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 μM), 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 μM) is mediated by D2Rs in the same MSNs. Addition of guanosine 5′-O-(2-thiodiphosphate) (GDPβS) 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
(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 Spiegelman 2012). In the companion article (Liang et al. 2014), we demonstrated CIE-induced alterations in the function and expression of GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs). Here we studied the modulatory influence of DA and selective D1R and D2R ligands on GABA<sub>A</sub>R-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<sub>A</sub>R currents, without effects on synaptic GABA<sub>A</sub>Rs. Furthermore, DA (10 nM) potentiates the tonic GABA<sub>A</sub> R current (I<sub>tonic</sub>) in MSNs, whereas higher [DA] (0.03–1 μM) depress this current. These bidirectional effects of DA on I<sub>tonic</sub> are mediated by D1Rs (potentiation) and D2Rs (depression), respectively. We also show that modulation of I<sub>tonic</sub> 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<sub>tonic</sub> in CIE rats, an EtOH challenge in these rats should result in unopposed D2R-mediated depression of I<sub>tonic</sub>. Coupled with the demonstrated increases in glutamatergic excitatory neurotransmission after CIE treatment (Jeanes et al. 2011; Marty and Spiegelman 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). Naïve 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, immersed with a razor blade, and glued to the base of a cutting chamber (Leica VT1200S) filled with cold (~4°C) artificial cerebrospinal fluid (ACSF) composed of (in mM) 125 NaCl, 2.5 KCl, 2 CaCl<sub>2</sub>, 2 MgCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, and 10 d-glucose (Sigma). The ACSF was continuously bubbled with a 95%-5% mixture of O<sub>2</sub> and CO<sub>2</sub> to ensure adequate oxygenation of slices and a pH of 7.4.

Patch electrode filling solutions contained (in mM) 135 Cs-glucuronate and 135 CsCl, 2 MgCl<sub>2</sub>, 1 CaCl<sub>2</sub>, 11 ethylene glycol-bis(β-aminooethyl ether)-N,N',N''-tetraacetic acid (EGTA), 10 HEPES, 2 ATP-K<sub>2</sub>, and 0.2 GTP-Na<sub>2</sub>; pH adjusted to 7.25 with CsOH. Whole cell patch-clamp recordings with CsCl- or Cs-glucuronate-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 ± 0.5°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 Spiegelman 2012); those that did not possess these characteristics were discarded. Next, GABA<sub>A</sub>R-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-glucuronate), and recordings began at least 10 min after membrane breakthrough to ensure adequate dialysis by intracellular 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-μs 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 (naïve, CIV, or CIE) that the rats received. All summary values are presented as means ± 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<sub>A</sub>R current. We first examined the effects of DA application on isolated GABA<sub>A</sub>R currents in NAcc MSNs from naïve 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; Yin and Gonzales 2000). Application of DA at increasing concentrations (0.01–1 μM) had a biphasic effect on the picrotoxin (GABA<sub>A</sub>R cl– channel blocker)-sensitive tonic current (I<sub>tonic</sub>). In all MSNs tested, DA (10 nM) produced a significant increase of I<sub>tonic</sub>, while higher [DA] (0.03–1 μM) had a significant depressive effect on I<sub>tonic</sub> (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<sub>tonic</sub> was fully reversible upon washout, irrespective of the [DA] applied (e.g., Fig. 1B).

Potentiation and depression of I<sub>tonic</sub> 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
Van Tol 1994; Undieh 2010), we tested for DA effects on $I_{\text{tonic}}$ in the presence of D1R or D2R antagonists. To that end, we recorded GABA$_A$R current responses to DA first at 10 nM and then at 1 $\mu$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 $I_{\text{tonic}}$ (Fig. 2A). In the continued presence of SKF 83566, application of DA (10 nM) did not increase $I_{\text{tonic}}$, whereas subsequent application of DA (1 $\mu$M) had a significant (>30%) depressant effect on $I_{\text{tonic}}$ (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 $I_{\text{tonic}}$ while D2Rs depress $I_{\text{tonic}}$. To confirm this, we measured GABA$_A$R current responses to DA first at 10 nM and then at 1 $\mu$M during continuous application
of the selective D2R antagonist sulpiride at 30 nM (Seeman and Van Tol 1994). Sulpiride application alone had no discernible effects on $I_{\text{tonic}}$ (Fig. 2, B and E). However, in the continued presence of sulpiride, application of DA (10 nM) significantly increased $I_{\text{tonic}}$ by $>20\%$ (Fig. 2E). During the subsequent application of DA (1 $\mu$M), the $I_{\text{tonic}}$ 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. 2F). 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 $I_{\text{tonic}}$ by DA are mediated by D1Rs and D2Rs, respectively. D2R-mediated effects on $I_{\text{tonic}}$ are blocked in recordings with intrapipette guanosine 5'-O-(2-thiodiphosphate). Modulation of $I_{\text{tonic}}$ magnitude by DA could be due to modulation of postsynaptic GABA$_A$R function by postsynaptic DA receptors or to DA-induced changes in ambient GABA concentrations ([GABA]). To help differentiate between these two possibili-
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) (GDPβS), 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 $I_{\text{ tonic}}$ are independent of the GABAAR 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 τ1: 102.4 ± 3.1, decay τ2: 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 GDPβS-containing patch pipettes (Fig. 3B). 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 τ1: 108.1 ± 22.3, decay τ2: 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 $I_{\text{ tonic}}$ 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 $I_{\text{ tonic}}$ 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 $I_{\text{ tonic}}$ 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 τ1: 111.6 ± 3.9, decay τ2: 136.5 ± 36.4, charge transfer: 103.2 ± 20.3; all expressed as % of baseline). The response to quinpirole is abolished. C: when the GABAAR 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 + GDPβS internal: n = 6; CsCl internal + PTX: n = 4. *P < 0.05, 1-way ANOVA. ΔHolding current = [holding current during quinpirole] − [holding current at baseline].
expressed as % of baseline). However, there was a small (92.3 ± 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 $I_{\text{tonic}}$ during recordings from MSNs of CIV rats (Fig. 4). When comparing recordings from CIE rats, DA (30 nM) still produced a significant depression of $I_{\text{tonic}}$ (Fig. 4B), while EtOH (10–60 mM) no longer potentiated $I_{\text{tonic}}$ (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 $\tau_1$: 100.6 ± 3.6, decay $\tau_2$: 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 ± 2.2% of baseline) in mIPSC frequency after DA application.

**CIE treatment does not alter $I_{\text{tonic}}$ response to selective D1R and D2R agonists.** To further assess whether CIE treatment alters dopaminergic modulation of $I_{\text{tonic}}$, we compared GABA$_{A}$R 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 antagonist SKF 83566 (0.1 µM) reversed this effect (Fig. 5A), confirming selectivity of D1R activation for $I_{\text{tonic}}$ 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 $\tau_1$: 110.9 ± 12.5, decay $\tau_2$: 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 $\tau_1$: 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 $\tau_2$, which was significantly ($P < 0.05$, paired $t$-test) increased (174.6 ± 23.1% of baseline).

Application of quinpirole suppressed $I_{\text{tonic}}$ 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 $\tau_1$: 100.0 ± 8.0, decay $\tau_2$: 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 $\tau_1$: 111.9 ± 1.8, decay $\tau_2$: 147.3 ± 24.8, frequency: 99.2 ± 1.2; expressed as % of baseline). Surprisingly, mIPSC charge transfer was signifi-

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**Fig. 5.** CIE treatment does not alter D1R- and D2R-mediated modulation of $I_{\text{tonic}}$. A: representative recording (CsCl-based patch electrode) of selective D1R agonist (SKF 38393, 0.1 µM) potentiation of the PTX-sensitive $I_{\text{tonic}}$ (dashed lines) in a NAcc MSN from a CIE-treated rat. Note that coapplication of the selective D1R antagonist SKF 83566 (30 nM) together with quinpirole reverses this depression. Horizontal brackets indicate the 100-s recording periods used for $I_{\text{tonic}}$ and mIPSC analysis. C: summary graph illustrates comparable potentiation of $I_{\text{tonic}}$ 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 $I_{\text{tonic}}$ 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 ± 1.8% of baseline), which was in contrast to the large decreases in $I_{\text{tonic}}$ observed in these recordings.

Importantly, comparison of D1R-mediated potentiation of $I_{\text{tonic}}$ between CIE and CIV rats did not reveal significant group differences (Fig. 4C). Similarly, D2R-mediated suppression of $I_{\text{tonic}}$ 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$_A$R-mediated $I_{\text{tonic}}$.

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 μM) preferentially modulates extrasynaptic GABA$_A$Rs that mediate the tonic current, without significant effects on synaptic GABA$_A$Rs. Studies in murine MSNs in the dorsal striatum have also suggested preferential modulation of $I_{\text{tonic}}$ 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), $I_{\text{tonic}}$ in NAcc MSNs is mediated by several different combinations of extrasynaptic GABA$_A$R subunits that demonstrate considerable alterations after CIE treatment (companion article, Liang et al. 2014). These low-conductance, high-affinity extrasynaptic GABA$_A$Rs 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 $I_{\text{tonic}}$ 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 $I_{\text{tonic}}$ 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 $I_{\text{tonic}}$ (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 postsynaptic mechanism by which extrasynaptic GABA$_A$Rs are modulated by DAR activation in MSNs. A postsynaptic mechanism of action was previously demonstrated for the D3R-mediated suppression of GABA$_A$R currents in the NAcc (Chen et al. 2006). Also, functional postsynaptic G protein-dependent cross talk between GABA$_A$Rs and GABA$_A$Rs that mediate $I_{\text{tonic}}$ has been recently demonstrated in thalamic, dentate gyrus, and cerebellar neurons (Connelly et al. 2013).

The preferential DA responsiveness of extrasynaptic GABA$_A$Rs 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 $I_{\text{tonic}}$ 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$_A$Rs versus synaptic GABA$_A$Rs. In the NAcc as in other forebrain regions, extrasynaptic GABA$_A$Rs include, together with the ubiquitous β subunits, various combinations of α4, α5, and δ subunits. Isoform-specific phosphorylation of individual GABA$_A$R 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$_A$Rs (Brandon et al. 1999; Kia et al. 2011; Werner et al. 2011). More studies are needed to precisely identify the extrasynaptic GABA$_A$R subunit combinations affected by DAR activation.

The observed selective potentiation of postsynaptic GABA$_A$Rs 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$_A$Rs contributes to the action potential-dependent evoked IPSCs measured in the above studies, GABA$_A$Rs giving rise to action potential-independent synaptic mIPSCs were separable from the extrasynaptic GABA$_A$Rs giving rise to $I_{\text{tonic}}$ in our recordings. Recent studies in the NAcc of mice have confirmed both D1R-mediated potentiation and D2R-mediated depression of extrasynaptic α4βδ GABA$_A$R 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$_A$Rs 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$_{i/o}$ family of G proteins to stimulate adenyl 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 Gq, 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 striatogniral (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 \( I_{\text{tonic}} \) enhancement (\( n = 14 \)), and conversely all MSNs tested with a selective D2R agonist or 0.03–1 \( \mu \text{M} \) DA responded with a decrease in \( I_{\text{tonic}} \) (\( n = 40 \)). The probability that our “blind” patch recordings selectively targeted a particular population of MSNs is very small, suggesting instead that the ability of DA and selective DAR agonists to bidirectionally modulate the activity of extrasynaptic GABAARs 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 striatogniral 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 \( I_{\text{tonic}} \) 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 \( I_{\text{tonic}} \) 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 GABAAR current reversal potential is depolarizing, making baseline \( I_{\text{tonic}} \) excitatory. Indeed, excitatory synaptic activation of single action potentials in MSNs is reduced during application of L655,703, which preferentially antagonizes \( I_{\text{tonic}} \) in MSNs (Ade et al. 2008). In contrast, when MSNs are in their depolarized “up” state of action potential discharge, the GABAAR current reversal potential is hyperpolarizing, making baseline \( I_{\text{tonic}} \) inhibitory. Therefore, low basal levels of extracellular DA (\( \leq 10 \text{nM} \)) would be expected to exert an excitatory influence on MSNs at their “down” state by D1R-mediated potentiation of \( I_{\text{tonic}} \), but when MSNs are in their depolarized “up” state D1R-mediated potentiation of \( I_{\text{tonic}} \) should have a hyperpolarizing inhibitory effect. Furthermore, phasic increases in DA release resulting in higher [DA] (\( \geq 30 \text{nM} \)) would be expected to block \( I_{\text{tonic}} \) via D2Rs. If phasic DA release were time-locked with activation of excitatory glutamatergic inputs in the NAcc, the D2R-mediated block of \( I_{\text{tonic}} \) 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 \( I_{\text{tonic}} \) 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 \( I_{\text{tonic}} \), thereby accentuating the throughput of strong glutamatergic inputs.

Neither the magnitude of \( I_{\text{tonic}} \) nor the relative effectiveness of D1R- and D2R-mediated modulation of \( I_{\text{tonic}} \) is altered by CIE treatment (Fig. 5 and Liang et al. 2014). The observed lack of CIE-induced changes in modulation of \( I_{\text{tonic}} \) 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 nondrinking control animals (Budygin et al. 2007; Diana et al. 1992, 1993; Weiss et al. 1996; Yoshimoto et al. 1996). However, EtOH potentiation of \( I_{\text{tonic}} \) 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 \( I_{\text{tonic}} \). 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 CIE rats compared with control rats. These neuroadaptative 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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No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


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