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Plasticity of GABA_A receptor-mediated neurotransmission in the nucleus accumbens of alcohol-dependent rats

Jing Liang, A. Kerstin Lindemeyer, Asha Suryanarayanan, Edward M. Meyer, Vincent N. Marty, S. Omar Ahmad, Xuesi Max Shao, Richard W. Olsen and Igor Spigelman
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Selective modulation of GABAergic tonic current by dopamine in the nucleus accumbens of alcohol-dependent rats

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Liang J, Marty VN, Mulpuri Y, Olsen RW, Spigelman I. Selective modulation of GABAergic tonic current by dopamine in the nucleus accumbens of alcohol-dependent rats. *J Neurophysiol* 112: 51–60, 2014. First published April 9, 2014; doi:10.1152/jn.00564.2013.—The nucleus accumbens (NAcc) is a key structure of the mesolimbic dopaminergic reward system and plays an important role in mediating alcohol-seeking behaviors. Alterations in glutamatergic and GABAergic signaling were recently demonstrated in the NAcc of rats after chronic intermittent ethanol (CIE) treatment, a model of alcohol dependence. Here we studied dopamine (DA) modulation of GABAergic signaling and how this modulation might be altered by CIE treatment. We show that the tonic current (I_{tonic}) mediated by extrasynaptic γ -aminobutyric acid type A receptors (GABA_ARs) of medium spiny neurons (MSNs) in the NAcc core is differentially modulated by DA at concentrations in the range of those measured in vivo (0.01–1 μ M), without affecting the postsynaptic kinetics of miniature inhibitory postsynaptic currents (mIPSCs). Use of selective D1 receptor (D1R) and D2 receptor (D2R) ligands revealed that I_{tonic} potentiation by DA (10 nM) is mediated by D1Rs while I_{tonic} depression by DA (0.03–1 μ M) is mediated by D2Rs in the same MSNs. Addition of guanosine 5'-O-(2-thiodiphosphate) (GDP β S) to the recording pipettes eliminated I_{tonic} decrease by the selective D2R agonist quinpirole (5 nM), leaving intact the quinpirole effect on mIPSC frequency. Recordings from CIE and vehicle control (CIV) MSNs during application of D1R agonist (SKF 38393, 100 nM) or D2R agonist (quinpirole, 2 nM) revealed that SKF 38393 potentiated I_{tonic} to the same extent, while quinpirole reduced I_{tonic} to a similar extent, in both groups of rats. Our data suggest that the selective modulatory effects of DA on I_{tonic} are unaltered by CIE treatment and withdrawal.

ventral striatum; ethanol; withdrawal; neuroadaptation; tonic current

ALCOHOL (ethanol, EtOH), consumed in moderation, has numerous beneficial effects on health (French and Zavala 2007; Gunzerath et al. 2004), yet excessive alcohol drinking can lead to alcohol dependence and loss of control over alcohol consumption, with serious detrimental health consequences (Room et al. 2005). Chronic alcohol exposure causes marked changes in reinforcement mechanisms and motivational state that are thought to contribute to the development of cravings and relapse during protracted withdrawal (Koob and Le Moal 2008). Relapse to alcohol is a critical problem in treating alcoholism, and effective treatments are yet to be found.

The nucleus accumbens (NAcc) is a key neural substrate for the rewarding actions of many drugs of abuse, including alcohol (Koob et al. 1998; Wise 2004). Enhancement of dopa-

mine (DA) release in several brain regions, most prominently in the NAcc, is a common property of alcohol and other drugs of abuse (Di Chiara and Imperato 1985, 1988). Action potential discharge in the ventral tegmental area (VTA) DA neurons mediates DA release in the NAcc (Sompers et al. 2009); this release is highly regulated by presynaptic auto- and heteroreceptors (Zhang and Sulzer 2012). DA concentrations ([DA]) reported in the NAcc of awake rats range from low nanomolar basal levels to high submicromolar concentrations during phasic DA release (Budygin et al. 2001; Owesson-White et al. 2012; Robinson et al. 2009; Segovia and Mora 2001; Yim and Gonzales 2000). Transient increases in extracellular [DA] measured with fast scanning voltammetry techniques are more frequent after administration of drugs of abuse such as alcohol and cocaine (Robinson et al. 2009; Stuber et al. 2005) and become time-locked to cues predicting reward (Day et al. 2007; Phillips et al. 2003; Stuber et al. 2005). Indeed, the mere expectation of EtOH evokes DA release in the NAcc (Katner et al. 1996; Melendez et al. 2002; Weiss et al. 1993). Basal DA release is decreased in alcohol-dependent animals, but an EtOH challenge evokes DA release comparable to if not greater than that in nondependent control animals (Budygin et al. 2007; Diana et al. 1992, 1993; Weiss et al. 1996; Yoshimoto et al. 1996). Also, EtOH-induced DA release is greater in rats bred for alcohol preference (Bustamante et al. 2008), and alcohol cue-evoked DA release in the ventral striatum is greatest in men with a greater genetic risk for alcoholism (Oberlin et al. 2013).

Upon release, DA affects signal transmission in the NAcc via activation of various pre- and postsynaptically localized DA receptor (DAR) subtypes. The D1 class of DA receptors (D1Rs and D5Rs) is richly expressed on GABAergic medium spiny neurons (MSNs) that project to the VTA, while the D2 class of DARs (D2Rs, D3Rs, and D4Rs) are preferentially expressed on MSNs projecting to the ventral pallidum (Beaulieu and Gainetdinov 2011; Sesack and Grace 2010). In addition, there is a population of NAcc MSNs that express both D1Rs and D2Rs (Lu et al. 1998; Perreault et al. 2010, 2011). DARs are also variably expressed on GABAergic and cholinergic interneurons, as well as on glutamatergic terminals projecting to the NAcc. There is also evidence for both D1Rs and D2Rs on striatal astrocytes (Bal et al. 1994; Hosli and Hosli 1987; Miyazaki et al. 2004; Zanassi et al. 1999).

On the basis of recordings in behaving animals it has been suggested that the action potential discharge of MSNs depolarized by excitatory glutamatergic inputs is the time when NAcc neurons execute their behaviorally relevant functions

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(O'Donnell et al. 1999). The patterns of spike discharge are thus sculpted mainly by a combination of glutamatergic excitation, GABAergic inhibition, and modulatory influences of DA. Recently, we demonstrated alterations in the intrinsic electrical membrane properties and enhanced glutamatergic synaptic transmission of MSNs in the NAcc core of rats during protracted withdrawal from chronic intermittent ethanol (CIE) treatment, a model of alcohol dependence (Marty and Spigelman 2012). In the companion article (Liang et al. 2014), we demonstrated CIE-induced alterations in the function and expression of GABA_A receptors (GABA_ARs). Here we studied the modulatory influence of DA and selective D1R and D2R ligands on GABA_AR-mediated currents in NAcc core MSNs in CIE rats and vehicle control (CIV) rats after protracted withdrawal with the goal of obtaining a better understanding of alterations in neurotransmission within the mesolimbic reward system in alcohol dependence.

Our studies reveal that at physiologically relevant concentrations DA selectively affects tonic GABA_AR currents, without effects on synaptic GABA_ARs. Furthermore, DA (10 nM) potentiates the tonic GABA_AR current (I_{tonic}) in MSNs, whereas higher [DA] (0.03–1 μM) depress this current. These bidirectional effects of DA on I_{tonic} are mediated by D1Rs (potentiation) and D2Rs (depression), respectively. We also show that modulation of I_{tonic} by selective D1R and D2R agonists is unaffected by CIE treatment and long-term withdrawal. However, because of the loss of EtOH potentiation of I_{tonic} in CIE rats, an EtOH challenge in these rats should result in unopposed D2R-mediated depression of I_{tonic} . Coupled with the demonstrated increases in glutamatergic excitatory neurotransmission after CIE treatment (Jeanes et al. 2011; Marty and Spigelman 2012; Szumlinski et al. 2007), we predict that EtOH-evoked DA release should produce larger increases in the “up-state” excitability of MSNs in alcohol-dependent compared with nondependent animals.

METHODS

The Institutional Animal Care and Use Committee approved all animal experiments. Male Sprague-Dawley rats (Harlan, weighing 190–220 g upon arrival) were housed in the vivarium under a 12:12-h light-dark cycle and had free access to food and water. Rats were administered a chronic intermittent EtOH (CIE) regimen: for the first 5 doses rats received 5 g/kg EtOH as a 25% (wt/vol) solution once every other day and for the following 55 doses 6 g/kg of EtOH 30% (wt/vol) once every day. The CIE control group received water (20 ml/kg). Naive rats age-matched to the CIV/CIE groups (weighing 300–350 g upon arrival) were housed under similar vivarium conditions for 1–2 mo prior to experiments.

Transverse brain slices (400 μm thick) at the level of the NAcc were obtained with standard techniques. Briefly, rats were decapitated under isoflurane anesthesia and brains were quickly removed, trimmed with a razor blade, and glued to the base of a cutting chamber (Leica VT1200S) filled with cold ($\sim 4^{\circ}\text{C}$) artificial cerebrospinal fluid (ACSF) composed of (in mM) 125 NaCl, 2.5 KCl, 2 CaCl_2 , 2 MgCl_2 , 26 NaHCO_3 , and 10 D-glucose (Sigma). The ACSF was continuously bubbled with a 95%–5% mixture of O_2 and CO_2 to ensure adequate oxygenation of slices and a pH of 7.4.

Patch electrode filling solutions contained (in mM) 135 Cs-gluconate or 135 CsCl, 2 MgCl_2 , 1 CaCl_2 , 11 ethylene glycol-bis(β -aminoethyl ether)- N,N,N',N' -tetraacetic acid (EGTA), 10 HEPES, 2 ATP- K_2 , and 0.2 GTP- Na_2 ; pH adjusted to 7.25 with CsOH. Whole cell patch-clamp recordings with CsCl- or Cs-gluconate-based pipette

solutions were obtained with a Multiclamp 700B amplifier and a Digidata 1440A A/D converter (Molecular Devices, Sunnyvale, CA) from cells in the NAcc core region during perfusion (1 ml/min) with oxygenated ACSF at $34 \pm 0.5^{\circ}\text{C}$. Most cells were putatively identified as MSNs within 30 s of membrane breakthrough by the presence of a characteristic delay in action potential generation with depolarizing current pulses (Marty and Spigelman 2012); those that did not possess these characteristics were discarded. Next, GABA_AR-mediated currents were separated pharmacologically by application of TTX (0.5 μM), CNQX (10 μM), APV (40 μM), and CGP54626 (1 μM) in the ACSF. Cells were subsequently voltage-clamped at -70 mV (CsCl) or 0 mV (Cs-gluconate), and recordings began at least 10 min after membrane breakthrough to ensure adequate dialysis by intrapipette contents. Picrotoxin, DA, and various D1R and D2R ligands were applied after appropriate dilution in the ACSF. The concentrations of the DAR ligands were chosen to maximize selectivity of their actions at a given DAR subtype (Ohlstein and Berkowitz 1985; Seeman and Van Tol 1994).

The recordings were low-pass filtered (Clampfit software, Molecular Devices) at 2 kHz and analyzed with the aid of the Mini Analysis Program (Synaptosoft, Fort Lee, NJ). Miniature inhibitory postsynaptic currents (mIPSCs) were detected with threshold criteria of 5-pA amplitude and 20-fC charge transfer. Frequency of mIPSCs was determined from all automatically detected events in a given recording period. Tonic current magnitudes were obtained from the mean baseline current of a given recording period. For kinetic analysis of mIPSCs, only single events with a stable baseline, sharp rising phase, and exponential decay were chosen. Double and multiple-peak mIPSCs were excluded. The mIPSC kinetics were obtained from analysis of the averaged chosen single events (>120 events/100-s recording period) aligned with half rise time in each cell.

The investigators performing the recordings and analysis were blind to the treatment (naive, CIV, or CIE) that the rats received. All summary values are presented as means $\pm$ SE. Group differences were evaluated by unpaired Student's *t*-test or one-way analysis of variance (ANOVA), where appropriate. $P < 0.05$ was considered statistically significant.

RESULTS

Selective concentration-dependent effects of DA on tonic GABA_AR current. We first examined the effects of DA application on isolated GABA_AR currents in NAcc MSNs from naive rats (Fig. 1). We chose to use [DA] in the range reported in the NAcc of awake rats at basal conditions and during phasic DA release (Budygin et al. 2001; Owesson-White et al. 2012; Robinson et al. 2009; Segovia and Mora 2001; Yim and Gonzales 2000). Application of DA at increasing concentrations (0.01–1 μM) had a biphasic effect on the picrotoxin (GABA_AR Cl^- channel blocker)-sensitive tonic current (I_{tonic}). In all MSNs tested, DA (10 nM) produced a significant increase of I_{tonic} , while higher [DA] (0.03–1 μM) had a significant depressant effect on I_{tonic} (Fig. 1, A and B). In contrast, DA (0.01–1 μM) had no significant effect on the average amplitude, charge transfer (area), or frequency of mIPSCs (Fig. 1, D–F). The rise time and decay kinetic parameters of mIPSCs were similarly unaffected by DA (0.01–1 μM) application (data not shown). The effect of DA on I_{tonic} was fully reversible upon washout, irrespective of the [DA] applied (e.g., Fig. 1B).

Potentiation and depression of I_{tonic} by DA are mediated by D1Rs and D2Rs, respectively. Since the effects of DA at its different receptor subtypes are determined in part by differences in the affinity for DA (Rankin et al. 2010; Seeman and

Fig. 1. Concentration-dependent effects of dopamine (DA) on the tonic GABA type A receptor (GABA_{A}) current (I_{tonic}). *A*, *bottom*: continuous voltage-clamp recording of pharmacologically isolated GABA_{A} currents in a nucleus accumbens (NAcc) core medium spiny neuron (MSN) from a naive rat. The picrotoxin (PTX, $50 \mu\text{M}$)-sensitive I_{tonic} is indicated by the dashed lines. Note the increase in I_{tonic} during DA (10 nM) application and the subsequent decrease during DA (30 nM) application. *Top*: the miniature inhibitory postsynaptic currents (mIPSCs) averaged over the indicated 100-s recording intervals are unaffected by DA applications. *B*: in another MSN recording from a naive rat, application of DA (100 nM) reversibly decreases I_{tonic} (*bottom*) without affecting mIPSCs (*top*). *C*: summary graph of concentration-dependent DA effects on I_{tonic} . * $P < 0.05$ ($n = 2$ – 6 neurons from 2 rats, 1-way ANOVA). *D*–*F*: summary graphs of DA effects on mIPSC amplitude (*D*), area (*E*), and frequency (*F*).

Van Tol 1994; Undieh 2010), we tested for DA effects on I_{tonic} in the presence of D1R or D2R antagonists. To that end, we recorded GABA_{A} current responses to DA first at 10 nM and then at $1 \mu\text{M}$ during continuous application of the selective D1R antagonist SKF 83566 at 10 nM (Ohlstein and Berkowitz 1985). SKF 83566 application alone had no discernible effects on I_{tonic} (Fig. 2A). In the continued presence of SKF 83566, application of DA (10 nM) did not increase I_{tonic} , whereas

subsequent application of DA ($1 \mu\text{M}$) had a significant ($>30\%$) depressant effect on I_{tonic} (Fig. 2, A and C). These pharmacological manipulations had no effect on mIPSC amplitude or charge transfer, with only a small ($<3\%$) effect on mIPSC frequency (Fig. 2D). These data suggested that D1Rs mediate potentiation of I_{tonic} while D2Rs depress I_{tonic} . To confirm this, we measured GABA_{A} current responses to DA first at 10 nM and then at $1 \mu\text{M}$ during continuous application

Fig. 2. D1 receptor (D1R)-mediated potentiation and D2 receptor (D2R)-mediated depression of I_{tonic} . **A**: selective D1R antagonist SKF 83566 (10 nM) blocks DA (10 nM) potentiation of I_{tonic} but not DA (1 μM) depression of I_{tonic} . **B**: in the presence of the selective D2R antagonist sulpiride, DA (10 nM and 1 μM) application potentiates I_{tonic} . Horizontal brackets indicate the 100-s recording periods used for I_{tonic} and mIPSC analysis. **C** and **D**: summary graphs of SKF 83566 effects on DA modulation of I_{tonic} (**C**) and mIPSCs (**D**). * $P < 0.05$ ($n = 6$ neurons from 2 rats, 1-way ANOVA vs. control). **E** and **F**: summary graphs of sulpiride effects on DA modulation of I_{tonic} (**E**) and mIPSCs (**F**). * $P < 0.05$ ($n = 4$ or 5 neurons from 3 rats, 1-way ANOVA vs. control).

of the selective D2R antagonist sulpiride at 30 nM (Seeman and Van Tol 1994). Sulpiride application alone had no discernible effects on I_{tonic} (Fig. 2, **B** and **E**). However, in the continued presence of sulpiride, application of DA (10 nM) significantly increased I_{tonic} by >20% (Fig. 2**E**). During the subsequent application of DA (1 μM), the I_{tonic} remained significantly potentiated above baseline (Fig. 2, **B** and **E**). During these pharmacological manipulations, only the amplitude of mIPSCs during the combined sulpiride + 10 nM DA application was significantly increased (<8%), while the frequency of mIPSCs was unaffected by application of sulpiride

or sulpiride-DA combinations (Fig. 2**F**). The charge transfer, rise time, and decay kinetic parameters of mIPSCs were similarly unaffected by sulpiride-DA application (not shown). Together, these data suggested that potentiation and depression of I_{tonic} by DA are mediated by D1Rs and D2Rs, respectively.

D2R-mediated effects on I_{tonic} are blocked in recordings with intrapipette guanosine 5'-O-(2-thiodiphosphate). Modulation of I_{tonic} magnitude by DA could be due to modulation of postsynaptic GABA_AR function by postsynaptic DA receptors or to DA-induced changes in ambient GABA concentrations ([GABA]). To help differentiate between these two possibili-

Fig. 3. D2R-mediated decrease in I_{tonic} is mediated by a postsynaptic G protein-dependent mechanism. **A**: representative recording of GABA_A currents in the NAcc MSNs from a naive rat with patch pipettes filled with a CsCl-based control internal solution. Voltage was clamped at -70 mV. Bath application of quinpirole (5 nM) produces a large, reversible decrease (ΔI_{hold}) in the baseline holding current (dashed line) without affecting mIPSCs. Horizontal brackets indicate the 100-s recording periods used for I_{hold} and mIPSC analysis. **B**: during recording with pipettes containing guanosine 5'-O-(2-thiodiphosphate) (GDP β S), the response to quinpirole is abolished. **C**: when the GABA_A currents are blocked with PTX, the remaining holding current is unaffected by quinpirole. **D**: summary graph comparing the quinpirole effect in the 3 recording conditions. CsCl internal: $n = 4$; CsCl + GDP β S internal: $n = 6$; CsCl internal + PTX: $n = 4$. * $P < 0.05$, 1-way ANOVA. Δ Holding current = [holding current during quinpirole] – [holding current at baseline].

ties we measured responses to the application of a selective D2R agonist, quinpirole at 5 nM (Seeman and Van Tol 1994), in recordings from naive rat NAcc MSNs with patch pipettes containing a control internal solution and compared them to recordings with pipettes containing guanosine 5'-O-(2-thiodiphosphate) (GDP β S), which antagonizes G protein signaling within the recorded neurons (Schiffmann et al. 1995; Yan et al. 1997). For these recordings, we used CsCl-based intrapipette solutions and voltage-clamped the MSNs at -70 mV to demonstrate that the effects of DAR activation on I_{tonic} are independent of the GABA_A Cl^- current reversal potential. In control recordings, quinpirole (5 nM) application produced large reversible decreases in the basal holding current (Fig. 3A). Quinpirole had no significant effect on any of the postsynaptic mIPSC kinetic parameters (rise time: 90.8 ± 3.6 , amplitude: 98.1 ± 10.2 , decay τ_1 : 102.4 ± 3.1 , decay τ_2 : 108.5 ± 13.1 , charge transfer: 103.2 ± 20.3 ; all expressed as % of baseline). There was a trend toward decreased mIPSC frequency: 88.5 ± 7.3 of baseline, which did not reach statistical significance (paired t -test). In contrast, the quinpirole effect on the tonic holding current was almost completely abolished in recordings with GDP β S-containing patch pipettes (Fig. 3, B and D). In these recordings, quinpirole also had no significant effect on any of the postsynaptic mIPSC parameters (rise time: 112.7 ± 14.3 , amplitude: 99.9 ± 11.3 , decay τ_1 : 108.1 ± 22.3 , decay τ_2 : 79.0 ± 39.5 , charge transfer: 105.0 ± 27.1 ; expressed as % of baseline), but we did observe a significant ($P < 0.01$, paired t -test) decrease in mIPSC frequency (59.1 ± 7.4 of baseline), consistent with activation of presynaptic D2Rs (Delle Donne et al. 1997; Zhang and Sulzer 2012). We also showed that during recordings with control CsCl-based pipettes application of

quinpirole (5 nM) during continuous bath application of picrotoxin had no effect on the remaining holding current (Fig. 3, C and D). Together, these data suggested that DA-mediated modulation of I_{tonic} requires the activation of postsynaptic G protein-coupled DARs, which is separate from the D2R-mediated presynaptic effects on mIPSC frequency.

DA-mediated depression of I_{tonic} is unaffected by CIE treatment. We next compared the D2R-mediated depressant effect of DA (30 nM) in NAcc MSN recordings from CIV- and CIE-treated rats. In the same recordings we also measured the effect of acute EtOH (10 and 60 mM) application during coapplication with DA (Fig. 4A). Similar to recordings from naive rats, DA (30 nM) application produced a significant depression of I_{tonic} in the MSNs from CIV rats without significant changes in postsynaptic mIPSC kinetic parameters (rise time: 103.9 ± 3.0 , amplitude: 98.8 ± 0.7 , decay τ_1 : 111.6 ± 3.9 , decay τ_2 : 136.5 ± 36.4 , charge transfer: 103.2 ± 20.3 ;

Fig. 4. Alterations in DA and EtOH effects on I_{tonic} after chronic intermittent ethanol (CIE) treatment. **A**: representative recordings of GABA_A currents in the NAcc MSNs from vehicle (CIV, top)- and CIE (bottom)-treated rats. DA (30 nM) application produces a visible depression of the picrotoxin-sensitive I_{tonic} (dashed lines). Subsequent coapplication of EtOH (10 and 60 mM) with DA increases I_{tonic} . Horizontal brackets indicate the 100-s recording periods used for I_{tonic} and mIPSC analysis. **B**: summary graph compares I_{tonic} depression by DA (30 nM) in CIV- and CIE-treated rats. Note the similar effect of DA on I_{tonic} from the 2 groups of rats. * $P < 0.05$ ($n = 6$ neurons from 2 rats/group, t -test vs. control). **C**: summary graph compares I_{tonic} potentiation by EtOH (10 and 60 mM) in the presence of DA (30 nM) in CIV- and CIE-treated rats. Note the loss of EtOH potentiation of I_{tonic} in NAcc MSNs from CIE-treated rats. * $P < 0.05$ (1-way ANOVA vs. control).

expressed as % of baseline). However, there was a small ($92.3 \pm 1.9\%$ of baseline) but significant ($P < 0.02$, paired t -test) decrease in mIPSC frequency after DA application, which was not observed in the initial set of DA application recordings (cf. Fig. 1).

During continuous application of DA, application of EtOH, first at 10 mM and then at 60 mM, resulted in significant concentration-dependent increases in I_{tonic} during recordings from MSNs of CIV rats (Fig. 4). When comparing recordings from CIE rats, DA (30 nM) still produced a significant depression of I_{tonic} (Fig. 4B), while EtOH (10–60 mM) no longer potentiated I_{tonic} (Fig. 4C). In these recordings from CIE rat MSNs, DA application again had no significant effect on postsynaptic mIPSC kinetic parameters (rise time: 97.4 ± 6.0 , amplitude: 99.0 ± 0.4 , decay τ_1 : 100.6 ± 3.6 , decay τ_2 : 112.9 ± 23.5 , charge transfer: 103.2 ± 20.3 ; expressed as % of baseline), although we again observed a significant ($P < 0.01$, paired t -test) decrease ($84.4 \pm 2.2\%$ of baseline) in mIPSC frequency after DA application.

CIE treatment does not alter I_{tonic} response to selective D1R and D2R agonists. To further assess whether CIE treatment alters dopaminergic modulation of I_{tonic} , we compared GABA_AR current responses to application of the selective D1R agonist SKF 38393 at 0.1 μM or separately to application of the selective D2R agonist quinpirole at 2 nM (Seeman and Van Tol 1994). Application of SKF 38393 produced an enhancement of the tonic current in the MSNs of CIE and CIV rats (Fig. 5). Subsequent coapplication of the selective D1R antag-

onist SKF 83566 (0.1 μM) reversed this effect (Fig. 5A), confirming selectivity of D1R activation for I_{tonic} potentiation. In recordings from CIV rats, SKF 38393 (0.1 μM) application had no significant effect on any mIPSC parameters (rise time: 105.2 ± 8.7 , amplitude: 106.7 ± 3.5 , decay τ_1 : 110.9 ± 12.5 , decay τ_2 : 133.7 ± 33.9 , charge transfer: 105.8 ± 7.8 , frequency: 101.8 ± 1.0 ; expressed as % of baseline). A similar lack of effects was observed in recordings from CIE rats (rise time: 106.8 ± 12.1 , amplitude: 100.5 ± 2.1 , decay τ_1 : 135.8 ± 17.7 , charge transfer: 111.8 ± 5.5 , frequency: 99.2 ± 1.2 ; expressed as % of baseline), with the exception of mIPSC decay τ_2 , which was significantly ($P < 0.05$, paired t -test) increased ($174.6 \pm 23.1\%$ of baseline).

Application of quinpirole suppressed I_{tonic} in MSNs from CIV and CIE rats, and this suppression was reversed by coapplication of the D2R antagonist sulpiride (30 nM) (Fig. 4B). Quinpirole application in recordings from CIV rat MSNs had no significant effect on postsynaptic mIPSC kinetic parameters (rise time: 116.9 ± 7.0 , amplitude: 101.5 ± 0.7 , decay τ_1 : 100.0 ± 8.0 , decay τ_2 : 114.4 ± 28.3 , charge transfer: 107.9 ± 8.7 ; expressed as % of baseline), although there was a very small, but significant ($P < 0.05$, paired t -test), decrease in mIPSC frequency (98.6 ± 0.6 of baseline), consistent with previous observations. A mostly similar lack of effects was observed in recordings from CIE rat MSNs (rise time: 114.4 ± 7.1 , amplitude: 101.3 ± 1.1 , decay τ_1 : 111.9 ± 1.8 , decay τ_2 : 147.3 ± 24.8 , frequency: 99.2 ± 1.2 ; expressed as % of baseline). Surprisingly, mIPSC charge transfer was signifi-

Fig. 5. CIE treatment does not alter D1R- and D2R-mediated modulation of I_{tonic} . **A**: representative recording (CsCl-based patch electrode) of selective D1R agonist (SKF 38393, 0.1 μM) potentiation of the PTX-sensitive I_{tonic} (dashed lines) in a NAcc MSN from a CIE-treated rat. Note that coapplication of the selective D1R antagonist SKF 83566 (0.1 μM) together with SKF 38393 reverses this potentiation. **B**: representative recording of selective D2R agonist (quinpirole, 2 nM) depression of I_{tonic} in a NAcc MSN from a CIV-treated rat. Note that coapplication of the selective D2R antagonist sulpiride (30 nM) together with quinpirole reverses this depression. Horizontal brackets indicate the 100-s recording periods used for I_{tonic} and mIPSC analysis. **C**: summary graph illustrates comparable potentiation of I_{tonic} by SKF 38393 (1 μM) in CIV and CIE rats. * $P < 0.05$ ($n = 4-6$ neurons from 2-4 rats/group, t -test vs. control). **D**: summary graph illustrates comparable depression of I_{tonic} by quinpirole (2 nM) in CIV and CIE rats. * $P < 0.05$ ($n = 9-14$ neurons from 4-6 rats/group, t -test vs. control).

cantly ($P < 0.001$, paired t -test) increased ($111.9 \pm 1.8\%$ of baseline), which was in contrast to the large decreases in I_{tonic} observed in these recordings.

Importantly, comparison of D1R-mediated potentiation of I_{tonic} between CIE and CIV rats did not reveal significant group differences (Fig. 4C). Similarly, D2R-mediated suppression of I_{tonic} was not different between CIE and CIV rats (Fig. 4D). These data suggested that CIE treatment does not modify the modulatory effect of DA on GABA_AR-mediated I_{tonic} .

DISCUSSION

Studies utilizing microdialysis and fast scanning voltammetry techniques demonstrated that [DA] measured in the NAcc of awake rats range from low nanomolar basal levels to high submicromolar concentrations during phasic DA release (Budygin et al. 2001; Owesson-White et al. 2012; Robinson et al. 2009; Segovia and Mora 2001; Yim and Gonzales 2000). Here we demonstrate that application of DA in NAcc slices at such physiologically relevant concentrations (0.01 – $1 \mu\text{M}$) preferentially modulates extrasynaptic GABA_ARs that mediate the tonic current, without significant effects on synaptic GABA_ARs. Studies in murine MSNs in the dorsal striatum have also suggested preferential modulation of I_{tonic} by DA receptor ligands compared with synaptic currents (Janssen et al. 2009). Analogous to the hippocampus (Glykys et al. 2008; Liang et al. 2007; Scimemi et al. 2005), I_{tonic} in NAcc MSNs is mediated by several different combinations of extrasynaptic GABA_AR subunits that demonstrate considerable alterations after CIE treatment (companion article, Liang et al. 2014). These low-conductance, high-affinity extrasynaptic GABA_ARs are activated by ambient GABA whose sources may include spillover from the synaptic cleft during synaptic transmission (Glykys and Mody 2007; however, see Bright et al. 2011) and channel-mediated release from astrocytes (Lee et al. 2010).

The selectivity of DA actions on I_{tonic} could result from DA modulation of extracellular [GABA]. Changes in extracellular [GABA] may result from changes in the amount of released GABA or its uptake. Presynaptic effects of DA on neuronal GABA release are well-established (Feuerstein 2008; Zhang and Sulzer 2012), and we did observe decreases in frequency of mIPSCs after DA (30 nM) or quinpirole application, without concomitant changes in postsynaptic mIPSC kinetic parameters. These data suggested that the observed DA actions on I_{tonic} might be mediated by changes in synaptic GABA release. GABA may also be released from astrocytes via Bestrophin 1 channels (Lee et al. 2010). Striatal astrocytes were demonstrated to express both D1Rs and D2Rs (Bal et al. 1994; Hosli and Hosli 1987; Miyazaki et al. 2004; Zanassi et al. 1999). Conceivably, activation of these DARs could modulate GABA release from astrocytes. Indeed, D1R activation was shown to increase extracellular [GABA] via nonvesicular transporter-mediated GABA release in striatal slices and primary cultures (Schoffelmeer et al. 2000). However, we demonstrated that blockade of postsynaptic G protein signaling in the recorded MSNs with GDPβS abolished D2R receptor-mediated decreases in I_{tonic} (Fig. 3), without affecting postsynaptic mIPSC kinetic parameters. Notably, during this blockade of postsynaptic G protein signaling in the recorded MSNs, mIPSC frequency could still be decreased by quinpirole application. These experiments provided concrete evidence for a postsyn-

aptic mechanism by which extrasynaptic GABA_ARs are modulated by DAR activation in MSNs. A postsynaptic mechanism of action was previously demonstrated for the D3R-mediated suppression of GABA_AR currents in the NAcc (Chen et al. 2006). Also, functional postsynaptic G protein-dependent cross talk between GABA_BRs and GABA_ARs that mediate I_{tonic} has been recently demonstrated in thalamic, dentate gyrus, and cerebellar neurons (Connelly et al. 2013).

The preferential DA responsiveness of extrasynaptic GABA_ARs in NAcc MSNs could result from preferential removal of extracellular synaptic DA by DA transporters localized around GABAergic synapses. However, extensive cellular and subcellular studies have failed to localize DA transporter to any synaptic active zones in the striatum, suggesting that striatal DA reuptake likely occurs outside of synaptic specializations once DA diffuses from the synaptic cleft (Hersch et al. 1997). Furthermore, the fact that I_{tonic} is also preferentially modulated by selective D1R and D2R agonists that are not subject to DA transporter-mediated removal makes this an unlikely possibility. More likely is the differential coupling of second messenger mechanisms to the unique subunit composition of extrasynaptic GABA_ARs versus synaptic GABA_ARs. In the NAcc as in other forebrain regions, extrasynaptic GABA_ARs include, together with the ubiquitous β subunits, various combinations of $\alpha 4$, $\alpha 5$, and δ subunits. Isoform-specific phosphorylation of individual GABA_AR subunits by protein kinase A (PKA) and protein kinase C (PKC) has been shown to differentially affect activation, pharmacological responsiveness, and membrane recycling of extrasynaptic versus synaptic GABA_ARs (Brandon et al. 1999; Kia et al. 2011; Werner et al. 2011). More studies are needed to precisely identify the extrasynaptic GABA_AR subunit combinations affected by DAR activation.

The observed selective potentiation of postsynaptic GABA_ARs by D1Rs and their selective depression by D2Rs is supported by previous studies in mice with genetic ablation of D1Rs or D2Rs (Centonze et al. 2003), which showed a loss of DA-dependent depression of IPSCs evoked in striatal interneurons of D2R-null mice. Differential D1R versus D2R regulation of IPSCs by DA was also demonstrated in recordings from rat prefrontal cortex neurons (Trantham-Davidson et al. 2004). It is noteworthy that while activation of both synaptic and extrasynaptic GABA_ARs contributes to the action potential-dependent evoked IPSCs measured in the above studies, GABA_ARs giving rise to action potential-independent synaptic mIPSCs were separable from the extrasynaptic GABA_ARs giving rise to I_{tonic} in our recordings. Recent studies in the NAcc of mice have confirmed both D1R-mediated potentiation and D2R-mediated depression of extrasynaptic $\alpha 4\beta\delta$ GABA_AR activity and also showed that such differential effects on MSN neuron excitability can modulate the behavioral response to psychostimulants (Maguire et al. 2014). The differential responsiveness of GABA_ARs to D1R or D2R activation is likely due to the activation of different intracellular signaling pathways by these DAR subtypes. The D1-class DARs (D1R and D5R) are generally known to activate the $G_{s/\text{olf}}$ family of G proteins to stimulate adenylyl cyclase (AC) and PKA activity, whereas the D2-class DARs (D2R, D3R, and D4R) couple to $G_{i/o}$ proteins, resulting in inhibition of AC and a decrease in PKA activity (Beaulieu and Gainetdinov 2011). Amplification of PKA signaling is mediated by the DA- and cAMP-regulated phosphoprotein (DARPP-32), which is highly expressed in MSNs,

where it acts as an integrator on modulatory responses to multiple neurotransmitters, including DA (Bateup et al. 2008; Nishi et al. 1997; Svenningsson et al. 2004). Coexpression of D1Rs and D2Rs may lead to formation of D1R:D2R heterodimers that, acting through $G\alpha_q$, can regulate downstream calcium signaling (Rashid et al. 2007).

Numerous studies have shown that striatal MSNs, including those in the NAcc core, form two principal subgroups that are defined by their selective expression of DAR subtypes and their projection targets (reviewed in Beaulieu and Gainetdinov 2011; Valjent et al. 2009). For example, murine NAcc MSNs projecting to the VTA selectively express D1Rs, thus resembling the striatonigral (direct pathway) MSNs, while MSNs projecting to the ventral pallidum express D2Rs and thus resemble the striatopallidal (indirect pathway) MSNs (Gangarossa et al. 2013). However, in addition to the two main subgroups there is a population of MSNs that express both D1Rs and D2Rs, with the highest incidence in NAcc (Lu et al. 1998; Perreault et al. 2010, 2011). Partial coexpression of D1R with D3R, particularly in the NAcc, has also been described (Le Moine and Bloch 1996). In our experiments, all recorded NAcc core MSNs tested with an application of a selective D1R agonist or 10 nM DA responded with I_{tonic} enhancement ($n = 14$), and conversely all MSNs tested with a selective D2R agonist or 0.03–1 μM DA responded with a decrease in I_{tonic} ($n = 40$). The probability that our “blind” patch recordings selectively targeted a particular subpopulation of MSNs is very small, suggesting instead that the ability of DA and selective DAR agonists to bidirectionally modulate the activity of extrasynaptic GABA_A Rs is common to all NAcc core MSNs. This is in agreement with the immunohistochemically and functionally demonstrated coexistence of D1Rs and D2Rs in virtually all embryonic rat striatal neurons after 3 wk in culture (Aizman et al. 2000) and with the functional and single-cell RNA amplification demonstrations of D1R, D2R, and D3R coexpression in retrogradely labeled single striatonigral rat MSNs (Surmeier et al. 1992). Taken together, these data suggest that there may be important species differences in the colocalization of DAR subtypes in direct and indirect pathway NAcc MSNs between rats and mice. Furthermore, the extensive colocalization of DARPP-32 with both D1R- and D2R-positive MSNs in the rat striatum (Rajput et al. 2009) provides a plausible intracellular substrate by which bidirectional modulation of I_{tonic} might be achieved. This conjecture would have to be thoroughly tested in future studies. It would also be of interest to determine whether there are differences in DA modulation of I_{tonic} between rat MSNs projecting to the VTA and those projecting to the ventral pallidum.

In MSNs at their hyperpolarized resting (“down”) state, similar to hippocampal interneurons (Song et al. 2011), the GABA_A current reversal potential is depolarizing, making baseline I_{tonic} excitatory. Indeed, excitatory synaptic activation of single action potentials in MSNs is reduced during application of L655,703, which preferentially antagonizes I_{tonic} in MSNs (Ade et al. 2008). In contrast, when MSNs are in their depolarized “up” state of action potential discharge, the GABA_A current reversal potential is hyperpolarizing, making baseline I_{tonic} inhibitory. Therefore, low basal levels of extracellular DA (≤ 10 nM) would be expected to exert an excitatory influence on MSNs at their “down” state by D1R-mediated potentiation of I_{tonic} , but when MSNs are in their

depolarized “up” state D1R-mediated potentiation of I_{tonic} should have a hyperpolarizing inhibitory effect. Furthermore, phasic increases in DA release resulting in higher [DA] (≥ 30 nM) would be expected to block I_{tonic} via D2Rs. If phasic DA release were time-locked with activation of excitatory glutamatergic inputs in the NAcc, the D2R-mediated block of I_{tonic} would then effectively accentuate the “up” state of affected MSNs. Evidence for the synchronized release of glutamate and DA includes the demonstration that dopaminergic terminals in the NAcc (but not the dorsal striatum) corelease glutamate, such that their selective optogenetic activation results in robust glutamate-mediated excitatory postsynaptic currents (EPSCs) in NAcc (but not dorsal striatum) MSNs (Stuber et al. 2010). Other studies have shown that cholinergic interneurons also corelease glutamate and that their selective optogenetic activation not only elicits EPSCs in MSNs (Higley et al. 2011) but also elicits DA release directly from DA terminals in the NAcc, this release being dependent on glutamatergic receptor activity (Cachope et al. 2012). Also, in vivo recordings of NAcc neuron activity coupled with fast scanning voltammetry measurements showed that phasic DA selectively modulates excitatory responses of NAcc neurons during cue-evoked sucrose-seeking behavior (Cacciapaglia et al. 2011). On the basis of these studies and our present findings, we propose that during basal DA release D1R-mediated potentiation of I_{tonic} will either increase or decrease the excitability of MSNs depending on their membrane potential. In contrast, D2R activation, which should be greatest during peak phasic DA release, will increase MSN excitability by blocking I_{tonic} , thereby accentuating the throughput of strong glutamatergic inputs.

Neither the magnitude of I_{tonic} nor the relative effectiveness of D1R- and D2R-mediated modulation of I_{tonic} is altered by CIE treatment (Fig. 5 and Liang et al. 2014). The observed lack of CIE-induced changes in modulation of I_{tonic} by selective DAR agonists is consistent with the reported absence of changes in D2Rs after a chronic EtOH diet (Tajuddin and Druse 1996). Also, in alcohol-dependent animals basal DA release is decreased, yet an EtOH challenge evokes comparable if not greater DA release than in nondependent control animals (Budygin et al. 2007; Diana et al. 1992, 1993; Weiss et al. 1996; Yoshimoto et al. 1996). However, EtOH potentiation of I_{tonic} is lost in CIE rats (Fig. 4 and Liang et al. 2014), and therefore an EtOH challenge in alcohol-dependent rats should result in unopposed D2R-mediated block of I_{tonic} . Coupled with the demonstrated increases in glutamatergic excitatory neurotransmission after CIE treatment (Jeanes et al. 2011; Marty and Spigelman 2012; Szumlanski et al. 2007), we predict that EtOH-evoked DA release should produce larger increases in the “up-state” excitability of MSNs in CIE rats compared with control rats. These neuroadaptive changes could contribute to the high levels of voluntary EtOH intake previously demonstrated in CIE rats (Rimondini et al. 2003). Analogous changes in the human NAcc could contribute to the development of cravings and relapse during protracted withdrawal in alcohol dependence.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.L., V.N.M., and I.S. conception and design of research; J.L., V.N.M., Y.M., and I.S. performed experiments; J.L., V.N.M., and I.S. analyzed data; J.L., V.N.M., R.W.O., and I.S. interpreted results of experiments; J.L., V.N.M., and I.S. prepared figures; J.L., V.N.M., Y.M., R.W.O., and I.S. edited and revised manuscript; J.L., V.N.M., Y.M., R.W.O., and I.S. approved final version of manuscript; V.N.M. and I.S. drafted manuscript.

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