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Dopamine DARPP-32-Dependent Modulation of Prefrontal Cortical Input and Intercellular Coupling in Mouse Accumbens Spiny and Aspyne Neurons

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Abstract

The roles of DARPP-32 in mediating dopamine (DA)-dependent modulation of corticoaccumbens transmission and intercellular coupling were examined in mouse accumbens (NAC) neurons by both intracellular sharp electrode and whole cell recordings. In wildtype (WT) mice bath application of the D2-like agonist quinpirole resulted in 73% coupling incidence in NAC spiny neurons, compared to baseline (9%), whereas quinpirole failed to affect the basal coupling (24%) in slices from DARPP-32 knockout (KO) mice. Thus, D2 stimulation attenuated DARPP-32-mediated suppression of coupling in WT spiny neurons, but this modulation was absent in KO mice. Further, whole cell recordings revealed that quinpirole reversibly decreased the amplitude of cortical-evoked EPSPs in spiny neurons of WT mice, but this reduction was markedly attenuated in KO mice. Bath application of the D1/D5 agonist SKF 38393 did not alter evoked EPSP amplitude in WT or KO spiny neurons. Therefore, DA D2 receptor regulation of both cortical synaptic (chemical) and local non-synaptic (dye coupling) communications in NAC spiny neurons is critically dependent on intracellular DARPP-32 cascades. Conversely, in fast-spiking interneurons, blockade of D1/D5 receptors produced a substantial decrease in EPSP amplitude in WT, but not in KO mice. Lastly, in putative cholinergic interneurons, cortical-evoked disynaptic IPSPs were attenuated by D2-like receptor stimulation in WT but not KO slices. These data indicate that DARPP-32 plays a central role in 1) modulating intercellular coupling, 2) cortical excitatory drive of spiny and aspiny GABAergic neurons, and 3) local feedforward inhibitory drive of cholinergic-like interneurons within accumbens circuits

Keywords

Excitatory and inhibitory synaptic transmission; non-synaptic gap junction-mediated morphological coupling; DA D1 & D2 receptor; mouse accumbens neurons; whole cell recording; sharp-electrode recording

Altered glutamate transmission from the prefrontal cortex (PFC) to the ventral striatum is often implicated in schizophrenia and drug addiction; disorders that involve dopamine (DA) dysfunctional states (Grace, 1991; 2000; Greengard, 1999; 2001; Albert et al., 2002; Saal et al., 2003). DA acting on D2-like receptors present on prefrontal cortical terminals (Levey et

al., 1993; Sesack et al., 1994; Delle Donne et al., 1997; Filoux et al., 1988; Jansson et al., 1998) plays an important role in regulating cortico-striatal/accumbal glutamate transmission in spiny projection neurons (Brown and Arbuthnott, 1983; O'Donnell and Grace, 1994; Flores-Hernandez et al., 1997; Cepeda et al., 2001; Bamford et al., 2004). One molecule involved in dopamine (DA) transmission, DA and cyclic-AMP Regulated Phosphoprotein-32 (DARPP-32), exerts complex actions on DA-dependent modulation in limbic prefrontal corticoaccumbens circuits (Greengard et al., 1999) and is decreased in brains of schizophrenia patients (Albert et al., 2002).

DARPP-32 is mainly expressed in striatal/accumbens spiny projection neurons, as well as in cortical pyramidal neurons (Berger et al., 1990; Gaspar et al., 1995; Ouimet et al., 1998; Albert et al., 2002; Narin et al., 2004; Wang et al., 2004), which are densely innervated by DA and contain DA D1- and D2-like receptor subtypes. Activation of DA D1-like receptors (D1 and D5) is reported to increase DARPP-32 phosphorylation in those neurons that express DARPP-32 proteins (Nishi et al., 1999). Studies in DARPP-32 knockout mice show that many of the physiological and behavioral effects of DA are mediated by DARPP-32 intracellular signaling pathways (Fienberg et al., 1998; Svenningsson et al., 2000; 2005). Electrophysiological responses to the DA D1/D5 agonist, including modulation of membrane excitability (Onn et al., 2003), long term potentiation and depression (Calabresi et al., 2000) and NMDA or GABA_A postsynaptic currents (Flores-Hernandez et al., 2000; 2002), are absent in striatal/accumbens spiny neurons of DARPP-32 knockout mice. Despite its established relationship with D1/D5 receptor-mediated mechanisms, DARPP-32 intracellular cascades are also obligatory for D2 receptor-mediated effects (Greengard et al., 1999; Nishi et al., 1999). Activation of D2-like receptors is reported to decrease DARPP-32 phosphorylation not only in the predominantly D2-containing striatopallidal projection neurons, but also in D1-containing striatonigral projection neurons (Lindskog et al., 1999). We have previously shown that DARPP-32 molecules are critical for synergistic excitation through postsynaptic D1-D2 receptor interaction in mouse striatal spiny projection neurons (Onn et al., 2003). This D1-D2 synergy in spiny neurons may be due to a direct drug action on DA receptor-coupled G-proteins (Hopf et al., 2003) and/or circuit interactions, i.e., DA-dependent modulation of other transmitter (e.g., glutamate) release within the accumbens (for review, see Nicola et al., 2000; Onn et al., 2000; Grace, 2002; Tepper et al., 2004; Surmeier et al., 2007; Wilson, 2007).

DA can also affect network interactions among neurons via actions on gap junctions and has been shown to occur within the rat striatum/accumbens (Cepeda et al., 1989; O'Donnell and Grace, 1993; Onn and Grace, 1994; 1995; 1999; 2000). Striatal/accumbens spiny neurons express the neuronal gap junction proteins connexin 32 and 36 (Micevych and Abelson, 1991; Rash et al., 2001; Meier et al., 2002; Venance et al., 2004). DA transmission via D1-like receptors is known to uncouple neurons through cAMP-dependent phosphorylation of specific connexins (Lasater, 1987; Rorig et al., 1995). We have demonstrated a DA D2 receptor dependent modulation (enhancement) of this non-synaptic, electrotonic, transmission in striatal/accumbens spiny projection neurons in anesthetized rats (Onn and Grace, 1994) and in rat brain slices (O'Donnell and Grace, 1993). This coupling is potently modulated by behaviorally (Moore and Grace, 2002) and psychiatrically relevant treatment regimens in intact animals, including amphetamine withdrawal (Onn and Grace, 2000), DA depletion (Onn and Grace, 1999) and antipsychotic drug administration (Onn and Grace, 1995). Given the potent modulation of coupling induced in intact anesthetized rats by psychiatrically relevant manipulations of the DA system, it is likely that modulation of gap junctional conductance plays a major role in the response of the mesocorticolimbic systems to decompensation, as occurs with drug dependency and schizophrenia (Grace, 1991; Greengard et al., 1999; Hyman and Malenka, 2001; Albert et al., 2002; Saal et al., 2003; Svenningsson et al., 2005).

In the present study we evaluated the incidence of intercellular coupling in WT and DARPP-32 KO accumbens core spiny neurons and its modulation by DA D1- and D2-like agonists using conventional intracellular sharp-electrode recording in brain slices from WT and DARPP-32 KO mice. Given that we are examining the impact of altering second and third messenger systems on cellular coupling, whole-cell current-clamp recordings, in which dialysis of essential constituents from the soma may occur, were employed to compare the results obtained by intracellular sharp electrode recordings. Secondly, cortically-evoked synaptic responses were examined in accumbens neurons of WT and KO mouse slices. The effects of DA D1- and D2-like receptor agonists and antagonists were examined in identified NAC spiny neurons and aspiny interneurons with fast spiking or cholinergic-like characteristics, which have been well characterized in the dorsal striatum (Aosaki et al., 1998; Koos and Tepper, 1999; Pisani et al., 2000; Bracci et al., 2001; Suzuki et al., 2001; Centonze et al., 2003; Tepper et al., 2004) but have not yet been studied in the NAC of rats or mice. Portions of these data were presented in abstract form (Onn et al., 2006b).

EXPERIMENTAL PROCEDURES

Animals and In Vitro Brain Slice Preparations

Adult male mice lacking the DARPP-32 gene were generated via gene-targeting in embryonic stem cells using standard techniques. Wild-type and mutant mice were generated by interbreeding of heterozygotes and were gene typed by southern analysis. The detailed protocol of producing such mutant mice is described elsewhere (Fienberg et al., 1998). All mice used in this study were handled in accordance with the USPHS Publication Guide for the Care and Use of Laboratory Animals, and the specific experimental protocols were approved by the Institutional Animal Care and Use committee of the Drexel University Medical College and the University of Pittsburgh.

Adult male mice (3–6 months of age) were deeply anesthetized with 8% chloral hydrate (400 mg/kg; i.p.) before they were decapitated and the brain was rapidly removed, blocked and attached onto the chamber of a DSK vibratome (Ted Pella, Japan) that was filled with chilled physiological saline saturated with 95%:5% O₂:CO₂; PH = 7.23. Tissue slices (350 μ m in thickness) were cut in a horizontal (oblique) plane to preserve corticoaccumbens connections (Charara and Grace, 2003; Onn et al., 2003) and immediately placed into incubation vials containing the oxygenated physiological saline solution at 34°C for at least 1 hour before intracellular recording. Recordings were performed in slices superfused with continuously oxygenated physiological saline at a flow rate of ~ 1 ml/min under the control of a peristaltic pump. The physiological saline solutions contained (in mM): 128 NaCl, 3 KCl, 2 CaCl₂, 2 MgSO₄, 24 NaHCO₃, 1.25 NaH₂PO₄, and 10 D-glucose.

Intracellular Sharp-Electrode Recordings and Labeling

Sharp electrodes were pulled from 1 mm o.d. Omegadot (WPI, Sarasota, FL) borosilicate glass tubing using a Flaming-Brown P-80/PC electrode puller and filled with 2 % biocytin (dissolved in 3 M potassium acetate, average electrode impedance ~110 M Ω as measured in situ). Intracellular recordings were performed using a NeuroData 383 intracellular preamplifier, with current injected across a bridge circuit integral to the preamplifier. All membrane potential values were adjusted by subtracting the tip potentials measured at the time point when electrodes were pulled out of the cells at the termination of recording. The biocytin-filled microelectrodes were lowered into the accumbens core region under visual control using a stereomicroscope (Nikon SMZ-2B; Melville, NY) by reference to anatomical landmarks on a mouse brain atlas. After impalement of a cell with stable membrane potentials, cells were injected with biocytin using 0.5–1 nA depolarizing current pulses delivered at 3.3 Hz (Onn et al., 2003, 2006a). Only one cell per slice was injected to ensure

an accurate correlation between pharmacological responses and cell morphology and location.

In Vitro Whole Cell Recordings and Labeling

Whole cell patch electrodes (5–7 M Ω) were pulled from 1.5 mm glass pipettes (WPI). Whole cell recordings were performed in a submersion chamber maintained at 31–32°C using patch pipettes filled with (in mM): 115 K-gluconate, 10 Hepes, 2 MgCl₂, 2 KCl, 2 Mg-ATP, 2 Na₂-ATP, 0.3GTP (pH 7.3; osmolality: 295 mOsm) containing 0.5% biocytin (Onn et al., 2006a). Cells were visualized using an Olympus B $\times$ 50WI (40 $\times$ infrared lens) under an infrared differential interference contrast microscopy (IR/DIC). Recordings of visualized accumbens (NAC) neurons were made in current clamp (bridge) mode with an Axoclamp-2B amplifier (Axon Instruments). The output signal was low-pass-filtered at 3 KHz, amplified and digitized at 15 KHz, and acquired using pClamp/Digidata 1322A software (Axon Instruments). Cells with evoked firing that showed signs of a washout effect associated with whole cell recordings after the initial gigohm seal was broken were discarded. Also excluded were neurons with unstable membrane potentials or access resistance (>10% changes) before drug application. During whole cell recordings, biocytin readily diffused from the patch recording pipette into the recorded neuron and was processed histochemically *post hoc* for cell location and morphology (Onn et al., 2006a).

Cortically-Evoked Excitatory and Inhibitory Postsynaptic Potentials

Whole cell recordings were performed from the NAC, while corticoaccumbens fibers were stimulated by placing a bipolar tungsten stimulating electrode in the white matter, to study DA agonist and antagonist effects on regulating glutamate release and thus excitatory postsynaptic potentials (EPSPs) in NAC neurons in slices from WT and DARPP-32 KO mice. Drugs used in the study were purchased from Sigma (St. Louis, MO): 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline disodium salt (CNQX), D-(-)-2-amino-5-phosphonovaleric acid (APV), SKF 38393 or SKF 81297, SCH 23390, bicuculline, picrotoxin, quinpirole and sulpiride, except the D4 antagonist (U 101958 or PD 101387 from RBI). Drugs were dissolved in the bath buffer from stock solutions made daily using a gravity-driven superfusion system under the control of a peristaltic pump, and equilibrated concentrations of the drugs were achieved in 1–2 min and drug effects were measured at 5 and 10 min. Antagonists, including APV (25–50 μ M), CNQX (10 μ M), picrotoxin (50 μ M) or bicuculline (10 μ M) were bath-applied, as were D1 and D2 receptor agonists (SKF 38393 and quinpirole) and antagonists (SCH 23390 and sulpiride) at final concentrations of 5–10 μ M, the dose range used in previous studies of rat spiny neurons (O'Donnell and Grace, 1994; Cepeda et al., 2001; Charara and Grace, 2003) and shown to differentiate between the response in WT and DARPP-32 KO animals (Onn et al., 2003).

Single pulse (0.2 Hz) stimulation (pulse duration: 0.2 ms) was applied at the range of stimulus intensities from 0.01–0.5 mA, except in a few cases (> 0.5 mA) in neurons with apparent high spike threshold. Monosynaptic responses were indicated by observing linearly increasing amplitudes of evoked EPSPs/IPSPs with constant onset latency, with varied stimulation intensities delivered by a Grass S-88 stimulator and constant current isolator to a bipolar stimulation electrode in the white matter for stimulating corticoaccumbens fibers. When studying orthodromic responses, stimulation intensity was adjusted to elicit spike discharge in approximately 20–50% of the trials to minimize saturation effects. Either polarity of extracellular stimulus was used accordingly, but in a given cell the same polarity of stimulus was used to evoke responses before and after the drug application for EPSP comparisons. In order to ensure accuracy in comparing EPSP amplitudes between neurons, an input-output curve was generated to demonstrate that the responses were stimulus intensity-dependent. These EPSP comparisons were made at the maximum EPSP amplitude

(i.e., peak minus resting potential) that was sufficient to elicit occasional spike discharge. Glutamate (Glu)-mediated synaptic responses were verified by their sensitivity to the presence of Glu antagonists (50 μ M D-APV and 10 μ M CNQX) and GABAergic inhibitory potentials (IPSPs) were verified by the sensitivity to bath application of a chloride channel blocker (50 μ M picrotoxin) or a GABA_A receptor antagonist (10 μ M bicuculline). The amplitude of the IPSPs was measured from the voltage corresponding to the resting membrane potential to the most hyperpolarized potential. As previously reported (Onn et al., 2003), there were no differences in action potential amplitude and spike threshold between WT and KO animals, so this was not assessed further. The input resistance of the recorded neurons was calculated by measuring the membrane voltage deflections produced in response to 1 s duration constant hyperpolarizing current pulses (range: 50–400 pA). Rheobase current, defined as the amplitude of current required to generate the first spike discharge or the first bursts of spikes (for fast-spiking interneurons), was used to assess the membrane excitability in conjunction with the assessment of pre-synaptic actions.

Histochemical reactions for biocytin to reveal morphological coupling in brain slices

At the end of recordings, brain sections were postfixed in 4% paraformaldehyde overnight and processed for histochemical reactions and staining of biocytin that was injected (from the sharp electrode) or diffused from the patch recording pipette into the recorded neuron. The brain slices with biocytin-injected or-filled neurons were then cryoprotected in 20% sucrose solution before subjecting to a freeze-thaw procedure to facilitate the penetration of the immunoreagents used for histochemical staining for biocytin in the cells recorded. In brief, brain sections were reacted with 10% avidin-biotin complex solution (Vector ABC kit; Vector) overnight in a cold room under shaking. The ABC complex was then reacted for 30 min with DAB solution (Sigma, 0.05%) and hydrogen peroxidase (0.003%). The slices were then cleared with 100% dimethyl sulfoxide (DMSO) for 20 min and mounted in DMSO for microscopic examination (Grace and Llinas, 1985). The biocytin-filled cells in DMSO-mounted slices were photographed using a Zeiss microscope before being subjected to a subsequent dehydrating/defatting process with an alcohol series and xylene to allow mounting of the slices with protex for long-term storage and examination.

Statistics

All data were expressed as mean $\pm$ standard error of the mean (S.E.M.). Synaptic changes induced by drug administration in brain slices were evaluated based on the percentage change from baseline values (an average of 4–5 test responses before and after drugs). Student's paired and unpaired t-tests were performed when appropriate. In general, an unpaired t-test was used for between group comparisons, whereas a paired Student's t-test was used for within-subject comparisons. The incidence of coupling was defined as the percentage of neurons coupled to one or more neurons revealed by single cell injection of biocytin. A nonparametric Fisher exact test was used to compare the incidence of coupling between the experimental groups. Statistical significance was set at $p < 0.05$ for all comparisons.

RESULTS

DA modulation of dye coupling between NAC spiny neurons in WT and KO corticoaccumbens slices

Sharp electrode intracellular recording and labeling of mouse NAC spiny neurons—Combined in vitro intracellular recording and labeling techniques were used to examine the effects of DA D1/D5 (SKF 38393) and D2/D3/D4 (quinpirole) receptor agonists on dye coupling (i.e., labeling more than 1 cell after single cell biocytin intracellular injection). All neurons recorded and labeled were located in the posterior

accumbens (NAC) core region and were morphologically identified as spiny neurons (N = 77; 32 WT and 45 KO neurons; Figure 1). In order to limit potential artifactual labeling, neurons were scored as dye coupled only if the following criteria were met: 1) a clear absence of extracellular spillage of dye, 2) labeling of neurons in which the somata were clearly separated to ensure absence of transfer between injured somata, 3) and specificity with respect to cell types labeled, with no labeling of surrounding glia (Onn and Grace, 1994;1995;1999). Moreover, given the blockade of coupling with the gap junction blocker carbenoxolone and the potent pharmacological and phenotypic modulation of dye coupling, it is unlikely that artifact coupling had a substantial impact on the results. Biocytin staining in WT mouse slices revealed 9% (1/11) basal coupling between spiny neurons in mouse horizontal slices, consistent with low basal coupling revealed in rat striatal/NAC spiny neurons in brain slices (Cepeda et al., 1989; O'Donnell and Grace, 1993; Venance et al., 2004) and in anesthetized rats (Onn et al., 1994;1995). Following bath administration of the D2-like agonist quinpirole (10 μ M) the incidence of coupling increased to 73% of neurons tested (8/11, Figure 1B), which is an 8-fold increase in coupling incidence (Fisher exact test, p value = 0.008, Figure 1E). In contrast, D1/D5 agonist administration (SKF 38393, 10 μ M) did not significantly alter the incidence of dye coupling (1/9; 11%; p = 0.7) compared to pre-drug conditions (1/11; 9%). Furthermore, following treatment with the gap junction blocker carbenoxolene (100 μ M, 10 min; Travaglia et al., 1995; Moore and Grace, 2002), the D2-like agonist quinpirole did not result in coupling (0/5; 0%), which is consistent with gap-junction mediated dye coupling between spiny neurons (Venance et al., 2004).

Injection of single neurons in KO mouse slices revealed 24 % (4/17) baseline coupling compared to WT (1/11, p = 0.66; N.S., Figure 1E). However, in KO spiny neurons, neither D1- nor D2-like agonist administration significantly affected dye coupling (Fig 1E: D1 agonist: 33 %; 2/6; D2 agonist: 33%, 3/9; p = 0.5; N.S.), relative to basal coupling in KO mouse slices (4/17, vs 2/6; p = 0.632; vs 3/9; p = 0.661). These results indicate that DARPP-32 signaling cascades are involved in the D2-enhancing coupling in WT spiny neurons, but this modulation was absent in DARPP-32 KO mice.

Whole cell patch clamp recording and labeling of spiny neurons—Whole cell patch clamp recordings are known to affect properties that depend on diffusible intracellular messengers via dialysis into the electrode, and therefore have the potential to affect cellular coupling. A total of 47 spiny neurons were recorded and labeled in mouse NAC spiny neurons (WT: n = 30; KO: n = 17) using whole cell patch electrodes that contained 0.5% biocytin to reveal dye coupling between neurons. We found similar low levels of basal coupling between WT spiny neurons revealed by whole cell recordings (1/10) as by intracellular sharp electrode (1/11). We also found that the D2-mediated increase in coupling between WT spiny neurons as revealed by intracellular sharp electrodes was reliably replicated with the whole cell patch-electrode recordings. Thus, 55% coupling (5/9; vs 1/10; p = 0.05, Figure 1F) was revealed upon quinpirole administration in WT mouse slices, whereas D1/D5 agonist SKF 38393 stimulation did not significantly affect coupling (3/11; 27%; vs 1/10; p = 0.3; Figure 1F). Thus, both whole cell and sharp electrode recordings consistently revealed the D2-mediated increased coupling in WT spiny neurons and this D2-mediated modulation was absent in KO spiny neurons.

Interestingly, no cases of coupling were revealed by whole cell recording from KO mouse slices (i.e., 0% for 5 cells under basal conditions, 6 cells after D1/D5 agonist application and 6 cells after D2-like agonist application). This was extrapolated as zero percent coupling (out of a total of 17 cells recorded) in KO mouse slices recorded using whole cell electrodes, compared to a 30% incidence (9/32; p = 0.01) revealed in KO mouse slices by sharp electrodes. These data suggest that dialysis of intracellular constituents through low-impedance patch electrodes may impact coupling in DARPP-32 KO spiny neurons.

DA regulation of cortical-evoked EPSPs in NAC spiny neurons in WT and KO mouse slices

DA D2/D4 receptors are present in the accumbens (NAC; Sibley, 1995) and located on presynaptic terminals (Svingos et al., 2000) from the cortex (Tarazi et al., 1998). We thus examined the modulation of cortical-evoked excitatory postsynaptic potentials (EPSPs) in mouse NAC spiny neurons by bath-applied D2-like (D2/D3/D4) receptor agonist (quinpirole, 5 & 10 μ M) and a selective D2 antagonist (l-sulpiride, 5 μ M) or D4 antagonist (PD 101387 and U101958; 10 μ M; Onn et al., 2006a) during whole cell recordings. Excitatory and inhibitory synaptic potentials (EPSPs/IPSPs) were elicited in NAC neurons by stimulating PFC afferent fibers in the corpus callosum of horizontal slices to minimize potential artifacts due to current spread from local (intra-accumbens) stimulation sites. As EPSP responses were stimulus intensity-dependent, these comparisons were made at the maximal subthreshold EPSP amplitude that was sufficient to occasionally trigger spike discharge (see Experimental Procedures).

Mouse spiny neurons were identified based on their physiological properties, i.e., resting membrane potential = -76.3 ± 2.1 mV (S.E.M; n = 33), input resistance = 81.3 ± 11 M Ω ; rheobase currents 65 ± 11 pA, and the discharge of a train of non-adapting spikes (15 ± 5 spikes) upon membrane depolarization (~ 250 pA depolarizing current injection). EPSPs evoked by stimulating the white matter were examined in 27 neurons (15 WT and 12 KO neurons) located in the posterior accumbens core that were histochemically verified post hoc as spiny neurons (Figure 3C). In 15 WT spiny neurons in which EPSPs were evoked (22 ± 2.8 mV) during pre-drug conditions, bath application of the D2 agonist quinpirole (10 μ M; n = 15) resulted in a $46 \pm 11\%$ reduction of cortical afferent-evoked EPSP amplitude in WT spiny neurons (Figure 2A3). In 8 cells tested prior to a final concentration of 10 μ M, 5 μ M quinpirole (5 min) resulted in a $32 \pm 9\%$ reduction ($p < 0.05$). Thus, approximately 53% (8/15) WT mouse spiny neurons responded to quinpirole (10 μ M) with $>50\%$ reductions in the amplitude of cortical-evoked EPSPs. In each case, the EPSP amplitude recovered to 60–80% of EPSP baseline or with spike discharge (Figure 2A4) upon drug washout (10–15 min of buffer rinse). This synaptic depression by D2 agonist application occurred without significantly altering input resistance ($+17 \pm 8$ M Ω ; $p < 0.3$; N.S.) or membrane potential ($+1.6 \pm 1.7$ mV; $p < 0.4$; N.S.) in WT spiny neurons, due to the high degree of variability across all cells before and after D2 agonist application in WT slices (Onn et al., 2003). Given numerous studies investigate the D2 presynaptic action, identification of the potential presynaptic mechanism was not the purpose of present study that was examining the DA modulation occurred via DARPP-32 cascades. Nevertheless, our data are consistent with a D2 presynaptic action on PFC afferent terminals in the rat NAC/striatal spiny neurons (Brown and Arbuthnott, 1983; O'Donnell and Grace, 1994; Cepeda et al. 2001; Bamford et al., 2004).

KO spiny neurons had resting membrane potentials and input resistance (-78.1 ± 1.5 mV; 72 ± 7.6 M Ω ; n = 24) similar to those in WT spiny neurons ($p = 0.45$; $p = 0.22$, respectively). Cortical stimulation-evoked EPSPs in KO spiny neurons (n = 24, 25 ± 1.8 mV; Figure 2B) were similar in amplitude to those observed in the WT (n = 30, 22 ± 2.8 mV; $p < 0.2$, Figure 2A). Twenty seven percent (8/30) of WT spiny projection neurons responded to cortical stimulation with large amplitude (>20 mV) EPSPs. However, sixty percent of spiny neurons in DARPP-32 KO mice exhibited large amplitude evoked EPSPs, i.e., 14 KO neurons (vs 8 WT neurons; X^2 test, one-tailed; $p < 0.048$) with EPSP amplitude >20 mV (range: 21 to 26 mV); the 10 remaining neurons in KO (vs 22 WT neurons) spiny neurons exhibiting small amplitude evoked responses (i.e., 10 to 19 mV). Although the differential amplitude EPSPs in spiny neurons may be due to a multitude of factors, it is interesting to note that they fell within the “small” versus “large” amplitude in a manner that is consistent with that predicted from the two different anatomical bouton sizes of corticostriatal fibers (Lei et al., 2004).

The D2-induced reduction of EPSP amplitude observed in WT mice ($46 \pm 11\%$ reduction, above) was significantly reduced in KO mice ($16 \pm 6\%$ reduction, $n = 14$; $p < 0.01$; Figure 2D1). Thus, the inhibitory D2 receptor modulation of corticoaccumbens transmission was absent in KO spiny neurons. Application of the D2 antagonist sulpiride ($5 \mu\text{M}$) to WT mouse slices did not produce a significant change ($15 \pm 3\%$) in EPSP amplitude ($n = 5$; Figure 2C4), nor application of the D4 selective antagonists PD 101387 and U101958 ($5\text{--}10 \mu\text{M}$, Merchant et al., 1996; Onn et al., 2006) to WT slices ($11 \pm 3\%$; $n = 5$). In the presence of D2 or D4 antagonists (sulpiride; $5 \mu\text{M}$; PD 101387, $10 \mu\text{M}$, respectively), quinpirole failed to suppress EPSP amplitude (before drug: $17 \pm 3.6 \text{ mV}$ vs after: $16 \pm 3.1 \text{ mV}$, $n = 5$ for each antagonist). Thus, in WT spiny neurons pre-treated with a D2 or D4 antagonist, subsequent application of quinpirole failed to induce synaptic depression. This is significantly different ($p < 0.03$) from a $46 \pm 11\%$ reduction produced by quinpirole alone (without antagonist pre-treatment), thus confirming the specificity of D2/D4 receptor-mediated pharmacological actions on reducing cortical-evoked EPSPs in mouse NAC spiny neurons.

In contrast, bath-applied SKF 38393 did not alter cortical-evoked EPSP amplitude in slices from either WT ($3 \pm 0.1\%$; $n = 7$) or KO ($3 \pm 0.03\%$; $n = 8$; $p = 0.99$ vs WT) spiny neurons (Figure 2D2). In WT mouse slices, bath-applied D1 agonist SKF38393 did not produce a change (0%) in evoked synaptic responses in 5 out of 7 neurons examined, with 1 cell showing a 37% increase and the other showing a 23% decrease. Evoked cortical responses in KO spiny neurons did not show any significant changes to the D1/D5 agonist ($n = 8$; 4 cells showing no changes, $\pm 5\%$, 2 cells of 15% increase and 2 cells of 12% decrease). Given the high degree of variability in these parameter when averaged across all cells before and after D1 agonist application, the D1/D5 agonist did not produce any consistent changes in input resistance, compared to pre-drug conditions (within-subject comparison; $p < 0.5$) or compared to KO cases (between-subject comparison; WT: $+16 \pm 11 \text{ M}\Omega$; vs KO: $+5 \pm 3.6 \text{ M}\Omega$; $p < 0.61$; N.S.). Significant difference was noted in D1-induced membrane potential changes between WT and KO cases (WT: $+4.6 \pm 1.8 \text{ mV}$; vs KO: $-2.5 \pm 1.3 \text{ mV}$, $p < 0.01$; Figure 2D2), similar to the results obtained by intracellular sharp electrode recordings (Onn et al., 2003).

DA D1 Regulation of PFC Excitatory Input in NAC Aspyneurons of WT and KO mice

Fast-Spiking (FS) GABA Interneurons—Thirty two (20 WT and 12 KO) NAC neurons were morphologically verified as medium-sized aspyneurons (Figure 3B) and were found to exhibit similar membrane potentials ($-79 \pm 3.0 \text{ mV}$) and input resistances ($81 \pm 27 \text{ M}\Omega$) as observed in spiny neurons ($-76.3 \pm 2.1 \text{ mV}$ and $81.3 \pm 11 \text{ M}\Omega$, respectively; $n = 20$, $p < 0.16$; Figure 3D). All of the aspyneurons recorded in WT and KO mouse slices were silent at rest, but fired trains of spikes during membrane depolarization (15 ± 3 spikes) at $\sim 250 \text{ pA}$, the threshold current (Figure 4A1-2; B1-2). However, spiny neurons exhibited a threshold (rheobase) current of $65 \pm 11 \text{ pA}$ ($50\text{--}75 \text{ pA}$; $n = 27$). The number of spikes produced in these aspyneurons was similar to that (15 ± 5 spikes) in spiny neurons at this amplitude of current injection. This is consistent with the response characteristics reported for FS GABA interneurons in the rat dorsal striatum (Kawaguchi et al., 1995; Koos and Tepper, 1999; Bracci et al., 2002; Centonze et al., 2003; Koos et al., 2004).

Accumbens FS GABA interneurons, like spiny GABA projection neurons, receive direct cortical inputs (Ramanathan et al., 2002) and can be easily activated by cortical stimulation (Parthasarathy and Graybiel, 1997). Monosynaptic EPSPs were evoked in both WT and KO FS neurons (Figure 4A3, B3). The maximal amplitude of the evoked EPSP in WT FS interneurons was similar to spiny neurons ($23 \pm 2.1 \text{ mV}$, range: $17\text{--}33 \text{ mV}$ vs spiny neurons:

22.4 ± 2.8 mV, range: 10–36 mV; $p < 0.4$; Figure 3D). However, unlike that observed in spiny projection neurons (see above), application of the D2-like agonist quinpirole (5 & 10 μ M) did not affect the EPSP amplitude (−11%; $n = 5$; Figure 4A4-5), or passive membrane properties (e.g., membrane potential, input resistance, firing pattern, or threshold current; Figure 4A1-2) in WT FS interneurons. Similarly, application of the D1/D5 agonist SKF 38393 (10 μ M), while depolarizing the cell membrane by 3–5 mV, did not produce significant changes (− 9.5% of pre-drug baseline) in cortical-evoked EPSPs in WT FS neurons. An absence of D1-like or D2-like agonist effects on cortical EPSPs in WT FS interneurons is similar to that reported in rat striatal FS interneurons (Bracci et al., 2002).

In 7 other WT FS interneurons bath application of the D1-like antagonist SCH 23390 resulted in 63 ± 15 % decreases in EPSP amplitude in WT slices (from 22.5 ± 2.1 mV to 11.3 ± 2.6 mV; $p < 0.05$; Figure 4B4). The reduced response to cortical input to FS neurons, upon D1/D5 receptor blockade, occurred in concert with decreased membrane excitability in FS neurons as indicated by increased current intensity required to emit spike firing (Figure 4B1-2). This together with the D1 agonist data (above) suggests that D1/D5 modulation of the cortical-evoked EPSPs in accumbens FS neurons was already saturated at baseline. Application of the D1/D5 antagonist SCH 23390 (5 μ M) to KO FS neurons ($n = 6$; Figure 4C1) did not attenuate cortical-evoked EPSPs (− 7.2 ± 1%; $p < 0.00001$, vs WT: − 63 ± 15%; Figure 4B3, B4). In 3 of these KO FS interneurons (after washout of the D1 antagonist) plus 6 additional KO neurons (3 for each drug), subsequent application of the D2-like agonist quinpirole (5 μ M; $n = 6$; Figure 4C2) or SKF 23390 (10 μ M; $n = 6$) did not significantly modify EPSPs (−5.3 ± 2% or 1.3 ± 0.7 %, respectively). Therefore, the ability of tonic D1/D5 stimulation to increase (maintain) the EPSPs evoked by cortical stimulation was absent in FS neurons in KO brain slices (Figure 4D). Thus, in contrast to an inhibitory D2/D4 receptor regulation of cortical input to WT *spiny* neurons (Figure 2D1), a tonic D1/D5-mediated enhancement in cortical-evoked EPSPs was observed in *aspiny* FS neurons of WT but not KO mouse slices.

Cholinergic-like Interneurons—Nineteen (13 WT and 6 KO) accumbens neurons were recorded that exhibited characteristics consistent with those described for striatal cholinergic interneurons (Aosaki et al., 1998; Pisani et al., 2000; Suzuki et al., 2001). These large-sized interneurons (Figure 3A1-5) had 1–2 thick primary dendrites that branch into fine processes and had a significantly more depolarized membrane potential (−60.8 ± 1.3 mV) and higher input resistance (151.6 ± 58.0 M Ω) than medium-sized aspiny fast spiking (FS) and spiny GABA neurons (Figure 3D). Cholinergic-like interneurons exhibited spike firing in response to intracellular depolarizing current pulses at the lowest intensity (50 pA; Figure 5) used in the present study. This activity consisted of oscillatory rhythmic spike discharge, with spikes preceded by a slow depolarization; the evoked activity lasted throughout the entire current pulse of 1 sec duration (Figure 5A2, B1, C1). In neurons with cholinergic-like firing activity, the cortical-evoked EPSPs were significantly smaller in amplitude (2.7 ± 4.2 mV; $n = 8$) than those in aspiny FS neurons (23 ± 2.1 mV, $P < 0.001$; Figure 3D). However, prominent IPSPs (4.3 ± 2.5 mV; 3–8 mV; $n = 8$) with a peak latency at 9.1 ± 0.7 ms ($n = 8$) were evoked in these neurons by cortical fiber stimulation (Figure 5A3, B3, C3). These evoked IPSPs were completely blocked by bicuculline (10–30 μ M; $n = 6$; Figure 5D) and thus represented GABAergic potentials.

Administration of D1/D5 agonist (SKF 81297; 10 μ M) or antagonist (SCH 23390; 5 μ M) did not significantly affect IPSPs in cholinergic-like neurons of WT (Figure 5A4; D1 agonist: 95 ± 7 % or D1 antagonist: 91 ± 3%; Figure 5A3) or KO slices (agonist: 89 ± 5 %; antagonist: 101 ± 7%; not shown). However, administration of the D2 receptor agonist (quinpirole; 5 or 10 μ M) in WT mouse slices potently attenuated these IPSPs (50 ± 5%; $n = 13$): 5 μ M inhibited IPSPs by 39 ± 5 % and 10 μ M by 54 ± 6 %, both significantly reduced

from pre-drug baseline ($p < 0.05$; Figure 5B4 & E). However, a similar D2 agonist quinpirole application in KO brain slices (Figure 5C3,C4) failed to reduce these IPSPs ($93 \pm 10\%$ of pre-drug conditions; $n = 6$; Figure 5E). The cortical-evoked disynaptic IPSPs in KO mouse slices (Figure 5C2) were significantly larger in amplitude (7.1 ± 2.0 mV; range: 6–15 mV) and longer in duration (321 ± 23 ms; $n = 6$) than those evoked in WT mice (Figure 5A, B; 4.3 ± 2.5 mV; range: 3–8 mV; 156 ± 14 ms; $n = 8$; $p < 0.043$). Thus, a D2-selective attenuation of feedforward inhibitory neuron input to cholinergic neurons was observed in WT, but not KO, slices. This is consistent with D2-like presynaptic suppression of cortically-evoked IPSPs reported in rat striatal cholinergic neurons (Pisani et al., 2000; Suzuki et al., 2001; Centonze et al., 2003).

DISCUSSION

DARPP-32 transgenic mice were used to evaluate the roles of DARPP-32 in DA-dependent modulation of synaptic and non-synaptic (morphological, tracer, coupling) in accumbens (NAC) neurons recorded in horizontal brain slices of WT and KO mice. DA D2 receptor activation was found to enhance coupling between mouse NAC spiny (projection) neurons; however, this effect was substantially attenuated in mice lacking DARPP-32. Similarly, DA D2-mediated suppression of corticoaccumbens synaptic excitation, which is prominent in the WT mouse spiny neurons, is also reduced in KO mouse spiny neurons. The fact that cortical glutamatergic input is known to increase coupling via a nitric oxide-dependent mechanism (O'Donnell & Grace, 1997) suggests that these two factors may be related. Thus, an absence of D2-mediated attenuation of corticoaccumbens transmission in the KO mice could contribute to the small increase in baseline coupling in this preparation. In contrast, corticoaccumbens excitation appears to be regulated via a different pharmacology in other striatal neuron types. Thus, in FS GABA interneurons, cortical excitatory potentials are attenuated by D1 blockade in WT mouse slices but not in the KO. This D1-mediated facilitation in mouse FS neurons appears already saturated, since further D1 stimulation fails to further augment corticostriatal inputs, similar to that reported in rat FS neurons (Bracci et al. 2002). Lastly, in neurons exhibiting a cholinergic-like activity pattern, D2 stimulation suppressed cortical-evoked GABA_A-mediated disynaptic IPSPs in WT mouse slices but not in the KO, again consistent with an absence of D2 modulation of corticostriatal transmission. Thus, distinct DA receptor subtypes appear to play unique roles in the regulation of prefrontal cortical input to mouse spiny (projection) neurons *versus* aspiny FS interneurons: D1/D5 receptors tonically *facilitates* cortical input and neuronal excitability in FS interneurons, whereas D2-like receptors *inhibit* corticoaccumbens – driven excitation and inhibition in mouse spiny projection neurons and cholinergic interneurons, respectively. Thus, DARPP-32 appears to play an essential role in linking DA modulation of corticoaccumbens transmission and circuit interaction in the accumbens; an effect that is attenuated in the KO animal.

D2-like receptor suppression of cortical evoked EPSPs in mouse NAC spiny neurons

D2 stimulation resulted in an average 46% reduction in cortical afferent-evoked EPSPs in 53% of WT spiny neurons, as observed previously in the NAC (O'Donnell and Grace, 1994) and striatum (Flores-Hernandez et al., 1997; Bamford et al., 2004). In contrast, in DARPP-32 knockout mouse NAC spiny neurons D2-receptor-mediated attenuation was reduced; furthermore, 14 out of 24 KO spiny neurons (compared to 8 out of 30 WT neurons) exhibited large amplitude cortical-evoked EPSPs, similar to the result reported in D2 receptor knockout mice (Cepeda et al., 2001). Taken together, these data provide further support for a role of D2 receptor modulation of striatal function via DARPP-32 cascades. Given that DARPP-32 KO mice are reported to have normal DA levels, DA receptors (Fienberg et al., 1998) and electrical-evoked DA release (Zachariou et al., 2002), a reduction

of the D2-like attenuation in KO spiny neurons in the NAC core region is most likely due to the absence of DARPP-32 in the corticoaccumbens fibers (Berger et al., 1990; Gaspar et al., 1995; Wang et al., 2004).

D1 receptor stimulation did not produce a significant alteration in cortical-evoked EPSPs in WT mouse NAC *core* spiny neurons, although D1-mediated suppression of cortical-evoked excitatory potentials may occur in NAC *shell* spiny neurons (Pennartz et al., 1992). Other studies in NAC *core* spiny neurons have suggested a potential presynaptic attenuation of corticoaccumbens transmission during administration of high doses (i.e., 100 μ M) of D1/D5 agonist (Nicola et al., 1996). The absence of D2 agonist effects observed in this study could be attributed to the use of slices from immature (e.g., P13 -40 day) rats in which functionality of D2 receptors (i.e., receptor density and signaling process) may not have achieved adult levels in most limbic cortical and striatal regions (Tepper et al., 1998; Tarazi and Baldessarini, 2000; Tseng and O'Donnell, 2006). Our D2 data obtained in slices prepared from adult mice (present study) and rats (O'Donnell and Grace, 1994) and in NAC *core* spiny neurons are consistent with neurochemical studies showing D2 selective attenuation of glutamate release in the NAC (Kalivas and Duffy, 1997) and D2 specific acceleration of stimulated depletion of glutamate from corticostriatal terminals (Bamford et al. 2004a,b). Indeed, anatomical studies have shown the presence of D2 receptors on majority of glutamatergic fibers (see Introduction), but only 1–2 % of glutamate terminals in NAC showing presynaptic D1 receptors (Hara and Pickel, 2005). Based on the substantial electrophysiological, neurochemical and anatomical literature, our results are consistent with the presence of presynaptic D2 DA receptors in regulating cortico-accumbens afferent fibers in mouse NAC core spiny neurons.

D1 and D2 modulation of excitatory and inhibitory inputs in fast-spiking (FS) and cholinergic-like interneurons

FS interneurons—FS GABA interneurons comprise only 1–2 % of the total NAC neurons and can be easily activated by cortical stimulation. D1 receptor blockade attenuated the amplitude of cortical-evoked EPSPs in FS neurons of WT, but not KO, mice, suggesting that tonic D1 receptor stimulation facilitates cortical inputs to WT FS neurons. The source of the D1 modulation is not clear. Its absence in the KO mouse suggests that it involves a DARPP-32-dependent process most likely in the corticoaccumbens fibers (Berger et al., 1990; Gaspar et al., 1995; Lei et al., 2004; Wang et al., 2004). Corticoaccumbens pyramidal neurons express D1-like receptors and use DARPP-32 signaling processes (Berger et al., 1990; Gaspar et al., 1995; Ouimet et al., 1998; Albert et al., 2002; Narin et al., 2004; Wang et al., 2004), although D1-ir axon terminals were only detected in ~2 % of axon terminals in the accumbens (Hara and Pickel, 2005). However, this may be sufficient given that FS interneurons comprise only 1–2 % of total population of striatal neurons (Kawaguchi et al., 1995; Koos and Tepper, 1999; Koos et al., 2004).

Cholinergic-like interneurons—Large aspiny interneurons comprise only 1–2 % of the total accumbens neuron population (Meredith et al., 1989; Kawaguchi et al., 1995) and receive glutamatergic inputs from the neocortex and thalamus (Lapper and Bolam, 1992). Although cortical stimulation did not produce prominent excitation (EPSPs) in WT cholinergic-like neurons, in part due to their depolarized membrane potentials, cortically-evoked disynaptic IPSPs were readily observed, and D2-like receptor stimulation potently attenuated the evoked IPSPs in these neurons. This is consistent with studies in the rat dorsal striatum showing that D2 agonists inhibit local GABA release and reduces spontaneous and evoked disynaptic inhibitory potentials in striatal cholinergic neurons (Suzuki et al., 2001; Centonze et al., 2003) via a D2 presynaptic mechanism (Pisani et al., 2005). FS GABAergic interneurons express D2 receptors in their cell bodies and axon terminals (Delle Donne et

al., 1997; Aizman et al., 2000) that could account for the observed D2-mediated attenuation of GABAergic IPSPs in WT cholinergic-like neurons. However, FS interneurons do not express DARPP-32 proteins (Ouimet et al., 1998). Thus, the absence of this D2 modulation of GABAergic IPSPs in KO cholinergic neurons would suggest that the IPSPs are due to inhibition exerted by extensive local axon collaterals of spiny projection neurons which express DARPP-32 proteins (Ouimet et al., 1998; Greengard, 1999; Narin et al. 2004) and play critical roles in both lateral inhibition and feedback inhibition to local circuit neurons in striatal/accumbens microcircuits (Czubayko and Plenz, 2002; Trunstall et al., 2002; Guzman et al., 2003; Koos et al., 2004; Venance et al., 2004; see Wilson 2007 for review).

According to this arrangement, one would predict that the cortex should provide a tonic D1/D5-dependent excitation of GABAergic FS interneurons, while at the same time presynaptically attenuating this local inhibitory input to spiny projection neurons (Flores-Hernandez et al., 2000). On the other hand, D2 receptor stimulation would selectively suppress cortical excitation, while enhancing coupling, in subsets (~ 50%) of NAC spiny neurons and would remove the GABA feedback inhibition from spiny neurons (Guzman et al., 2003; Taverna et al., 2005), potentially offsetting the direct D2-mediated inhibition of cholinergic neuron activity (Stoof et al., 1987). However, if the modulatory influence of DARPP-32 is not present, this DA stabilizing influence would be lost in KO mice (Fienberg et al. 1998). Thus, the interaction of DA modulation of corticoaccumbens excitation and DARPP-32 modulation of local network interactions appears to exert a critical stabilizing influence on the striatal network.

Morphological coupling in mouse NAC core spiny neurons

D2 stimulation was found to increase coupling only in the WT spiny neurons, that is consistently revealed by both intracellular sharp electrode and whole cell recordings. These data suggest that DARPP-32 may down-modulate coupling via D1 stimulation (O'Donnell and Grace, 1993), with D2 stimulation potentially working at least in part by reversing this attenuation. But how DARPP-32 is involved in D2-dependent coupling is not obvious. Studies have shown that D2 stimulation will effectively alter the response of D1-DARPP-32 mechanisms both by DA autoreceptor stimulation (which will decrease DA-D1 receptor activation; Bamford et al., 2004) or via postsynaptic attenuation of adenylate cyclase (Greengard et al., 1999; Lindskog et al., 1999), which is implicated in modulating coupling (Lasater, 1987; Rorig et al., 1995). Indeed, the lack of D2-mediated attenuation of corticoaccumbens excitation and the higher basal coupling in the KO animals may be functionally linked. Thus, as described above, the increase in evoked EPSP amplitude in KO animals and the lack of down-modulation by D2 receptors could yield a higher level of glutamate-nitric oxide-driven coupling (O'Donnell & Grace, 1997) in the KO animals. Alternately, the higher basal coupling may be a compensatory response to the inability of the KO animals to exert DA-mediated increased coupling, thus maintaining a basal level of interaction while losing the ability to modulate the coupling. Interestingly, when whole-cell recordings were employed, coupling was observed among accumbens spiny neurons in WT mice, whereas no coupling was observed in slices from the KO animals. Although not immediately apparent why this would be the case, given that patch recordings are known to dialyze soluble constituents of the intracellular milieu, and gap junctions are influenced by Ca^{+2} and Ca^{+2} -dependent kinases or phosphatases (Lampe and Lau, 2000), it is possible that in the absence of DARPP-32, dialysis of these constituents more potently affects coupling. Therefore, it is possible for DARPP-32 to interact with D2 modulation of dye coupling at several levels, including modulation of corticoaccumbens glutamate transmission and alteration of intracellular cAMP/calcium dynamics. Which of these has primary involvement remains to be elucidated.

By providing a low-resistance channel between neurons, coupling has the ability to synchronize slow membrane events such as up states among accumbens neurons (O'Donnell and Grace, 1995; Onn and Grace, 2000) or permit the exchange of small compounds such as second messengers (Harris, 2007). Therefore, gap junctions can provide a means for neurons that have inhibitory synaptic connections, such as accumbens spiny neurons, to exhibit activity- and neurochemical-mediated synchronization of function at a network level when the lateral inhibition between GABAergic spiny neurons is suppressed (Guzman et al., 2003). Our findings of a reduced DA-dependent modulation of corticoaccumbens transmission and electrical coupling suggests that DARPP-32 plays an essential role in allowing the DA system to effectively modulate network synchrony within the accumbens. This is particularly evident in the KO animals, in which coupling appears to exist at a level above baseline in the WT, but in a condition where it cannot be further modulated by DA modulation either directly or via an action on corticoaccumbens transmission. It is apparent that DARPP-32 is central to homeostasis within this system (Fienberg et al., 1998; Greengard, 2001; Svenningsson et al., 2005). Therefore, therapeutically targeting the DARPP-32 cascade may provide a unique site that can potently modulate corticoaccumbens information flow without directly antagonizing a specific neurotransmitter system, thus circumventing disruptive compensatory processes. This could be particularly effective in disorders known to involve disrupted corticoaccumbens information flow, such as schizophrenia, drug abuse, or affective disorders (Grace, 1991; 2000; Greengard 1999; 2001; Weinberger et al., 2001).

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FIG. 1.

Bath application of the D2-like agonist quinpirole, but not the D1-like agonist, enhanced dye coupling between spiny neurons in WT mouse brain slices but not in KO slices. (A–D) Representative labeling after single cell intracellular injection of biocytin during sharp electrode recordings in 350 μ m-thick brain slices prepared from WT (A & B) versus KO (C & D) mice. In each brain slice, a single cell was recorded and injected with the tracer biocytin during recordings. (A & B) Brain slices from WT mice showing singly-labeled (A) and dye-coupled (B) spiny neurons after bath-applied D1 (SKF 38393, 10 μ M; A) or D2 (quinpirole, 10 μ M, B) agonist. (C & D) In KO mouse brain slices, pairs of dye-coupled spiny neurons were recovered after either bath-applied D1-like agonist (C) or D2-like agonist (D). An intrasomatal gap between coupled neurons was confirmed in every case of coupling, including the case shown in D that was seen at a high magnification, arguing against spurious dye transfer (O'Donnell and Grace, 1993; Onn and Grace, 1994). (E & F) Summary of the DA agonist effect on the incidence of dye coupling in WT spiny neurons (gray) or KO spiny neurons (red) revealed by high-impedance sharp-electrode (E) or by

whole cell recordings (F). Both recording techniques consistently revealed significant increases in the incidence of coupling during D2 receptor stimulation in the WT cases (Fisher exact test; Sharp electrode; 8/11 vs 1/11; $p^{**} = 0.008$; Whole cell recording; 5/9 vs 1/10; $p^* = 0.05$), and the absence of D2 modulation in the KO cases (Sharp electrode: 3/9 vs 4/17; $p = 0.661$; Whole cell recording; 0/6 vs 0/5). Scale bars: A & B = 20 μm ; C & D: 50 μm

FIG. 2.

Bath application of the D2-like agonist quinpirole suppressed cortical-evoked synaptic potentials in physiologically identified NAC spiny neurons in WT (A), but not KO (B) mice. (A1) Membrane voltage traces recorded in an identified WT spiny neuron in response to a series of current pulses (range: -250 pA to +350 pA, each step of 50 pA, red trace at 300 pA). A current-voltage plot revealed a pre-drug input resistance of 73 M Ω . NAC spiny neurons exhibited very hyperpolarized resting membrane potentials and were quiescent, but were able to fire non-adapting spikes upon injection of long duration (1s) depolarizing current pulses. (A2) This spiny neuron responded to 0.5 mA cortical fiber stimulation (arrow) with maximal amplitude EPSPs (in red, 23 mV in this cell; 4 repetitions) and 1 spike (in black, in 1 out of 5 trials) that yielded 20 % probability of spike discharge. Downward arrows marked stimulus artifacts that were removed for the clarity purpose. (A3 & A4) A 43% reduction in the maximal EPSP amplitude (A3: 13 mV) was noted in this spiny neuron after 10 min exposure to quinpirole, which exhibited a nearly full recovery with spike discharge (A4; asterisk) after 15 min washout. (B1) Membrane voltage traces produced in a KO spiny neuron, in response to current pulses (-300 pA to +300 pA), revealed a pre-drug input resistance of 69 M Ω . (B2) Monosynaptic EPSPs (in red), leading to a spike (marked

by asterisk), were evoked in this KO spiny neuron by cortical fiber stimulation (arrow), with increasing stimulation current intensities (0.1–0.5 mA). (B3 & B4) The maximal EPSP amplitude (average of 4–5 repetitions) was evoked at 0.5 mA in this KO spiny neuron, before (B3; 24 mV) and after (B4: 22 mV) quinpirole application (10 min). (C1 & C2) A DIC image of a patch electrode attached to a small (~12 μ m) accumbens neuron (asterisk) in a WT mouse slice that responded to cortical stimulation with monosynaptic EPSPs (C2: 22 mV) and a spike (asterisk) in the presence of D4 antagonist U101958. (C3) In the presence of D4 antagonist (U101958; 10 μ M), quinpirole did not modify EPSPs: before (red trace: 19 mV) vs 10 min after the drug (black trace: 19 mV). (C4) Similar pretreatment with the D2/D3 antagonist sulpiride (5 μ M) did not alter EPSPs (in red: 13 mV) and blocked the effect of quinpirole (in blue; 13 mV). Each trace in C3 & C4 is an average of 4–5 repetitions. Spikes marked by asterisks are truncated. (D1 & D2) Effects of quinpirole (10 μ M; n = 15 WT; n = 14 KO) and SKF 38393 (10 μ M; n = 7 WT; n = 8 KO) on RMP (resting membrane potential) and maximal EPSPs in WT and KO spiny neurons. Statistical significance (*p < 0.05; **p < 0.01) was indicated for the between-subject comparisons (between WT and KO cases) using unpaired Student's t-test. All scale bars in A2–4, B2–4, C2–4 = 100 ms, except in A1 and B1 (= 500 ms).

FIG. 3.

Mouse accumbens interneurons exhibited distinct somatodendritic morphology (A, B) and basic membrane properties (D) from spiny projection neurons (C) during whole cell recordings. Note that no cases of tracer coupling were revealed between NAC interneurons under all drug conditions examined, consistent with the result that morphological coupling thus far has not been demonstrated in rat striatal/accumbens interneurons in brain slices (Cepeda et al., 1989; O'Donnell and Grace, 1993; Venance et al., 2004). (A1–5) Large, cholinergic-like, aspiny interneurons (LAN) had a large soma (~25 μm in diameter) and 1–2 stout primary dendrites that branch into 3–5 smooth fine dendrites. Biocytin-filled large aspiny neurons (A) exhibited cholinergic-like spike firing characteristics during whole cell recordings (Figure 5). The WT neurons, respectively, in A1 & A2 showed that quinpirole suppressed cortical-evoked IPSPs (Figure 5B) and SKF 38393 did not (Figure 5A4). The KO neuron in A5 showed that quinpirole failed to attenuate cortical-evoked IPSPs (Figure 5C). (B1–4) Biocytin-filled medium-sized (~10–15 μm) aspiny neurons during whole cell recordings exhibiting fast spiking (FS) characteristics in WT (B1) and KO mouse slices (B2, B3 & B4). The WT neuron in B1 showed the quipirole effect in cortical-evoked IPSPs (Figure 5B), whereas the KO neuron in B2 showed no quinpirole effect (Figure 5C). A primary neuron (recorded) was observed in addition to 3–5 neighboring neurons (B3), that were however incompletely filled (labeled) and thus not considered as coupling (Onn and Grace, 1994; 1999). In general, dendritic processes of mouse FS interneurons were less completely stained by biocytin than spiny projection neurons (C) during long-duration (> 50 min) whole cell recordings. (D) Bar graph summarizes basic membrane properties of 3 major cell subtypes in the WT mouse accumbens. Cholinergic-like large aspiny neurons (LAN) in WT mice exhibit significantly more depolarized resting membrane potential

(RMP, $p < 0.05$ vs FS & MSN), higher input resistance (IR, $p < 0.001$ vs FS & MSN) and smaller cortical EPSPs ($p < 0.003$ vs FS; or < 0.003 vs MSN) than medium-sized spiny (MSN) and aspiny fast-spiking (FS) neurons. * $P < 0.05$, * * $P < 0.003$, *** $P < 0.001$. Scale bars in A, B, C = 50 μm .

FIG. 4.

In WT fast-spiking (FS) interneurons cortical-evoked EPSPs were not altered by bath application of the D2-like agonist quinpirole (A), but were attenuated by application of the D1-like receptor antagonist SCH 23390 (B). (A1 & A2) FS GABA interneurons fired fast spikes in bursts before (A1) and after (A2) quinpirole application, with little effect on firing pattern, membrane excitability, or input resistance. The threshold current intensity of intracellular depolarizing pulses for eliciting fast spike discharge was 300 pA, before (A1: red trace) and after (A2: red trace) quinpirole. Input resistance (81 M Ω , A1 vs 67 M Ω , in A2) was measured by membrane deflections produced by hyperpolarizing current pulses (of 1 sec duration) against current intensities. (A3) Monosynaptic EPSPs (20 mV) were evoked by cortical stimulation (at 0.2 Hz) in every FS interneuron recorded in both WT and KO mice (not shown). (A4 & A5) The D2 agonist quinpirole (10 μ M; 10 min) did not affect the EPSP amplitude in WT FS neurons (A4: before; 20 mV vs A5: after; 20 mV). (B1 & B2) In a WT FS neuron, the D1-like antagonist SCH 23390 (10 μ M) decreased membrane excitability, as shown in the depolarizing current-evoked spike discharge at 200 pA before (B1: red trace) vs at 300 pA after (B2: red trace) drug application (10 min), but did not affect input resistance (B1: 83 M Ω vs B2: 84 M Ω). (B3 & B4) Maximal EPSPs were evoked in a WT FS interneuron (B3) that responded to SCH 23390 (10 μ M, 5 min) with a 70% reduction in EPSP amplitude (B4, blue trace), compared to before drug (B4, red trace), that recovered to pre-drug baseline after 10–15 min washout. The asterisk in B3 denotes an antidromic spike (truncated) that was elicited at the highest intensity (i.e., 0.5 mA in this case). (C1 & C2) In KO mouse fast spiking interneurons, nearly identical EPSP amplitudes were observed before (red trace) vs after the D1 antagonist SCH 23390 (blue trace; C1) or after the D2 agonist quinpirole (blue trace; C2). (D) Bar graph summarizing the effect of SCH 23390 on cortical-evoked EPSPs in FS interneurons of WT and KO slices, indicating that D1/D5 receptor blockade significantly attenuated cortical EPSPs in FS neurons in WT, but not in KO mouse brain slices. A1, A2, B1, B2 scale bars = 20 mV; 1 s; A3–5, B3–4, C1–2 scale bars = 10 mV; 100 ms.

FIG. 5. Cortical-evoked GABAergic IPSPs were attenuated by the D2-like agonist quinpirole in cholinergic-like neurons in WT (A, B), but not in KO (C) mouse brain slices. (A1) A DIC-image of a large (~20 × 18 μm) neuron (asterisk) attached to a patch pipette (open arrow) during whole cell recording that exhibited cholinergic-like firing activity and characteristics. These neurons had comparatively depolarized membrane potentials (−60 mV) and were mostly quiescent in brain slices. They responded with oscillatory spike discharge (A2, B1, C1) to the lowest intensity of intracellular depolarizing current pulses (i.e., 50 pA) used in the present study. Each spike in cholinergic-like neurons was preceded by a slow depolarization, with spike activity elicited continuously without accommodation throughout

the entire duration (1s) of membrane depolarization. In addition, cholinergic-like neurons responded to hyperpolarizing current pulses with a characteristic sag in the membrane potential (dashed arrows in A2) and a rebound depolarization that often led to spike discharge (A2, open arrowheads) after the hyperpolarizing current step. The current-voltage plot revealed a high input resistance (e.g., 137 M Ω) in this cholinergic-like neuron. Cholinergic-like neurons responded to cortical stimulation (upward arrows) with prominent GABAergic IPSPs (peak marked by asterisk) that were preceded by small amplitude (<2 mV) EPSPs. Upward arrows mark the stimulus artifacts that were removed for the clarity purpose. (A3 & A4) D1 antagonist SCH 23390 application (A3; 10 μ M, 10 min) did not affect the cortical-evoked GABAergic inhibitory potentials (before: red; after: blue) in WT slices. Further, D1 agonist application (A4; before: red; after: blue; an average of 5 repetitions) also failed to alter the evoked inhibitory potential. (B1 & C1) Similar current-voltage relationship was noted in WT vs KO cholinergic-like interneurons. (B2 & C2) Disynaptic IPSPs in both WT (B2) and KO (C2) cholinergic neurons showed a progressive increase in amplitude in response to gradually increasing intensities (0.2–0.7 mA) of cortical fiber stimulation. (B3 & B4) In the WT neuron, IPSPs (11 mV; average of 5 repetitions; B3) were evoked (at 0.5 mA) before quinpirole. Approximately 55% (B4, green trace) and 90% (B4, blue trace) of IPSPs were attenuated at 5 and 10 min, respectively, after quinpirole application. (C3 & C4) In the KO neuron (shown in Figure 3A5), IPSPs of 10 mV amplitude were evoked before (C3) and after (C4) quinpirole application, revealing no attenuation in the evoked IPSPs at 5 min (green trace) or at 10 min (blue trace). (D) The GABA_A receptor antagonist bicuculline (10 μ M; n = 6) attenuated cortical-evoked IPSPs (blue: before, vs black: after the antagonist). Spontaneous oscillatory spike discharge (green trace) was observed in this neuron approx 20 minutes into the BIC administration. (E) Summary of the quinpirole dose-dependent effects on cortical IPSPs in WT large aspiny (cholinergic-like) interneurons and its loss in KO neurons. Statistical significance (*p < 0.05) was noted for the quinpirole's effect on cortical IPSPs in WT large aspiny (cholinergic-like) interneurons. Scale bar in A1: 100 μ m. A2, B1, C1 scale bars = 20 mV; 500 ms. B2–4 & C2–4 scale bars = 20 mV; 100 ms. A3, A4 scale bars = 20 mV; 200 ms.