 mM), were placed ~100 μm from recorded cells. Pressure of 20 p.s.i. was applied to rapidly eject dopamine. The duration of pressure application (10–150 ms) was adjusted to obtain dopamine-evoked outward currents of ~50 pA in each cell.

Drugs

Quinpirole, (RS)-baclofen, CGP54626, U73122, 2-aminoethoxydiphenylborane (2-APB), cyclopiazonic acid (CPA), and (S)-3,5-dihydroxyphenylglycine (DHPG) were obtained from Tocris Bioscience. Heparin and KN-62 were purchased from Calbiochem. All other chemicals used in electrophysiology experiments were from Sigma-RBI.

Data Analyses

In analyzing quinpirole/baclofen-induced outward currents, holding currents during two ~5-min periods, one immediately before quinpirole/baclofen application and one after reversal of quinpirole/baclofen-induced currents, were fitted to a single straight line, and this line was subtracted from recorded currents to correct for gradual shift in holding current levels frequently observed during whole-cell recordings. Quinpirole/baclofen-induced currents thus obtained were normalized by the membrane capacitance in each cell to estimate current density (expressed in pA/pF). The membrane capacitance was estimated from the fast component of double-exponential fit to the decay of capacitative transients (filtered at 10 kHz and digitized at 20 kHz) evoked by 10-mV hyperpolarizing voltage steps. *In vivo* saline/ethanol treatments did not affect the membrane capacitance thus estimated in cells reported in this study (naïve: 57.5 ± 1.2 pF, $n = 63$; saline-treated: 57.3 ± 1.1 pF, $n = 50$; ethanol-treated: 59.0 ± 1.4 pF, $n = 39$; $F_{2,149} = 0.52$, $p = 0.59$, one-way ANOVA). The difference between the initial peak current amplitude and the current amplitude at the end of continuous agonist application was divided by the peak current amplitude to obtain the magnitude of desensitization (expressed in %).

Data are expressed as mean ± SEM. Statistical significance was determined by Student's *t*-test or ANOVA followed by Bonferroni *post hoc* test. The difference was considered significant at $p < 0.05$.

RESULTS

D_2 Receptor-Mediated Inhibition is Enhanced after Repeated *In Vivo* Ethanol Exposure

To test if *in vivo* ethanol exposure alters D_2 autoreceptor-mediated inhibition, we performed whole-cell voltage-clamp recordings from naïve C57BL/6J mice and from mice that received injections of saline or ethanol (2 g/kg, i.p.) three times daily for 7 days. Recordings were made in midbrain slices prepared 1 day after the final injection. Putative dopamine neurons were identified electrophysiologically (see Subjects and Methods section). Bath application of the D_2 agonist quinpirole (300 nM) produced outward currents that reached peak amplitude in 1–2 min. These currents gradually desensitized during 10 min of continuous quinpirole application and were rapidly reversed by the D_2 antagonist sulpiride (1–2 μM) (Figure 1a). Application of sulpiride by itself elicited no measurable currents (three cells each from saline- and ethanol-treated mice), suggesting the absence of effective dopamine tone in brain slice preparations used in this study. Quinpirole-induced currents exhibited larger peak amplitude and smaller desensitization in ethanol-treated mice compared with naïve or saline-treated mice (peak amplitude: $F_{2,34} = 6.23$, $p < 0.01$; desensitization: $F_{2,22} = 15.7$, $p < 0.0001$; one-way ANOVA) (Figures 1a–c). Furthermore, concentration-response curves for peak currents produced by quinpirole (3 nM–30 μM) revealed an approximately twofold increase in quinpirole sensitivity, with no change in the maximal response, in ethanol-treated mice compared with saline-treated mice (Figure 1d). Therefore, D_2 receptor-GIRK

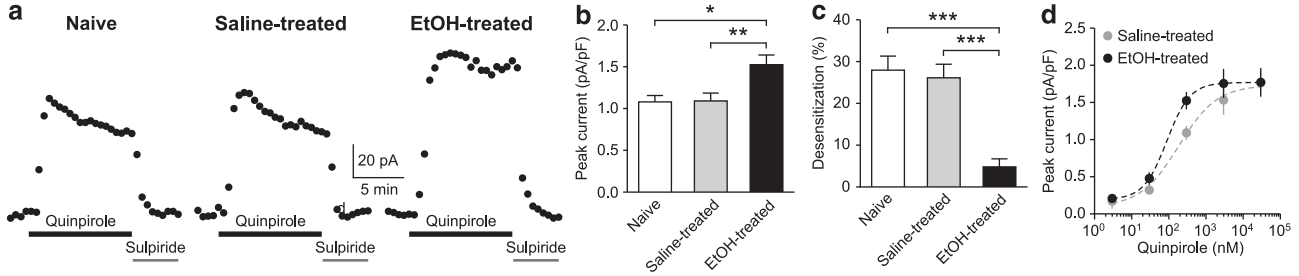

Figure 1 D_2 receptor-mediated outward currents are increased and exhibit less desensitization after *in vivo* ethanol exposure. (a) Examples of quinpirole-induced outward currents ($V_h = -62$ mV) in naïve, saline-treated, and ethanol-treated mice. Quinpirole (300 nM) was bath applied for 10 min. Subsequent application of sulpiride (1 μM) reversed quinpirole-induced currents. (b) Summary bar graph showing the peak amplitude of quinpirole-induced outward currents in naïve ($n = 12$ from 8 mice), saline-treated ($n = 15$ from 12 mice), and ethanol-treated mice ($n = 10$ from 9 mice). (c) Summary bar graph plotting the amount of desensitization of quinpirole-induced currents in naïve ($n = 8$ from 5 mice), saline-treated ($n = 10$ from 8 mice), and ethanol-treated mice ($n = 7$ from 7 mice). (d) Concentration-response curves for quinpirole-induced outward currents in saline- and ethanol-treated mice. Each point represents an average of data from 4 to 15 cells. Averaged data points are fitted to a logistic equation. The estimated EC_{50} values are 100 and 43 nM for saline- and ethanol-treated mice, while the estimated maximal responses are 1.73 and 1.76 pA/pF for saline- and ethanol-treated mice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, Bonferroni *post hoc* test. Error bars indicate SEM.

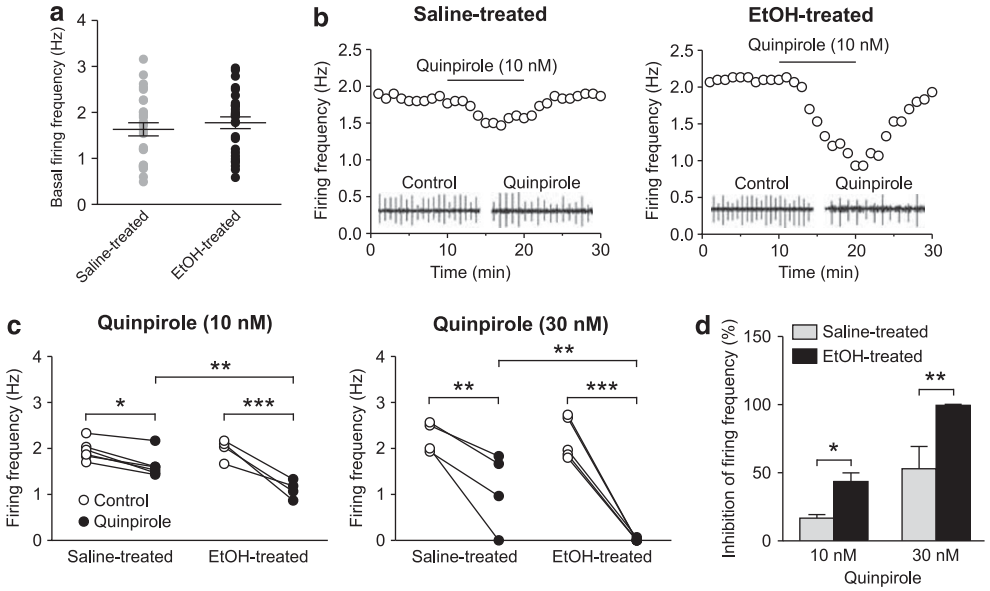

Figure 2 Dopamine neuron firing is more sensitive to D_2 inhibition in ethanol-treated mice. (a) Basal firing frequency in individual cells is plotted from saline- and ethanol-treated mice ($n = 23$ from 11 mice in saline group and $n = 25$ from 14 mice in ethanol group). (b) Representative time graphs illustrating quinpirole-induced inhibition of dopamine neuron firing in saline- and ethanol-treated mice. Quinpirole (10 nM) was applied at the time indicated by the horizontal bar. Each point represents average firing frequency during a 60-s period. Example traces (10 s) of dopamine neuron firing in control and in quinpirole are shown in the inset. (c) Summary graphs plotting the firing frequency in control and in quinpirole for individual cells in saline- and ethanol-treated mice. Left: 6 cells from 5 mice in saline group and 4 cells from 4 mice in ethanol group were tested for 10 nM quinpirole (*in vivo* treatment: $F_{1,8} = 3.30$, $p = 0.11$, quinpirole: $F_{1,8} = 74.3$, $p < 0.0001$, *in vivo* treatment × quinpirole: $F_{1,8} = 15.9$, $p < 0.01$; mixed two-way ANOVA). Right: 4 cells from 3 mice in saline group and 5 cells from 4 mice in ethanol group were tested for 30 nM quinpirole (*in vivo* treatment: $F_{1,7} = 4.48$, $p = 0.07$, quinpirole: $F_{1,7} = 89.9$, $p < 0.0001$, *in vivo* treatment × quinpirole: $F_{1,7} = 9.13$, $p < 0.05$; mixed two-way ANOVA). (d) Summary bar graph demonstrating that quinpirole caused larger firing inhibition in ethanol-treated mice. Data are from the same cells as in c (*in vivo* treatment: $F_{1,15} = 23.5$, $p < 0.001$, quinpirole concentration: $F_{1,15} = 37.3$, $p < 0.0001$, *in vivo* treatment × quinpirole concentration: $F_{1,15} = 1.71$, $p = 0.21$; two-way ANOVA). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Bonferroni *post hoc* test. Error bars indicate SEM.

channel coupling is more potent and also becomes resistant to desensitization after repeated ethanol exposure.

We next examined the effect of quinpirole on the firing activity of VTA dopamine neurons monitored with a loose-patch configuration. The basal firing frequency was not altered by *in vivo* ethanol treatment ($t_{54} = 0.17$, $p = 0.46$; unpaired *t*-test) (Figure 2a). Furthermore, sulpiride (1–2 μM) had no effect on the firing frequency (saline-treated: 1.69 ± 0.43 Hz in control *vs* 1.65 ± 0.43 Hz in sulpiride, $n = 5$, $t_4 = 1.36$, $p = 0.25$; ethanol-treated: 1.75 ± 0.25 Hz in control *vs* 1.72 ± 0.25 Hz in sulpiride, $n = 6$, $t_5 = 1.04$, $p = 0.35$; paired *t*-test), consistent with the lack of effect of sulpiride in whole-cell voltage-clamp recordings described above. However, bath application of low concentrations of quinpirole (10 and 30 nM) caused significantly larger inhibition of firing in ethanol-treated mice (Figures 2b–d). Here, quinpirole was tested only in cells that exhibited firing frequency in the range of 1.5–3 Hz, because the magnitude of D_2/GIRK-mediated inhibition of firing has been shown to depend on baseline firing frequency (Putzier *et al*, 2009; Werkman *et al*, 2001). The firing frequency in the presence of quinpirole was significantly lower in ethanol-treated mice compared with saline-treated controls, although the firing frequency before quinpirole application was comparable in the two groups in these experiments (Figure 2c). A higher concentration of quinpirole (100 nM) invariably abolished dopamine neuron firing in eight cells tested (four cells each from saline- and ethanol-treated mice). These results demonstrate that dopamine neuron firing is more sensitive to D_2 inhibition after repeated ethanol exposure.

In Vivo Ethanol Exposure Does Not Affect $GABA_B$ Receptor-Mediated Inhibition

D_2 receptors and $GABA_B$ receptors most likely share the same downstream signaling pathway (Beckstead *et al*, 2004; Labouebe *et al*, 2007; Luscher and Slesinger, 2010). Thus, we next tested the $GABA_B$ agonist baclofen, which has been shown to produce desensitizing GIRK-mediated currents with an EC_{50} of ~10 μM in VTA dopamine neurons while causing non-desensitizing GIRK-mediated currents in VTA GABA neurons (Cruz *et al*, 2004; Labouebe *et al*, 2007). Bath application of baclofen (10 μM) induced large outward currents associated with desensitization in both saline- and ethanol-treated mice (Figure 3a). Reponses elicited by baclofen (1 and 10 μM) were comparable in saline- and ethanol-treated mice in terms of both peak outward current amplitude (*in vivo* treatment: $F_{1,33} = 0.42$, $p = 0.52$, baclofen concentration: $F_{1,33} = 126$, $p < 0.0001$, *in vivo* treatment × baclofen concentration: $F_{1,33} = 0.47$, $p = 0.50$; two-way ANOVA) and amount of desensitization (*in vivo* treatment: $F_{1,12} = 0.20$, $p = 0.67$, baclofen concentration: $F_{1,12} = 74.0$, $p < 0.0001$, *in vivo* treatment × baclofen concentration: $F_{1,12} = 0.0029$, $p = 0.96$; two-way ANOVA) (Figures 3b and c). Thus, $GABA_B$ receptor-mediated inhibition is not affected by ethanol treatment. This finding suggests that the enhancement of D_2 inhibition after *in vivo* ethanol

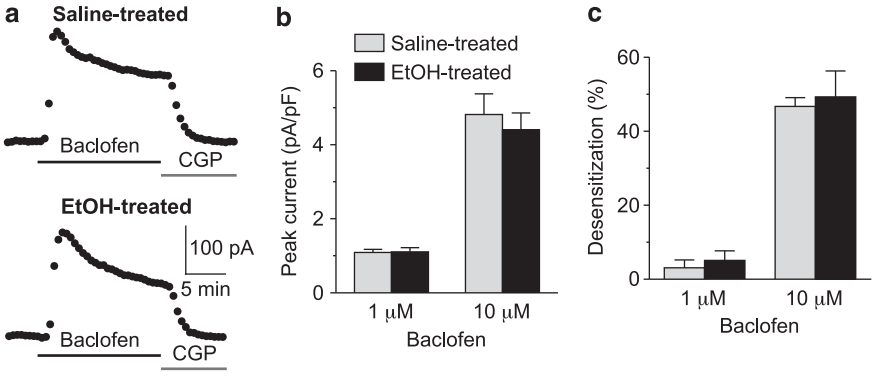

Figure 3 $GABA_B$ receptor-mediated outward currents are not altered by *in vivo* ethanol exposure. (a) Examples of baclofen-induced outward currents in saline- and ethanol-treated mice. Baclofen (10 μM) was bath applied for 15 min, and then the $GABA_B$ antagonist CGP54626 (200 nM) was applied to reverse baclofen-induced currents. (b) Summary bar graph showing the peak outward current amplitude produced by baclofen (1 and 10 μM) in saline- and ethanol-treated mice (1 μM: $n = 14$ from 11 mice in saline group and $n = 8$ from 6 mice in ethanol group; 10 μM: $n = 8$ from 7 mice in saline group and $n = 7$ from 6 mice in ethanol group). (c) Summary bar graph depicting the amount of desensitization of baclofen-induced currents in saline- and ethanol-treated mice (1 μM: $n = 3$ from 3 mice in saline group and $n = 3$ from 2 mice in ethanol group; 10 μM: $n = 5$ from 4 mice in saline group and $n = 5$ from 5 mice in ethanol group). Error bars indicate SEM.

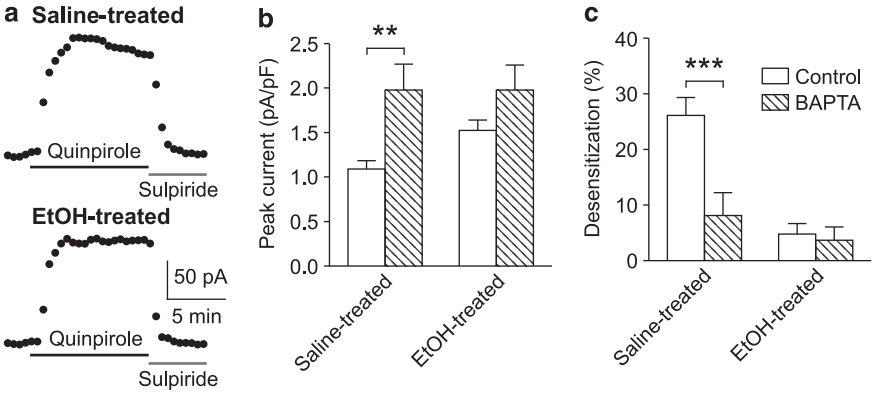

Figure 4 Chelating cytosolic Ca^{2+} occludes the enhancement of D_2 receptor-mediated currents caused by ethanol exposure. (a) Examples of quinpirole-induced currents in the presence of BAPTA in saline- and ethanol-treated mice. Quinpirole (300 nM) and sulpiride (1 μM) were applied as indicated by horizontal bars. (b) Summary bar graph showing the effect of BAPTA on the peak amplitude of quinpirole-induced currents in saline group (control: $n = 15$ from 12 mice, BAPTA: $n = 7$ from 4 mice) and in ethanol group (control: $n = 10$ from 9 mice, BAPTA: $n = 6$ from 3 mice). (c) Summary bar graph depicting the effect of BAPTA on desensitization of quinpirole-induced currents in saline group (control: $n = 10$ from 8 mice, BAPTA: $n = 6$ from 4 mice) and in ethanol group (control: $n = 7$ from 7 mice, BAPTA: $n = 5$ from 3 mice). $^{**}p < 0.01$, $^{***}p < 0.001$, Bonferroni *post hoc* test. Error bars indicate SEM.

exposure results from regulation at the level of D_2 receptors and not at downstream signaling components ($G_{i/o}$ proteins or GIRK channels).

Regulation of D_2 Receptor-Mediated Inhibition by a Ca^{2+}-Dependent Desensitization Mechanism

It has been shown that buffering cytosolic Ca^{2+} with BAPTA suppresses desensitization of D_2 receptor-mediated outward currents in dopamine neurons (Beckstead and Williams, 2007). Therefore, we loaded recorded cells with BAPTA (5 mM) through the whole-cell pipette to test its effect on quinpirole-induced currents in saline- and ethanol-treated mice. Intracellular BAPTA significantly increased the peak amplitude of quinpirole-induced outward currents and reduced the amount of desensitization in saline-treated mice (Figure 4). In contrast, BAPTA failed to significantly affect quinpirole-induced currents in ethanol-treated mice, although the peak current amplitude was slightly increased. Furthermore, Ca^{2+} chelation with BAPTA eliminated the difference in quinpirole responses between saline- and ethanol-treated mice (peak amplitude, BAPTA: $F_{1,34} = 14.3$, $p < 0.001$, *in vivo* treatment: $F_{1,34} = 1.48$, $p = 0.23$, BAPTA × *in vivo* treatment: $F_{1,34} = 1.49$, $p = 0.23$; desensitization, BAPTA: $F_{1,24} = 8.38$, $p < 0.01$, *in vivo* treatment: $F_{1,24} = 15.3$, $p < 0.001$, BAPTA × *in vivo* treatment: $F_{1,24} = 6.58$, $p < 0.05$; two-way ANOVA). These results suggest that attenuation of a Ca^{2+}-dependent desensitization mechanism is responsible for the enhancement of D_2 receptor-mediated inhibition after *in vivo* ethanol exposure.

We further explored the intracellular pathways mediating Ca^{2+}-dependent desensitization of D_2 receptor signaling in VTA dopamine neurons from naïve mice. D_2 receptor activation may cause release of Ca^{2+} from intracellular stores via the phospholipase C (PLC)/IP_3-mediated pathway

(Hernandez-Lopez *et al*, 2000; Hu *et al*, 2005; Takeuchi *et al*, 2002; Vallar *et al*, 1990). Thus, we examined the effects of pharmacological treatments that interfere with this signaling pathway on outward currents produced by 10-min perfusion of quinpirole (300 nM) (Figure 5). First, the PLC inhibitor U73122 (1 μM, intracellular dialysis through the whole-cell pipette) significantly diminished the desensitization of quinpirole-induced currents (Figures 5a and b). IP_3 receptor antagonists heparin (1 mg/ml, intracellular dialysis) or 2-APB (30 μM, bath application, >10 min pretreatment and present throughout quinpirole application) also significantly attenuated quinpirole-induced desensitization. Furthermore, bath application of CPA (10–20 μM, >10 min pretreatment and present throughout quinpirole application), which depletes intracellular Ca^{2+} stores (Seidler *et al*, 1989), suppressed quinpirole-induced desensitization. All of these treatments produced ~50% increases in the average peak amplitude of quinpirole-induced currents compared with the value obtained under control conditions (Figure 5c).

D_2 receptor-induced Ca^{2+} rises may lead to activation of CaMKII (Takeuchi *et al*, 2002) or protein kinase C (PKC) (Gordon *et al*, 2001). The CaMKII inhibitor KN-62 (10 μM, >1-h preincubation plus intracellular dialysis) and CaMKII inhibitory peptide (fragment 290–309; 25 μM, intracellular dialysis) significantly reduced the amount of quinpirole-induced desensitization, whereas PKC inhibitory peptide (fragment 19–31; 25 μM, intracellular dialysis) had no significant effect (Figures 5a and b). Altogether, these results suggest that PLC/IP_3/CaMKII-mediated pathway is responsible for Ca^{2+}-dependent desensitization of D_2 receptor signaling in dopamine neurons.

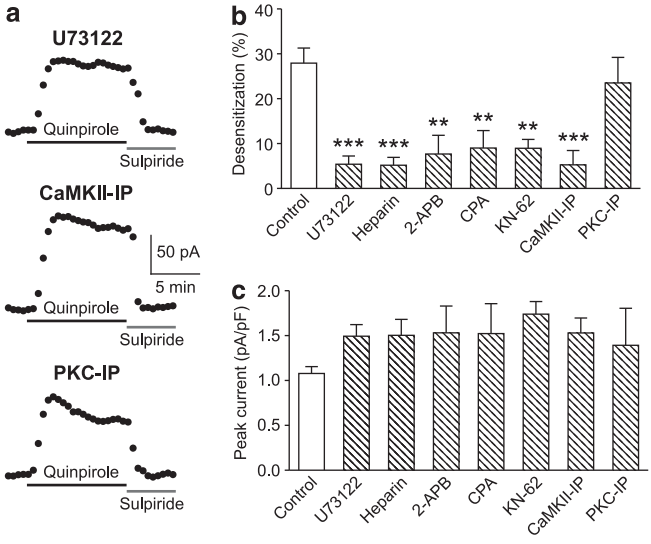

Figure 5 Blocking the PLC-IP_3-CaMKII cascade suppresses desensitization of D_2 receptor-mediated currents. (a) Examples of quinpirole-induced currents in cells loaded with U73122 (1 μM), CaMKII inhibitory peptide (fragment 290–309; 25 μM), or PKC inhibitory peptide (fragment 19–31; 25 μM). Quinpirole (300 nM) and sulpiride (1 μM) were applied as indicated by horizontal bars. (b, c) Summary bar graphs showing the effects of various intracellular Ca^{2+} signaling blockers on desensitization (b) and peak amplitude (c) of quinpirole-induced currents. Each drug was tested in 3–7 cells, while the control data are the same as those from naïve mice shown in Figures 1b and c (desensitization: $F_{7,35} = 9.47$, $p < 0.0001$; peak amplitude: $F_{7,41} = 1.61$, $p = 0.16$; peak amplitude: $F_{7,41} = 1.61$, $p = 0.16$; one-way ANOVA). $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* control, Bonferroni *post hoc* test. Error bars indicate SEM.

Cross-Desensitization of D_2 Receptor-Mediated Currents by Activation of mGluRs

Metabotropic glutamate receptors (mGluRs) are coupled to the generation of IP_3 in dopamine neurons (Cui *et al*, 2007). Thus, we next examined if mGluR activation causes cross-desensitization of D_2 receptor-mediated outward currents. In these experiments, pressure ejection of dopamine (100 μM) was made every 90 s. Transient outward currents thus evoked were abolished by sulpiride (1 μM; $n = 3$) (Figure 6a), confirming that they are mediated by D_2 receptors. Bath application of the mGluR agonist DHPG (1 μM) produced a reversible inhibition of dopamine-evoked currents (29 ± 4% inhibition, $n = 5$) (Figure 6b). To examine the role of IP_3-mediated Ca^{2+} release in the DHPG effect, we depleted intracellular Ca^{2+} stores with CPA (20 μM). Bath application of CPA by itself dramatically augmented dopamine-evoked currents (113 ± 32% increase, $n = 5$) (Figure 6c), suggesting that D_2 receptor signaling is constitutively desensitized by constant Ca^{2+} release from intracellular stores. DHPG failed to affect dopamine-evoked currents when the cells were pretreated with CPA (−4 ± 5% change). Therefore, mGluR activation leads to cross-desensitization of D_2 receptor signaling in a Ca^{2+} store-dependent manner.

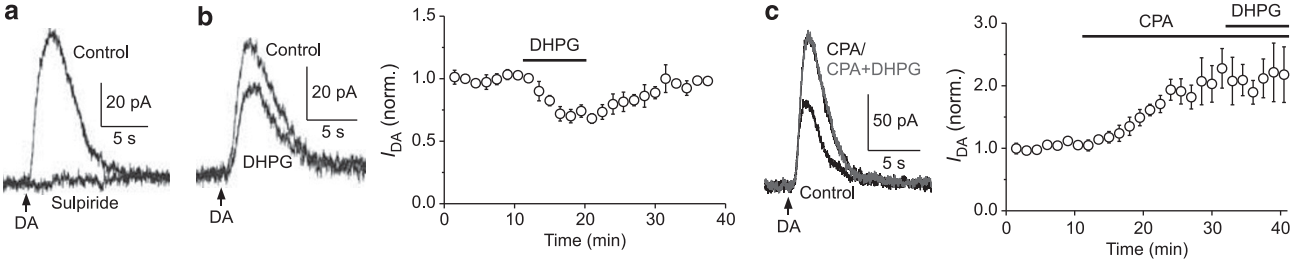

Figure 6 mGluR activation cross-desensitizes D_2 receptor-mediated outward currents. (a) Sample traces depicting that dopamine-induced outward currents were abolished by sulpiride (1 μM). Pressure ejection of dopamine (100 μM) was made at the time indicated by the arrow. (b) Sample traces and summary time graph showing inhibition of dopamine-evoked currents by bath application of DHPG (1 μM). (c) Sample traces and summary time graph demonstrating that the DHPG effect on dopamine-evoked currents was suppressed by pretreatment with CPA (20 μM). Note that CPA itself augmented dopamine-induced currents. Error bars indicate SEM.

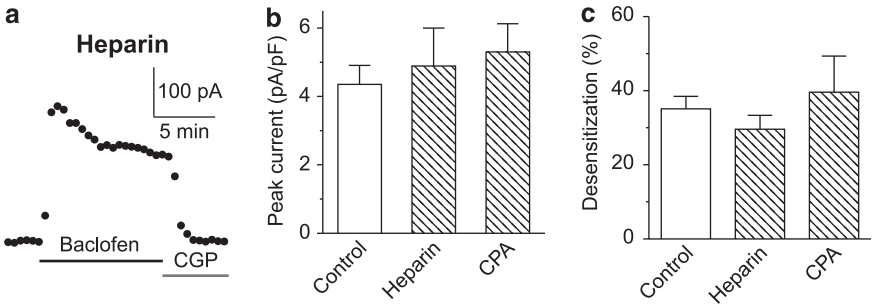

Figure 7 $GABA_B$ receptor-mediated currents are not regulated by Ca^{2+}-dependent desensitization. (a) An example of baclofen-induced current in a cell loaded with heparin (1 mg/ml). Baclofen (10 μM) and CGP54626 (200 nM) were applied as indicated by horizontal bars. (b, c) Summary bar graphs showing that both CPA and heparin failed to affect peak amplitude (b) and desensitization (c) of baclofen-induced currents. Baclofen was applied for 10 min in these experiments. Three to four cells were tested for each condition. Error bars indicate SEM.

$GABA_B$ Receptor Signaling is Not Regulated by Ca^{2+}-Dependent Desensitization

It has been shown that chelating intracellular Ca^{2+} with BAPTA does not affect desensitization of $GABA_B$ receptor-mediated outward currents in dopamine neurons (Beckstead and Williams, 2007). Consistent with this, blocking IP_3 receptors with heparin or depleting Ca^{2+} stores with CPA had no significant effects on the peak amplitude or the amount of desensitization of baclofen-induced outward currents (peak amplitude: $F_{2,8} = 0.38$, $p = 0.69$; desensitization: $F_{2,7} = 0.46$, $p = 0.65$; one-way ANOVA) (Figure 7). Thus, the Ca^{2+}-dependent mechanism mediating D_2 receptor desensitization does not regulate $GABA_B$ receptor signaling in dopamine neurons.

DISCUSSION

In this study, we found that repeated *in vivo* exposure to ethanol results in an enhancement of D_2 autoreceptor-mediated inhibition of VTA dopamine neurons. This enhancement results from downregulation of a Ca^{2+}-dependent desensitization mechanism comprising IP_3-induced Ca^{2+} release from intracellular stores and activation of CaMKII. In contrast, $GABA_B$ receptor-mediated inhibition is not regulated by this Ca^{2+}-dependent desensitization mechanism, and hence is not altered by repeated ethanol exposure. Upregulation of dopamine autoinhibition may have an important role in regulating the mesolimbic dopaminergic output during ethanol withdrawal *in vivo*.

Ca^{2+}-Dependent Desensitization of D_2 Receptor-Mediated Inhibition

A previous study has demonstrated that chelating cytosolic Ca^{2+} with BAPTA in dopamine neurons results in an increase in the amplitude of D_2 receptor-mediated outward currents together with a decrease in the amount of desensitization (Beckstead and Williams, 2007). In that study, desensitization was induced either via sustained stimulation of dendritic dopamine release, which caused long-term depression of D_2 IPSCs, or via prolonged application of dopamine. Our data also show that BAPTA significantly increased the peak amplitude of quinpirole-induced outward currents and suppressed their desensitization in VTA dopamine neurons from saline-treated mice. These observations indicate the role of cytosolic Ca^{2+} in regulating basal, as well as agonist induced, desensitization of D_2 receptor signaling. However, depletion of intracellular Ca^{2+} stores with CPA had no effect on D_2 receptor desensitization in the study by Beckstead *et al*, whereas CPA largely suppressed desensitization of quinpirole-induced currents in this study. Although the reason for this discrepancy is not clear, it may be accounted for, at least partially, by sampling of different populations of dopamine neurons (mostly from the substantia nigra in the study by Beckstead *et al vs* from the lateral VTA in our study). Indeed, it has been shown that different populations of dopamine neurons in different areas of the ventral midbrain have distinct functional and chemical properties, such as the expression levels of Ca^{2+}-binding proteins (Lammel *et al*, 2008; Neuhoff *et al*, 2002).

Our data suggest that D_2 receptor signaling is constitutively desensitized by constant Ca^{2+} release from intracellular stores under basal conditions, because Ca^{2+} store depletion by CPA, similarly to Ca^{2+} chelation by BAPTA, increased the peak amplitude of D_2-mediated outward currents. A number of studies have demonstrated that D_2 receptor activation leads to Ca^{2+} release from intracellular stores via the $G_{i/o}$/PLC/IP_3-mediated pathway (Hernandez-Lopez *et al*, 2000; Hu *et al*, 2005; Takeuchi *et al*, 2002; Vallar *et al*, 1990). In line with this, blockade of PLC or IP_3 receptors significantly attenuated quinpirole-induced desensitization. Thus, D_2 receptor-induced rises in cytosolic Ca^{2+} levels, in addition to basal levels, may mediate the desensitization observed during continuous D_2 receptor activation. Although a rise in Ca^{2+} levels following iontophoretic application of dopamine has not been observed in dopamine neurons (Beckstead and Williams, 2007), small but continuous Ca^{2+} release, which cannot be readily detected with fluorescence Ca^{2+} imaging, may be responsible for agonist-induced desensitization of D_2 receptor signaling. Moreover, prolonged mGluR activation by bath perfusion of the agonist DHPG, which would partially deplete Ca^{2+} stores via continuous IP_3-mediated Ca^{2+} release (Cui *et al*, 2007), cross-desensitized dopamine-induced currents in a Ca^{2+} store-dependent manner, further supporting the idea that continuous Ca^{2+} release can mediate desensitization of D_2 receptor signaling.

It has been reported recently that CaMKII phosphorylation causes desensitization of D_3 receptors (Liu *et al*, 2009) and also D_2 receptors (JQ Wang, personal communication), both

of which belong to the D_2-like dopamine receptor family coupled to $G_{i/o}$ G proteins. Although dopamine neurons express both D_2 and D_3 receptors, only D_2 receptors are coupled to GIRK activation in dopamine neurons (Beckstead *et al*, 2004; Davila *et al*, 2003). Furthermore, activation of D_2 receptors, as well as D_4 receptors, has been shown to activate CaMKII via $G_{i/o}$ and intracellular Ca^{2+} release (Gu and Yan, 2004; Takeuchi *et al*, 2002). Consistent with these previous findings, CaMKII inhibition suppressed quinpirole-induced desensitization in the current study. In contrast, PKC, which can also desensitize D_2 receptors (Morris *et al*, 2007; Namkung and Sibley, 2004), appears not to have a role in dopamine neurons.

D_2 and $GABA_B$ receptor-mediated outward currents in dopamine neurons are largely eliminated by genetic deletion of GIRK2 but not GIRK3 (Beckstead *et al*, 2004; Labouebe *et al*, 2007), indicating that these two receptors most likely share the same signaling targets. Furthermore, $GABA_B$ receptor activation has also been shown to cause IP_3-mediated Ca^{2+} release in certain cells (Michler and Erdo, 1989; Parramon *et al*, 1995). However, blockade of IP_3-mediated Ca^{2+} release failed to affect desensitization of baclofen-induced currents in this study, demonstrating that Ca^{2+}-dependent desensitization is selectively involved in the regulation of D_2, but not $GABA_B$, receptor signaling in dopamine neurons. It has been shown that $GABA_B$ receptor-GIRK channel coupling efficiency, as well as agonist-induced desensitization of $GABA_B$ receptor-mediated currents, are regulated by regulator of G protein signaling (RGS) proteins (Labouebe *et al*, 2007; Mutneja *et al*, 2005). These findings strongly suggest that these two $G_{i/o}$-coupled receptors are differentially regulated by distinct desensitization mechanisms in dopamine neurons.

Enhanced D_2 Autoinhibition and Hypodopaminergic State During Ethanol Abstinence

Repeated ethanol exposure resulted in a dramatic reduction in the amount of desensitization observed during quinpirole application. This attenuated desensitization was accompanied by an increase in the potency of quinpirole to activate GIRK channels. The maximal quinpirole-induced currents were comparable in saline- and ethanol-treated mice, suggesting that the expression levels of D_2 receptors and GIRK channels were not altered. It is of note that a similar increase in the potency of baclofen has been observed in VTA dopamine neurons after repeated *in vivo* exposures to morphine or γ-hydroxybutyrate (Labouebe *et al*, 2007). This increase in baclofen potency results from downregulation of RGS proteins, which control $GABA_B$ receptor desensitization as described above.

It is well known that repeated exposure to cocaine or amphetamine leads to a decrease in D_2 agonist-induced inhibition of VTA dopamine neuron firing (Henry *et al*, 1989; Marinelli *et al*, 2003; Wolf *et al*, 1993). Furthermore, repeated cocaine administration has been shown to cause upregulation of CaMKII expression in the VTA (Backes and Hemby, 2003; Licata *et al*, 2004). Therefore, differential regulation of the CaMKII-dependent desensitization mechanism may contribute to the difference in D_2 autoreceptor sensitivity, that is, increase *vs* decrease, following repeated intermittent administration of alcohol *vs* psychostimulants.

In vivo recording studies have found profound reductions in VTA dopamine neuron firing activity after withdrawal from repeated ethanol exposure ((Diana *et al*, 1993, 1995), but also see (Shen, 2003; Shen and Chiodo, 1993)). Our data show that the basal firing frequency of dopamine neurons measured in brain slices was not altered by *in vivo* ethanol exposure, as has been reported in previous brain slice recording studies (Brodie, 2002; Hopf *et al*, 2007; Okamoto *et al*, 2006). However, inhibition of firing produced by low concentrations of quinpirole was significantly augmented in ethanol-treated mice, consistent with the increased potency of quinpirole to activate GIRK channels. Although our data failed to detect functional dopamine tone in VTA slices, as evidenced by the lack of effect of the D_2 antagonist sulpiride, autoinhibitory regulation mediated by dopamine tone has been shown to have an important role in controlling the basal firing of dopamine neurons *in vivo* (Henry *et al*, 1989; Pucak and Grace, 1994; White and Wang, 1984). Therefore, increased D_2 autoreceptor-mediated inhibition may well lead to reduced dopamine neuron firing *in vivo* after withdrawal from repeated ethanol exposure.

In addition to dopamine-mediated autoinhibition, the activity of dopamine neurons is regulated by numerous neurotransmitter inputs that may undergo ethanol-induced adaptive changes (Morikawa and Morrisett, 2010). For example, it has been reported that VTA GABA neuron firing, as well as GABA release onto dopamine neurons, are increased following ethanol withdrawal (Gallegos *et al*, 1999; Melis *et al*, 2002), which would result in enhanced GABAergic inhibition of dopamine neurons. The relative contribution of different neurotransmitter inputs to dopamine neuron hypoactivity remains to be determined.

Ample evidence indicates the impact of stress on the dopaminergic system and its role in the development of addictive behaviors (Sinha, 2008; Ungless *et al*, 2010). Thus, repeated stress associated with daily ethanol intoxication and withdrawal may have played a role in causing the alterations in D_2 signaling in this study and reductions in dopamine neuron firing reported in previous studies (Diana *et al*, 1993, 1995).

The hypodopaminergic state observed in ethanol-withdrawn animals or in detoxified alcoholics is characterized by hypoactivity of dopamine neurons and the resulting decrease in dopamine release in the NAc and striatum, together with downregulation of postsynaptic D_2 receptors in those dopamine projection areas (Martinez *et al*, 2005; Melis *et al*, 2005; Volkow *et al*, 2007). It has been postulated that this hypodopaminergic state contributes to the emotional/motivational component of ethanol dependence, although activation of other neurotransmitter systems, such as those involving corticotropin-releasing factor or the endogenous κ-opioid dynorphin, has also been implicated (Koob, 2009; Wee and Koob, 2010). In support of the role of the hypodopaminergic state in dependence, ethanol-withdrawn animals have been shown to self-administer ethanol until NAc dopamine levels are restored to the pre-withdrawal baseline levels (Weiss *et al*, 1996). Furthermore, animals that exhibit high ethanol preference and consumption have low basal dopamine levels in the NAc (George *et al*, 1995; McBride *et al*, 1995). Our study suggests that promoting the Ca^{2+}-dependent desensitization mechanism that regulates D_2 autoinhibition in the VTA might alleviate

the hypodopaminergic state in alcohol-dependent individuals, thus aid in reducing compulsive alcohol drinking.

ACKNOWLEDGEMENTS

We thank Dr Giorgio Gorini for assistance with part of the study. This work was funded by NIH Grant R01 AA015521. BEB was supported by a National Research Service Award.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

Ahn KC, Bernier BE, Harnett MT, Morikawa H (2010). IP3 receptor sensitization during *in vivo* amphetamine experience enhances NMDA receptor plasticity in dopamine neurons of the ventral tegmental area. *J Neurosci* **30**: 6689–6699.

Backes E, Hemby SE (2003). Discrete cell gene profiling of ventral tegmental dopamine neurons after acute and chronic cocaine self-administration. *J Pharmacol Exp Ther* **307**: 450–459.

Beckstead MJ, Gantz SC, Ford CP, Stenzel-Poore MP, Phillips PE, Mark GP *et al* (2009). CRF enhancement of GIRK channel-mediated transmission in dopamine neurons. *Neuropsychopharmacology* **34**: 1926–1935.

Beckstead MJ, Grandy DK, Wickman K, Williams JT (2004). Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. *Neuron* **42**: 939–946.

Beckstead MJ, Williams JT (2007). Long-term depression of a dopamine IPSC. *J Neurosci* **27**: 2074–2080.

Boileau I, Assaad JM, Pihl RO, Benkelfat C, Leyton M, Diksic M *et al* (2003). Alcohol promotes dopamine release in the human nucleus accumbens. *Synapse* **49**: 226–231.

Brodie MS (2002). Increased ethanol excitation of dopaminergic neurons of the ventral tegmental area after chronic ethanol treatment. *Alcohol Clin Exp Res* **26**: 1024–1030.

Brodie MS, Shefner SA, Dunwiddie TV (1990). Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area *in vitro*. *Brain Res* **508**: 65–69.

Cruz HG, Ivanova T, Lunn ML, Stoffel M, Slesinger PA, Luscher C (2004). Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. *Nat Neurosci* **7**: 153–159.

Cui G, Bernier BE, Harnett MT, Morikawa H (2007). Differential regulation of action potential- and metabotropic glutamate receptor-induced Ca2+ signals by inositol 1,4,5-trisphosphate in dopaminergic neurons. *J Neurosci* **27**: 4776–4785.

Davila V, Yan Z, Craciun LC, Logothetis D, Sulzer D (2003). D3 dopamine autoreceptors do not activate G-protein-gated inwardly rectifying potassium channel currents in substantia nigra dopamine neurons. *J Neurosci* **23**: 5693–5697.

Diana M, Pistis M, Carboni S, Gessa GL, Rossetti ZL (1993). Profound decrement of mesolimbic dopaminergic neuronal activity during ethanol withdrawal syndrome in rats: electrophysiological and biochemical evidence. *Proc Natl Acad Sci USA* **90**: 7966–7969.

Diana M, Pistis M, Muntoni A, Gessa G (1996). Mesolimbic dopaminergic reduction outlasts ethanol withdrawal syndrome: evidence of protracted abstinence. *Neuroscience* **71**: 411–415.

Diana M, Pistis M, Muntoni AL, Gessa GL (1995). Ethanol withdrawal does not induce a reduction in the number of spontaneously active dopaminergic neurons in the mesolimbic system. *Brain Res* **682**: 29–34.

Erhardt S, Mathe JM, Chergui K, Engberg G, Svensson TH (2002). GABA(B) receptor-mediated modulation of the firing pattern of ventral tegmental area dopamine neurons *in vivo*. *Naunyn Schmiedebergs Arch Pharmacol* **365**: 173–180.

Ford CP, Mark GP, Williams JT (2006). Properties and opioid inhibition of mesolimbic dopamine neurons vary according to target location. *J Neurosci* **26**: 2788–2797.

Gallegos RA, Lee RS, Criado JR, Henriksen SJ, Steffensen SC (1999). Adaptive responses of gamma-aminobutyric acid neurons in the ventral tegmental area to chronic ethanol. *J Pharmacol Exp Ther* **291**: 1045–1053.

George SR, Fan T, Ng GY, Jung SY, O'Dowd BF, Naranjo CA (1995). Low endogenous dopamine function in brain predisposes to high alcohol preference and consumption: reversal by increasing synaptic dopamine. *J Pharmacol Exp Ther* **273**: 373–379.

Gessa GL, Muntoni F, Collu M, Vargiu L, Mereu G (1985). Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. *Brain Res* **348**: 201–203.

Gordon AS, Yao L, Jiang Z, Fishburn CS, Fuchs S, Diamond I (2001). Ethanol acts synergistically with a D2 dopamine agonist to cause translocation of protein kinase C. *Mol Pharmacol* **59**: 153–160.

Gu Z, Yan Z (2004). Bidirectional regulation of Ca2+/calmodulin-dependent protein kinase II activity by dopamine D4 receptors in prefrontal cortex. *Mol Pharmacol* **66**: 948–955.

Henry DJ, Greene MA, White FJ (1989). Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: repeated administration. *J Pharmacol Exp Ther* **251**: 833–839.

Hernandez-Lopez S, Tkatch T, Perez-Garci E, Galarraga E, Bargas J, Hamm H *et al* (2000). D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca2+ currents and excitability via a novel PLC[beta]1-IP3-calcineurin-signaling cascade. *J Neurosci* **20**: 8987–8995.

Hopf FW, Martin M, Chen BT, Bowers MS, Mohamedi MM, Bonci A (2007). Withdrawal from intermittent ethanol exposure increases probability of burst firing in VTA neurons *in vitro*. *J Neurophysiol* **98**: 2297–2310.

Hu XT, Dong Y, Zhang XF, White FJ (2005). Dopamine D2 receptor-activated Ca2+ signaling modulates voltage-sensitive sodium currents in rat nucleus accumbens neurons. *J Neurophysiol* **93**: 1406–1417.

Ikemoto S (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res Rev* **56**: 27–78.

Imperato A, Di Chiara G (1986). Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *J Pharmacol Exp Ther* **239**: 219–228.

Koob GF (2009). Neurobiological substrates for the dark side of compulsivity in addiction. *Neuropharmacology* **56**(Suppl 1): 18–31.

Koob GF, Volkow ND (2010). Neurocircuitry of addiction. *Neuropsychopharmacology* **35**: 217–238.

Labouebe G, Lomazzi M, Cruz HG, Creton C, Lujan R, Li M *et al* (2007). RGS2 modulates coupling between GABAB receptors and GIRK channels in dopamine neurons of the ventral tegmental area. *Nat Neurosci* **10**: 1559–1568.

Lacey MG, Mercuri NB, North RA (1987). Dopamine acts on D2 receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta. *J Physiol* **392**: 397–416.

Lammel S, Hetzel A, Hackel O, Jones I, Liss B, Roeper J (2008). Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. *Neuron* **57**: 760–773.

Licata SC, Schmidt HD, Pierce RC (2004). Suppressing calcium/calmodulin-dependent protein kinase II activity in the ventral tegmental area enhances the acute behavioural response to cocaine but attenuates the initiation of cocaine-induced behavioural sensitization in rats. *Eur J Neurosci* **19**: 405–414.

Liu XY, Mao LM, Zhang GC, Papasian CJ, Fibuch EE, Lan HX *et al* (2009). Activity-dependent modulation of limbic dopamine D3 receptors by CaMKII. *Neuron* **61**: 425–438.

Luscher C, Slesinger PA (2010). Emerging roles for G protein-gated inwardly rectifying potassium (GIRK) channels in health and disease. *Nat Rev Neurosci* **11**: 301–315.

Margolis EB, Lock H, Hjelmstad GO, Fields HL (2006). The ventral tegmental area revisited: is there an electrophysiological marker for dopaminergic neurons? *J Physiol* **577**(Part 3): 907–924.

Marinelli M, Cooper DC, Baker LK, White FJ (2003). Impulse activity of midbrain dopamine neurons modulates drug-seeking behavior. *Psychopharmacology (Berl)* **168**: 84–98.

Martinez D, Gil R, Slifstein M, Hwang DR, Huang Y, Perez A *et al* (2005). Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. *Biol Psychiatry* **58**: 779–786.

McBride WJ, Bodart B, Lumeng L, Li TK (1995). Association between low contents of dopamine and serotonin in the nucleus accumbens and high alcohol preference. *Alcohol Clin Exp Res* **19**: 1420–1422.

Melis M, Camarini R, Ungless MA, Bonci A (2002). Long-lasting potentiation of GABAergic synapses in dopamine neurons after a single *in vivo* ethanol exposure. *J Neurosci* **22**: 2074–2082.

Melis M, Spiga S, Diana M (2005). The dopamine hypothesis of drug addiction: hypodopaminergic state. *Int Rev Neurobiol* **63**: 101–154.

Michler A, Erdo SL (1989). Stimulation by phaclofen of inositol 1,4,5-triphosphate production in cultured neurons from chick tectum. *Eur J Pharmacol* **167**: 423–425.

Morikawa H, Morrisett RA (2010). Ethanol action on dopaminergic neurons in the ventral tegmental area: interaction with intrinsic ion channels and neurotransmitter inputs. *Int Rev Neurobiol* **91**: 235–288.

Morris SJ, Van II H, Daigle M, Robillard L, Sajedi N, Albert PR (2007). Differential desensitization of dopamine D2 receptor isoforms by protein kinase C: the importance of receptor phosphorylation and pseudosubstrate sites. *Eur J Pharmacol* **577**: 44–53.

Mutneja M, Berton F, Suen KF, Luscher C, Slesinger PA (2005). Endogenous RGS proteins enhance acute desensitization of GABA(B) receptor-activated GIRK currents in HEK-293T cells. *Pflugers Arch* **450**: 61–73.

Namkung Y, Sibley DR (2004). Protein kinase C mediates phosphorylation, desensitization, and trafficking of the D2 dopamine receptor. *J Biol Chem* **279**: 49533–49541.

Neuhoff H, Neu A, Liss B, Roeper J (2002). I(h) channels contribute to the different functional properties of identified dopaminergic subpopulations in the midbrain. *J Neurosci* **22**: 1290–1302.

Okamoto T, Harnett MT, Morikawa H (2006). Hyperpolarization-activated cation current (Ih) is an ethanol target in midbrain dopamine neurons of mice. *J Neurophysiol* **95**: 619–626.

Parramon M, Gonzalez MP, Herrero MT, Oset-Gasque MJ (1995). GABAB receptors increase intracellular calcium concentrations in chromaffin cells through two different pathways: their role in catecholamine secretion. *J Neurosci Res* **41**: 65–72.

Pucak ML, Grace AA (1994). Evidence that systemically administered dopamine antagonists activate dopamine neuron firing primarily by blockade of somatodendritic autoreceptors. *J Pharmacol Exp Ther* **271**: 1181–1192.

Putzier I, Kullmann PH, Horn JP, Levitan ES (2009). Dopamine neuron responses depend exponentially on pacemaker interval. *J Neurophysiol* **101**: 926–933.

Riegel AC, Williams JT (2008). CRF facilitates calcium release from intracellular stores in midbrain dopamine neurons. *Neuron* **57**: 559–570.

Rossetti ZL, Melis F, Carboni S, Gessa GL (1992). Dramatic depletion of mesolimbic extracellular dopamine after withdrawal from morphine, alcohol or cocaine: a common neurochemical substrate for drug dependence. *Ann NY Acad Sci* **654**: 513–516.

Seidler NW, Jona I, Vegh M, Martonosi A (1989). Cyclopiazonic acid is a specific inhibitor of the Ca2+-ATPase of sarcoplasmic reticulum. *J Biol Chem* **264**: 17816–17823.

Shen RY (2003). Ethanol withdrawal reduces the number of spontaneously active ventral tegmental area dopamine neurons in conscious animals. *J Pharmacol Exp Ther* **307**: 566–572.

Shen RY, Chiodo LA (1993). Acute withdrawal after repeated ethanol treatment reduces the number of spontaneously active dopaminergic neurons in the ventral tegmental area. *Brain Res* **622**: 289–293.

Sinha R (2008). Chronic stress, drug use, and vulnerability to addiction. *Ann NY Acad Sci* **1141**: 105–130.

Takeuchi Y, Fukunaga K, Miyamoto E (2002). Activation of nuclear Ca(2+)/calmodulin-dependent protein kinase II and brain-derived neurotrophic factor gene expression by stimulation of dopamine D2 receptor in transfected NG108-15 cells. *J Neurochem* **82**: 316–328.

Trevisan LA, Boutros N, Petrakis IL, Krystal JH (1998). Complications of alcohol withdrawal: pathophysiological insights. *Alcohol Health Res World* **22**: 61–66.

Ungless MA, Argilli E, Bonci A (2010). Effects of stress and aversion on dopamine neurons: implications for addiction. *Neurosci Biobehav Rev* **35**: 151–156.

Vallar L, Muca C, Magni M, Albert P, Bunzow J, Meldolesi J *et al* (1990). Differential coupling of dopaminergic D2 receptors expressed in different cell types. Stimulation of phosphatidylinositol 4,5-bisphosphate hydrolysis in LtK- fibroblasts, hyperpolarization, and cytosolic-free Ca2+ concentration decrease in GH4C1 cells. *J Biol Chem* **265**: 10320–10326.

Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Jayne M *et al* (2007). Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. *J Neurosci* **27**: 12700–12706.

Wanat MJ, Hopf FW, Stuber GD, Phillips PE, Bonci A (2008). Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of Ih. *J Physiol* **586**: 2157–2170.

Wee S, Koob GF (2010). The role of the dynorphin-kappa opioid system in the reinforcing effects of drugs of abuse. *Psychopharmacology (Berl)* **210**: 121–135.

Weiss F, Lorang MT, Bloom FE, Koob GF (1993). Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. *J Pharmacol Exp Ther* **267**: 250–258.

Weiss F, Parsons LH, Schulteis G, Hyytia P, Lorang MT, Bloom FE *et al* (1996). Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. *J Neurosci* **16**: 3474–3485.

Werkman TR, Kruse CG, Nievelstein H, Long SK, Wadman WJ (2001). *In vitro* modulation of the firing rate of dopamine neurons in the rat substantia nigra pars compacta and the ventral tegmental area by antipsychotic drugs. *Neuropharmacology* **40**: 927–936.

White FJ, Wang RY (1984). A10 dopamine neurons: role of autoreceptors in determining firing rate and sensitivity to dopamine agonists. *Life Sci* **34**: 1161–1170.

Wolf ME, White FJ, Nassar R, Brooderson RJ, Khansa MR (1993). Differential development of autoreceptor subsensitivity and enhanced dopamine release during amphetamine sensitization. *J Pharmacol Exp Ther* **264**: 249–255.

Zhang TA, Placzek AN, Dani JA (2010). *In vitro* identification and electrophysiological characterization of dopamine neurons in the ventral tegmental area. *Neuropharmacology* **59**: 431–436.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)