Published in final edited form as:

Brain Res. 2014 November 7; 1588: 1–16. doi:10.1016/j.brainres.2014.09.015.

BK Channels Mediate Dopamine Inhibition of Firing in a Subpopulation of Core Nucleus Accumbens Medium Spiny Neurons

Xincai Ji and Gilles E. Martin

University of Massachusetts Medical School, The Brudnick Neuropsychiatric Research Institute, Department of Psychiatry, 303 Belmont Street, Worcester, MA 01604

Abstract

Dopamine, a key neurotransmitter mediating the rewarding properties of drugs of abuse, is widely believed to exert some of its effects by modulating neuronal activity of nucleus accumbens (NAcc) medium spiny neurons (MSNs). Although its effects on synaptic transmission have been well documented, its regulation of intrinsic neuronal excitability is less understood. In this study, we examined the cellular mechanisms of acute dopamine effects on core accumbens MSNs evoked firing. We found that 0.5 μ M A-77636 and 10 μ M quinpirole, dopamine D1 (DR1s) and D2 receptor (D2Rs) agonists, respectively, markedly inhibited MSN evoked action potentials. This effect, observed only in about 25% of all neurons, was associated with spike-timing-dependent (STDP) long-term potentiation (tLTP), but not long-term depression (tLTD). Dopamine inhibited evoked firing by compromising subthreshold depolarization, not by altering action potentials themselves. Recordings in voltage-clamp mode revealed that all MSNs expressed fast (I_A), slowly inactivating delayed rectifier (I_{dr}), and large conductance voltage- and calcium-activated potassium (BKs) channels. Although A-77636 and quinpirole enhanced I_A , its selective blockade by 0.5 μ M phrixotoxin-1 had no effect on evoked firing. In contrast, exposing tissue to low TEA concentrations and to 10 μ M paxilline, a selective BK channel blocker, prevented D1R agonist from inhibiting MSN firing. This result indicates that dopamine inhibits MSN firing through BK channels in a subpopulation of core accumbens MSNs exclusively associated with spike-timing-dependent long-term potentiation.

Keywords

dopamine; nucleus accumbens; potassium channels; mice; BK channels

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Corresponding Author: Gilles E. Martin, University of Massachusetts Medical School, The Brudnick Neuropsychiatric Research Institute, Department of Psychiatry; 303 Belmont Street, Worcester, MA 01604. Tel: 508-856-4074, Fax: 508-856-2526, gilles.martin@umassmed.edu.

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Author contributions: X.J. acquired and analyzed data. G.E.M designed experiments, and wrote the manuscript.

Introduction

Several decades of research leave little doubt about the role of dopamine in mediating acute and chronic effects of drugs of abuse (Luscher and Malenka, 2011). It is well established that the complex action of dopamine results in part from the modulating electrical properties of GABAergic projecting MSNs of the nucleus accumbens, a region involved in drug reward and goal-directed behaviors (Olds, 1982; Wise and Hoffman, 1992) that receives strong inputs from the ventral tegmental area dopaminergic neurons (Humphries and Prescott, 2010). In contrast to this uncontroverted understanding, the role of dopamine in altering MSNs intrinsic neuronal excitability remains unclear. Although this question has received some attention over the years, a consensus on the mechanisms employed by dopamine remains elusive. It is generally agreed that, in the dorsal striatum, a brain region anatomically similar to the core accumbens subdivision (de Olmos and Heimer, 1999), dopamine regulates MSNs excitability by altering voltage-gated conductances controlling directly or indirectly action potential firing. Activation of dopamine D1Rs attenuates action potentials by inhibiting sodium channels (Surmeier et al., 1992). Dopamine also targets a number of voltage-gated N, P, and Q calcium channels (Surmeier et al., 1995) recruited during rapid action potential-driven depolarizations. Similarly, D2Rs depress MSNs excitability by inhibiting sodium and calcium channels, as shown in a number of studies (Greif et al., 1995; Hernandez-Lopez et al., 2000; Olson et al., 2005).

In contrast to the understanding of the action of dopamine on dorsal striatum, its action on nucleus accumbens MSNs excitability is much less understood. A study by Zhang et al., (1998) reported that dopamine D1Rs inhibited fast inactivating sodium currents, a phenomenon mediated by the cAMP-dependent protein kinase pathway, corroborating data found in the dorsal striatum. In addition to sodium channels, early intracellular recording studies indicated that D1Rs and D2Rs hyperpolarized and depolarized MSNs, respectively, by altering properties of potassium channels, although their exact identity remained unclear (Uchimura et al., 1986). These responses were small, however, usually no more than 6 mV, and subsequent studies had difficulty observing these effects despite the use of several different recording techniques (Harvey and Lacey, 1996; Harvey and Lacey, 1997; Nicola and Malenka, 1997; Pennartz et al., 1992). Recently, Perez et al (2006) found that the D2R agonist quinpirole inhibited most core accumbens MSNs firing, an effect they attributed to the enhancement of a slowly inactivating delayed rectifiers, although they did not directly identify this current. In this work, we present evidence that activation of D1Rs and D2Rs dramatically decreased core MSNs firing in only about 25% of all MSN tested. Contrary to previous findings, this inhibition is not mediated by slowly inactivating delayed rectifiers, but instead by BK channels. Our study reveals a novel mechanism employed by dopamine to alter evoked firing in a discrete subpopulation of MSNs.

Results

Dopamine Decreases Firing in tLTP but not in tLTD Medium Spiny Neurons

In a previous work (Ji and Martin 2012), using spike-timing dependent plasticity, a model of synaptic plasticity, we reported that the same pairing protocol induced tLTP or tLTD, indicating the putative existence of two populations of MSNs. Because tLTP and tLTD

MSNs presented similar basic biophysical properties, it was impossible to distinguish one population from the other at the outset of each recording session. Therefore, we sought to identify markers specific to each group. We explored the idea that MSNs associated with tLTP expressed D1Rs, while those associated with tLTD expressed D2Rs as shown in the dorsal striatum (Calabresi et al., 2007), a brain region anatomically very similar to the core nucleus accumbens (de Olmos and Heimer, 1999). As an assay, we tested the effects of 0.5 μ M A-77636 and 10 μ M quinpirole, specific D1Rs and D2Rs agonists, respectively, on MSNs evoked firing. We found that A-77636 dramatically inhibited firing only in some tLTP MSNs (Fig. 1D and F), while it had no effects on tLTD neurons (Fig. 1A and C). This inhibition was accompanied by a stable resting membrane potential and input resistance (Fig. 1E, left and middle graphs). Similarly, D2R agonist quinpirole inhibited firing of tLTP MSNs only (Ji and Martin, 2012).

Dopamine Receptor Target Subthreshold Conductances

Several explanations may account for D1Rs and D2Rs selective inhibition of tLTP MSNs firing. First, induction of LTP may change the sensitivity of dopamine receptors. However, this seems unlikely since MSNs firing is inhibited when no induction was performed (see Fig. 10A) in proportion similar to our previous study (Ji and Martin, 2012). Another explanation hinges on sodium and calcium channels. Indeed, these channels directly shape action potentials and are robustly inhibited by DA (Nicola et al., 2000). Characteristically, in our study, action potential overshoot (control: 37.2 ± 1.26 mV $n = 8$; A-77636: 36.1 ± 1.86 mV $n = 4$; quinpirole: 36.89 ± 2.01 mV $n = 3$), width (control: 1.4 ± 0.15 ms; A-77636: 1.35 ± 0.22 ms; quinpirole: 1.41 ± 0.15 ms), and threshold (control: -42.5 ± 1.25 mV; A-77636: -43.6 ± 1.38 mV; quinpirole: -44.5 ± 2.12 mV) of MSNs that nearly lost their ability to fire remained unchanged in presence of D1R and D2R agonists (Fig. 2 B, D and E). This suggested that D1Rs- and D2Rs-mediated inhibition of tLTP MSNs firing due to the action of these agonists on action potentials themselves was unlikely. However, the ability of MSNs to reach spike threshold was consistently compromised in presence of dopamine receptor agonists, as the first action potentials appear later after the start of the current pulse in presence of the agonists (Fig. 2F). These observations suggested that A-77636 and quinpirole targeted subthreshold currents and not ionic conductances directly shaping action potentials.

Fast Inactivating Conductances do not Mediate Dopamine Receptors' Effect on Firing

Early work showed that in response to a constant positive current pulse, MSNs depolarize slowly before reaching spike threshold, as shown in Fig. 2E (right panel). This slow ramping subthreshold depolarization is controlled in part by delayed rectifier potassium channels. Although these channels have received some attention in MSNs of the dorsal striatum (Nisenbaum and Wilson, 1995; Shen et al., 2004; Tkatch et al., 2000), less is known about their contribution and their regulation by dopamine receptors in core NAcc. Therefore, we sought to better characterize the contribution of the various potassium currents, before studying their roles in mediating the effects of dopamine on MSNs firing. Although recording outward currents in slices induces larger space clamp errors than in freshly isolated neurons, errors are mitigated by the fact that most channels directly involved in

regulating action potential firing are expressed primarily in the soma, a neuronal compartment that is well clamped compared to remote dendrites.

As in dorsal striatum MSNs, large depolarizing voltage steps from a holding potential (i.e. -90 mV) close the core accumbens MSNs resting membrane potential (-86 mV), evoked a complex response characterized by an early fast outward current (left panel, arrow head, Fig. 3A) that quickly decays before reaching a steady state at the end of the voltage step (open circles, 4Fig. 3A). We isolated the fast inactivating conductances (I_A) by subtracting currents evoked from resting potential held at -40 (Fig. 3A, right panel) and -90 mV (Fig. 3A, right panel). Representative traces shown in Fig. 3B indicate that these currents have a $V_{1/2}$ of -9 mV. On average, core nucleus accumbens MSNs I_A current $V_{1/2}$ was -8.47 mV (Fig. 3F, solid circles, $n = 8$), a value similar to that of the dorsal striatum MSNs (Tkatch et al., 2000), suggesting the expression of Kv4.2 subunits. As expected for I_A currents, they are also characterized by a very fast inactivation that takes place within 100 ms of the onset of the voltage step (Fig. 3C). The inactivation kinetics was best fitted with a single exponential (Fig. 3C, red line) when currents were recorded at 0 mV. The inactivation rate ranged from 26 to 36 ms with an average of 30.2 ± 2.3 ms (Fig. 3D). We examined the steady-state voltage dependence of I_A inactivation by evoking currents with a series of 10 mV incremental conditioning voltage steps from a holding potential of -90 mV to -40 mV followed by a short step to $+10$ mV (Fig. 3E). Averaged over 9 MSNs, the voltage dependence of inactivation was -49.89 mV, which is more depolarized than in dorsal striatum MSNs.

On the same neuron as in Fig. 3A, we measured I_A channels ability to recover from inactivation induced with a series of 800 ms conditioning voltage steps from a holding potential of -90 mV to -40 mV, followed by 50 ms test pulses to $+20$ mV (Fig. 4A) of increasing delay from 10 to 200 ms with 10 ms interval increments. Traces from a representative MSN show that I_A currents are inactivated by holding membrane potential at -40 mV before recovering when membrane was stepped from -90 mV to $+20$ mV. The recovery was biphasic, with an initial fast recovery phase followed by period during which I_A current amplitude slowly level off before reaching a steady state. When averaged over 8 MSNs, we found that the recovery kinetics was well fitted with a double exponential (Fig. 4B), with fast ($\tau_1 = 23 \pm 2.2$ ms) followed by a slow exponential ($\tau_2 = 167 \pm 13.4$ ms). We tested the effects of phrixotoxin-1 (100 nM), a toxin that selectively blocks Kv4.2 and Kv4.3 channels in oocytes and in cardiomyocytes (Chagot et al., 2004; Diochot et al., 1999) at concentrations as low as 30 nM but that had no effects on Kv1.4 subunits. In 5 MSNs, it decreased I_A current amplitude without affecting the slowly inactivating delayed rectifier current (Fig. 4C and D). Surprisingly, in a smaller subset of MSNs ($n = 4$), it had no effect (Fig. 4E and F), suggesting that I_A channels expression is more heterogeneous than previously thought. In light of phrixotoxin-1 effects on I_A currents, we explored whether this toxin could alter MSNs firing, and mediate the effects of dopamine, hoping to observe an increase of firing in about half of all MSNs. To ensure that changes observed in presence of the toxin could safely be attributed to the drug itself, we first evoked a series of action potentials by injecting ~ 200 pA depolarizing current pulses every 30 sec for up to 25 minutes in absence of the toxin, in a subset of 4 MSNs. In these conditions, the number of evoked action potentials was stable over the duration of the recordings (Fig. 5A). This was

accompanied by a stable RMP and input resistance (Fig. 5B). Surprisingly, phrixotoxin-1 failed to alter firing pattern in all MSNs tested ($n = 7$; Fig. 5D-F), suggesting that Kv4.2 I_A currents in core MSNs do not significantly contribute to subthreshold conductances, and therefore are unlikely to contribute to dopamine-mediated inhibition of firing in NAcc MSNs.

Slowly Inactivating Delayed Rectifiers Strongly Regulate MSN NAcc Evoked Firing

We next examined the effects of 4AP and TEA on outward currents and firing patterns at concentrations (0.5 and 10 mM respectively) that target slowly inactivating delayed rectifier while leaving I_A currents intact (Coetzee et al., 1999). Unlike phrixotoxin-1, 4AP/TEA strongly reduced the outward currents in all MSNs (Fig. 6A), while leaving intact I_A currents (data not shown). The graph in Fig. 6B shows the extent of the reduction of late outward current amplitude in the same neuron as in Fig. 6A. On average, 4-AP and TEA reduced the amplitude of the outward currents at the end of the depolarizing voltage pulses by $58 \pm 1.3\%$. Unlike phrixotoxin-1, TEA/4AP increased the number of action potentials from 6 to 10 (Fig. 6C) in presence of 4AP and TEA. In a group of 7 MSNs, TEA and 4-AP systematically increased firing from an averaged of 4.3 ± 0.04 to 7.2 ± 0.18 action potentials (Fig. 6E, right graph). Having shown that core accumbens MSNs intrinsic excitability was controlled in part by slowly inactivating delayed rectifiers, we questioned whether the effects of A-77636 and quinpirole on MSNs firing were mediated through these channels. To address this question, we next examined the effects of these agonists on outward currents. In control conditions, when slices were not exposed to drugs, outward currents were stable over a period of 20 min as shown in Fig. 7A and B, ensuring that any change of current amplitude in presence of agonists could safely be attributed to the drugs and not to random changes of current amplitudes over time. In the presence of A-77636, the selective DAR1 agonist, outward currents, evoked with a depolarizing voltage step to +20 mV from a holding potential of -90 mV, were insensitive to the agonist (Fig. 7D, F gray columns) in a majority of MSNs ($n = 8$). In contrast, in a smaller subset of 4 MSNs, exposure to A-77636 led to an increase of both I_A and I_{dr} currents (Fig. 7E, F open columns). Averaged over 4 cells, A-77636 increased outward I_A and I_{dr} currents to 113 ± 3.4 and $110 \pm 2.42\%$ of control, respectively (Fig. 7F). Although relatively modest, this increase was highly significant compared to control ($F(26,32) = 3.04$, $p < 0.001$ for I_A ; $F(26,32) = 2.34$, $p < 0.001$ for I_{dr}). In similar experimental conditions, quinpirole failed to alter outward current amplitudes in most MSNs, mirroring what we found with A-77636 (Fig. 7G). However, as with A-77636, quinpirole increased I_A and I_{dr} current amplitude in a subset of MSNs by 115 ± 3.34 and $113 \pm 3.06\%$ ($F(35,37) = 3.79$, $p < 0.001$). The increase was not uniform, however, between I_A and slowly inactivating delayed rectifier currents. Indeed, while it boosted I_A current amplitudes in one third all the neurons tested (5 out of 16), the proportion was lower for slowly inactivating currents (3 out of 16; Fig. 7E). These effects were blocked by 10 μ M SCH 23390 and 3 μ M L-741,626, selective dopamine D1Rs and D2Rs antagonists, respectively (data not shown). Having shown the interactions between dopamine receptors and delayed rectifiers in a subpopulation of MSNs, we sought to determine whether blockade of these currents would inhibit dopamine effects on MSNs firing. First, we exposed MSNs to TEA and 4-AP for 4 min before adding A-77636. As

shown previously, 4-AP/TEA increased MSNs firing. In presence of A-77636, the firing rate remained unchanged in all 8 MSNs tested (Fig. 8A).

BK Channels Mediate Dopamine Effects on Evoked Firing

Although these data appeared to implicate slowly inactivating delayed rectifiers in dopamine receptor-mediated inhibition of MSNs action potential firing, we sought to rule out the possible involvement of BK channels. Indeed, like delayed rectifiers, BK currents inactivate very slowly, are blocked by TEA, are activated within the same range of membrane potentials, and are enhanced by dopamine in neurons of the motor system (Kloppenburg et al., 1999). Although we described extensively the properties of BK channels in NAcc MSNs in a previous study (Martin et al., 2004), we never determined their overall contribution to the macroscopic outward currents. We exposed slices to 1 mM TEA, a concentration that blocks all BK channels without affecting slowly inactivating delayed rectifiers, in the presence and absence of A-77636. We used this low TEA concentration as it targets all BK channels regardless of their subunit composition. Thus, 1 mM TEA partially inhibited representative outward currents evoked from a holding potential of +40 mV to inactivate I_A channels. Symbols indicate where currents were measured (Fig. 9A). Exposing the same MSN to 10 mM TEA further reduced the amplitude of the outward currents, but left a portion of the outward current intact. On average, 1 and 10 mM TEA reduced current amplitudes by 23%, and 63% of control (Fig. 9B) when measured at +20 mV, but failed to fully inhibit outward currents. This result demonstrates that the contribution of BK channels to the macroscopic current is about half that of the slowly inactivating delayed rectifiers. In current-clamp recording mode, 1 mM increased moderately MSN evoked firing which jumped from 6 to 8 action potentials (Fig. 9C). In presence of this low TEA concentration (i.e. 1 mM), A-77636 failed to significantly reduce MSNs firing rate as shown in figure 9C (TEA + A-77636) where the number of action potentials dropped from 8 to 7, while leaving resting membrane potential (Fig. 9D, left graph) and input resistance (Fig. 9D, middle graph) unchanged. Thus, in 13 MSNs, the D1R agonist marginally reduced the number of action potentials in 3 neurons, but had no effect at all on the remaining 10 neurons. On average, the number of spikes in the presence of A-77636 dropped from 6.7 ± 0.6 to 5.2 ± 0.5 . This effect, while modest in comparison to A-77636 alone, was statistically significant ($F(24,25) = 1.51$; $p < 0.01$), suggesting that slowly inactivating delayed rectifier may marginally contribute to D1R-mediated inhibition of NAcc MSNs evoked firing. In light of these findings, the seemingly small (i.e., ~10%) increase of the slowly inactivating current amplitude shown in Fig. 7 must be reconsidered and put in perspective. Because this value was initially derived from measurements of the whole macroscopic currents, D1R- and D2R-mediated potentiation represents actually a more robust 44% and 52% increase, respectively, as it originates from BK currents whose fraction of the macroscopic current is only 23%, as detailed above (Fig. 9B).

To confirm the role of these channels and to identify their subunit composition, we exposed slices to either 0.5 μ M iberiotoxin (Ibtx) or 10 μ M paxilline before testing the effects of 0.5 μ M A-77636. While Ibtx blocks $\alpha\beta 1$ channels only, the alkaloid paxilline blocks both $\alpha\beta 1$ and $\alpha\beta 4$ BKs. In previous works, we have shown that both subunits are expressed in mice and rats NAcc MSNs and that they shape action potentials (Ji and Martin, 2012; Martin et

al., 2004), although their contribution to subthreshold depolarization is unclear. Despite the presence of Ibtx, A-77636 reduced MSNs firing in 3 out of 9 MSNs (Fig. 10A and B). In another subset of 10 MSNs, exposing slices to paxilline blocked nearly all the effects of A-77636 (Fig. 10D and E). Although a small decrease was observed as the number of action potentials dropped from 4.67 ± 0.5 in presence of Pax to 4.43 ± 0.3 APs in presence of Pax + A-77636 ($n = 10$), this effect was not significant. This result suggests that BK channels mediating the effects of dopamine receptors are likely composed of the $\beta 4$ auxiliary subunit.

Discussion

In this work, we provide new information regarding the various conductances underlying main outward currents, their respective contribution to the total currents, their involvement in controlling evoked action potential firing, and their role in mediating dopamine receptor-mediated inhibition of MSNs evoked firing. In particular, we showed that neither fast (I_A) nor slowly inactivating delayed rectifiers are responsible for dopamine action on MSNs firing. Instead, the dramatic reduction of MSN firing is likely caused by dopamine receptor-mediated enhancement of BK currents, a new finding in nucleus accumbens.

Early studies indicated that stimulation of D1R inhibited dorsal striatum MSNs intrinsic excitability (Surmeier et al., 2007). While supported by our findings, this view is at odds with the idea that inhibition of neuronal excitability is achieved through either sodium and/or calcium channels. Indeed, in the dorsal striatum, this effect is controlled by D1Rs's action on sodium channels ((Surmeier et al., 1992; Schiffmann et al., 1995; Zhang et al., 1998), and on inward rectifying conductances (Pacheco-Cano et al., 1996). We were unable to replicate these findings as action potentials appeared unchanged after dopamine exposure when MSNs stopped firing almost entirely. Similarly, we observed no shift in resting membrane potentials or input resistance that could have indicated the contribution of inward rectifying conductances. Instead, our work clearly points to BK channels and to a lesser extent slowly inactivating delayed rectifiers as mediating the effects of dopamine on firing. Regarding the effects of quinpirole, the D2R agonist, our findings are in line with previous findings showing that activation of this receptor inhibits MSNs firing in prefrontal cortex and striatum (Gulledge and Jaffe, 1998; Tseng and O'Donnell, 2004), although an increase of spiking was reported by coactivation of D1Rs and D2Rs in the nucleus accumbens (Hopf et al., 2003). Based on work in oocytes, it is believed that this effect is mediated in part by M currents (Ljungstrom et al., 2003). However, it is unlikely that this conductance is responsible for the effects we observed since KCNQ subunits, although weakly expressed in NAcc, are found in interneurons, not in MSNs (Cooper et al., 2001). In a recent study, Perez et al (2006) found that D2Rs reduced MSNs spiking, an effect they attributed in part to slowly inactivating delayed rectifiers based on the use of low (i.e., 10 μ M) 4-AP concentrations, a surprising conclusion considering that concentrations 5 to 10 times higher are typically required to block such conductance (Coetzee et al., 1999; Nisenbaum et al., 1994).

In our study, action potentials remained unchanged in neurons with firing severely inhibited by D1Rs and D2Rs agonists, an observation that appears to be at odds with the established role of BK channels in regulating neuronal excitability. Indeed, BK channels control

neuronal excitability in a number of preparations by modulating spike waveform (Poolos and Johnston, 1999; Shao et al., 1999). It is unclear why dopamine receptors did not alter action potential themselves but spike threshold instead. One possible explanation is that BK channels contributing to spike threshold and action potentials may have different subunit composition and only those involved in the former may be targeted by dopamine receptors. NAcc MSNs would possibly lend themselves to this idea considering that their BK channel expression is complex, with BK channels composed of either $\beta 1$ or $\beta 4$ auxiliary subunits (Martin et al., 2004). The impact of BK channel composition on spiking is illustrated by their seemingly opposite effects on hippocampal pyramidal neuron excitability. Thus, exposure of CA1 neurons to tedisamil, a blocker specific for cardiac BK channels (i.e. α and $\alpha\beta 1$ BKs) reduce firing by altering spike duration and afterhyperpolarization (Church and McLarnon, 2002). In contrast, in transgenic mice lacking the $\beta 4$ subunit, dendate granule cells firing was increased (Brenner et al., 2005). Some have argued that only when $\beta 4$ subunit is expressed does it confer the channel its ability to control action potential characteristics (Jaffe et al., 2011). Regardless of the mechanism, studies on neocortical pyramidal and guinea pig sympathetic neurons supports the idea that BKs role in controlling spike firing is not limited to targeting action potential themselves but extend to setting spike threshold as well (Benhassine and Berger, 2008; Locknar et al., 2004), as in our study.

The other intriguing observation concerns the regulation of MSNs firing by dopamine receptors in about ~25% of all MSNs recorded. This observation raises the possibility that inhibition of evoked firing by D1Rs and D2Rs occurs in the same MSNs. In the core and shell accumbens regions, MSNs are typically divided in two groups as in the dorsal striatum. Neurons from the first group project directly to the substantia nigra/ventral tegmental area, express substance P, and high levels of D1Rs. In contrast, neurons projecting first to the ventral pallidum belong to the indirect group and express enkephalin and mostly D2Rs (Lu et al., 1998). Some MSNs, however, are believed to express D1Rs and D2Rs, both in the dorsal and ventral striatum as shown with immunohistochemical (Le Moine and Bloch, 1995) and single-neuron RT-PCR studies (Surmeier et al., 1996). Although there is little controversy regarding the coexpression of D1R and D2Rs in MSNs, the percentage of MSNs varies widely between studies. Aizman et al (2000) reported that, after two weeks in culture, virtually all striatum MSNs co-expressed D1Rs and D2Rs. That finding contrasts with a number of studies carried out in brain slices from young or adult rats and monkeys. In situ hybridization and immunochemistry studies showed that less than 5 % of MSNs express both dopamine receptors (Aubert et al., 2000; Le Moine and Bloch, 1995). Also, single cell rt-PCR showed a markedly higher percentage (~25%) of coexpression in the dorsal striatum (Surmeier et al., 1996), a value that mirrors our electrophysiological data (Ji and Martin, 2012). In shell NAcc, Bertran-Gonzalez (2008) showed that while the vast majority of D1R and D2R are segregated in substance P- and enkephalin-expressing MSNs, a minority of about MSNs (~17%) expressed both D1R and D2Rs (Bertran-Gonzalez et al., 2008). Functionally, there is evidence that D1R and D2Rs form functional heteromers in a subset of enkephalin-expressing MSNs that may have functional characteristics distinct from neurons expressing either D1 or D2 receptor (Hopf et al., 2003; Perreault et al., 2011). Taken together, these data suggest that inhibition of firing by A-77636 and quinpirole may occur in a subpopulation of MSNs expressing enkephalin, and belonging to the indirect pathway.

Although evidence is still indirect, this population of MSNs may overlap with neurons co-expressing D1R and D2Rs. Certainly, conjecture about the role of this particular set of neurons is rather perilous. The recent development of genetic tools (i.e. immediate early genes activated during learning combined with fluorescent proteins, see Reijmers and Mayford 2009 for review), however, has been instrumental in studying the neurobiology of complex behavior. Astoundingly, this emerging field in neuroscience shows that only a very small percentage of neurons (i.e. ~ 3 %) are actually recruited during acquisition of a behavior (e.g. fear in amygdala). If we extend this conclusion to the nucleus accumbens, one can wonder what is the role of the remaining 97% of MSNs that perhaps include neurons identified in our study. Indeed, an overlooked facet of the nucleus accumbens physiology is that, beside its well-known role in drug addiction, it is involved in a number of other physiological functions (e.g. feeding, depression). Therefore, the information we possess seems too fragmentary to hypothesize about the role of this subpopulation of MSNs in relation with drug abuse.

Experimental Procedure

The C57B/6 mice used in these studies were housed in an animal care facility approved by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). Animal experimental procedures followed a protocol approved by the Institutional Animal Care and Use Committee of University of Massachusetts Medical School. This protocol adhered to the ethical guidelines described in The Care and Use of Laboratory Animals 8th Edition (Garber, 2011). All efforts were made to minimize animal suffering and to reduce the number of animals used. Mice were housed four per cage and maintained at constant temperature and humidity with a 12-h light–dark cycle. Water and food were provided ad libitum.

Animals and slice preparation

We used 21–30 day old male C57BL/6 mice to prepare slices from fresh brain tissue. Mice were deeply anesthetized with isoflurane, and killed by decapitation. We rapidly removed and transferred the brain in a cold (~ +0.5°C) oxygenated (95% O₂ and 5% CO₂) high-sucrose solution of the following composition (in mM): 125 Sucrose, 53 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂, 25 D-Glucose, 26 NaHCO₃, and kynurenic acid (0.18 mg/ml). We cut slices (300 µm) transversely with a Vibroslicer (VT1200, Leica MicroInstruments; Germany), and immediately transferred them in an incubation chamber containing a modified Earl's Balance Salt Solution (EBSS) maintained at 35° C. Slices were left to recuperate in the EBSS solution for 50 min to 1 hour before being moved into an artificial cerebrospinal fluid (ACSF; in mM): 126 NaCl, 2.5 KCl, NaH₂PO₄·H₂O, 1 MgCl₂, 2 CaCl₂, 26 NaHCO₃, 15 D-Glucose, at room temperature. We perfused submerged slices at room temperature (~21°C) with a carbogenated ACSF, at a constant rate of 1 – 2 ml /min in the recording chamber. We visualized neurons in infrared differential interference contrast (IR-DIC) videomicroscopy using a fully motorized microscope (Scientifica; Uckfield England) mounted with Olympus objectives (10x and 60x; Olympus Microscopy, Japan).

Electrophysiology

We filled borosilicate glass electrodes (1.5 mm OD, 4–6 M Ω resistance) with an internal solution containing (in mM): 120 K-methanesulfonate; 20 KCl; 10 HEPES; 2 K₂ATP, 2 K₂GTP, and 12 phosphocreatine. Following seal rupture, series resistance (R_s) typically between 10 and 20 M Ω was compensated (75–85%), and periodically monitored throughout recording sessions. Recordings with R_s changes larger than 20% were rejected. We acquired voltage and current traces in whole-cell patch-clamp with an EPC10 amplifier (HEKA Elektronik; Germany). In voltage-clamp recording mode, traces acquired with PatchMaster 2.15 (HEKA Elektronik; Germany), were sampled and filtered at 5 kHz, and 2 kHz respectively. All traces were subsequently analyzed off-line using FitMaster 2.15 (HEKA Elektronik; Germany). Leak and capacitive currents were subtracted online using P/4 procedures. To isolate I_A current, we added the following reagents to the external solution: 0.5 mM 4-aminopyridine (4-AP), 10 mM TEA, 1 μ M TTX. In current-clamp mode, current and voltage traces were digitized at 2 kHz and filtered at 20 kHz, respectively.

We evoked synaptic events by positioning a bipolar stimulation electrode (FHC; Bowdoin, ME) ventral of the anterior commissure, at the interface between core and shell accumbens regions. In control (10 min) and post-induction (up to 70 min) conditions, we evoked EPSPs at 0.05 Hz at resting membrane potential (RMP $\sim$ –86 mV; the junction potential was left uncompensated). We adjusted the stimulus intensity (100 μ s, 40–100 mA) to evoke subthreshold EPSPs between +10 and +15 mV amplitude. We generated tLTP and tLTD by pairing an EPSP and a postsynaptic AP evoked with a 5 ms/800 pA depolarizing pulse.

To study the action of dopamine receptor agonists on MSNs RMP, Rin, and firing pattern, we injected hyperpolarizing (–110 pA, 800 ms) and depolarizing ($\sim$ +260 pA but adjusted in each neuron to evoke about 6–8 APs) current steps with 10 sec intervals. This protocol, repeated every 30 sec before and during exposure to drugs, followed our usual plasticity stimulation paradigm (10 min control, AP-EPSP 20 ms 1 Hz, 10 min post induction period) to determine whether the recorded cell was “tLTP” or “tLTD”.

Analysis

We fitted current-voltage data with Boltzman's equation ($1/(1 + \exp((V - V_h)/V_c))$), where V is membrane potential, V_h is half activation voltage, and V_c is the slope factor, in Igor Pro 6.22 (WaveMetrics, Lake Oswego OR). We compared EPSPs maximum amplitude measured in a 20 ms time window, 10 ms after the stimulus artifact, with its baseline amplitude 10 ms before it. We performed this measurement on 30 consecutive EPSPs before, and 20 min after APs-EPSPs pairing. We expressed the difference of EPSP amplitude before and after induction as percent of control (100 %). Delayed rectifier potassium currents decay was measured using a single exponential in FitMaster 2.15. Statistical analysis, in Prism 5 (Graphpad, CA, USA) running on a Mac Power PC G5, was performed with Student's one sample or unpaired t tests, with $P < 0.05$ considered statistically significant. All averaged results are expressed as mean $\pm$ SEM values.

4-aminopyridine (4-AP), TEA were purchased from Sigma Aldrich, A-77636, quinpirole, paxilline, SCH 23390 and L-741,626 from Tocris Bioscience (Bristol, UK), and phrixotoxin-1, TTX from Alomone Labs (Jerusalem, Israel).

Acknowledgments

Funding: This work was supported by the National Institute of Alcohol Abuse and Addiction AA-020501

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- Dopamine inhibits evoked firing in a subpopulation of core accumbens medium spiny neurons
- Potassium channels controlling sub-threshold depolarization mediate this inhibition.
- More specifically, BK channels appear to be specifically targeted by dopamine.

Figure 1.

Differential effects of D1 receptor agonist A-77636 on tLTP- and tLTD-MSNs. **A.** Voltage traces in response to hyperpolarizing (-110 pA) and depolarizing (+260 pA) current pulses (800 ms) recorded at various interval before (control) and during exposure to A-77636. Note A77636's lack of effects on the neuron resting membrane potential, input resistance, and spiking. **B.** Graphs of resting membrane potential (RMP), input resistance (Rin) expressed as amplitude of voltage deflection in mV, and number of action potentials (right panel) of neuron shown in A, monitored every 30 sec before and during exposure to the drug. Again,

note the stability of all three parameters during the recording session. **C.** EPSPs traces measured every 20 sec at RMP before (control) and after AP-EPSP pairing (post-induction) from same neuron shown in A. The red lines represent cumulative EPSPs traces in control and post-induction conditions. **D.** Voltage traces of a difference MSN in response to hyperpolarizing (-110 pA) and depolarizing ($+270$ pA) current pulses recorded at various interval before (control) and during exposure to A-77636. Note the massive effect of the drug on spike firing. **E.** Graphs of resting membrane potential (RMP), input resistance (R_{in}) expressed as amplitude of voltage deflection in mV, and number of action potentials (right panel) of neuron shown in D, monitored every 30 sec before and during exposure to the drug. Again, note the stability of all three parameters during the recording session. **F.** EPSPs traces measured every 20 sec at RMP before (control) and after AP-EPSP pairing (post-induction) from neuron shown in D. The red lines represent cumulative EPSPs traces in control and post-induction conditions.

Figure 2.

Decrease of MSNs firing by dopamine receptors is not caused by alterations of action potential properties. **A.** Voltage traces in response to hyperpolarizing (–110 pA) and depolarizing (+250 pA) current pulses (800 ms) recorded before (Control, black traces) and 4 min after exposure to A-77636 (red traces). The broken line indicates action potential threshold. **B.** From traces shown in A, overlay of first action potentials before (black trace) and during exposure to A-77636 (red trace) shows that neither the amplitude, width nor threshold are affected by the D1R agonist. **C.** Voltage traces in response to hyperpolarizing

(-110 pA) and depolarizing ($+260$ pA) current pulses (800 ms) recorded before (Control, black traces) and 3.30 min after exposure to quinpirole (red traces). The broken line indicates action potential threshold. **D.** From traces shown in C, overlay of first action potentials before (black trace) and during exposure to quinpirole (red trace) shows that, as with D1 receptor agonist, neither the amplitude, width nor threshold are affected by quinpirole. **E.** Averaged action potentials width (left panel), overshoot (middle panel), and threshold (left panel), in control conditions (control), and in presence of either A-77636 or quinpirole. **F.** From the same neuron shown in panels C and D, voltage deflections evoked by hyperpolarizing (-110 pA) and depolarizing ($+260$ pA) current pulses recorded before (Control, black traces) and 3:30 and 5 min after exposure to quinpirole. As indicated by the broken line, the quinpirole reduces the ability of the MSN to reach spike threshold.

Figure 3.

MSNs outward currents are composed of a fast and slowly inactivating delayed rectifiers. **A.** Outward currents evoked by a series of incremental (+10 mV) depolarizing voltage steps from a holding potentials of -90 mV (Left panel) and -40 mV (right panel) in presence of 0.5 μ M TTX. Panel **B** shows isolated I_A currents obtained by subtracting currents of panel A recorded at -40 from those at -90 mV. Note the very fast inactivation of this current. **C.** Representative example of the single exponential fit of I_A current inactivation kinetics. **D.** Distribution of I_A inactivation kinetics in 6 MSNs, and the averaged value (green symbol). **E.** Voltage-dependent inactivation of the I_A currents. **F.** Plot of the averaged (n = 8) peak conductance of I_A currents as a function of test pulse. Fits were obtained with Boltzmann equation.

Figure 4.

Recovery from inactivation of core NAcc medium spiny neurons fast inactivating currents.

A. Following condition pulses at -40 mV, recovery was produced by holding potential at -90 mV for various periods of time before a test pulse to 0 mV was applied. In this representative example, I_A currents recover within 50 ms from the initial inactivation. **B.** Averaged I_A current recovery, expressed recovery as percent of current amplitude evoked by conditioning pulse, as function of time between end of conditioning and beginning of test pulse. Recovery kinetic was best fitted with a double exponential function (red line). **C.**

Exposure to 100 nM Phrixotoxin-1 Inhibits I_A currents in a MSN. Black and red circles indicate where current amplitude was measured. **D.** Averaged I_A currents amplitude as function of voltage in absence (control) and 6 min after phrixotoxin-1 exposure (red circles, phrixotoxin-1) in 5 MSNs. **E** and **F.** in another group of 4 MSNs, phrixotoxin-1 failed to alter I_A current amplitudes.

Figure 5.

I_A ion channel blocker phrixotoxin-1 does not alter MSNs firing pattern. **A.** Stability of MSNs RMP, Rin and firing frequency. Voltage traces in response to hyperpolarizing (-110 pA) and depolarizing ($+250$ pA) current pulses (800 ms) recorded at various time points in absence of the drug. **B.** RMP (left), Rin (middle), and number of action potentials (right) from the same neuron as in A and monitored every 30 sec for up to 25 min. **C.** Averaged RMP, Rin and AP# from 4 MSNs monitored every 30 sec for 25 min. **D.** Voltage traces in response to depolarizing ($+250$ pA) current pulses (800 ms) recorded at various time points

before (control) and at different times during exposure to (100 nM) phrixotoxin-1. **E.** RMP (left), Rin (middle), and number of action potentials (right) from the same neuron as in D and monitored every 30 sec for up to 10 min. **F.** Averaged RMP, Rin and AP# monitored every 30 sec for 10 min before and during 0.5 μ M phrixotoxin-1 exposure from 7 MSNs. All values are expressed as averaged $\pm$ SEM.

Figure 6.

Slowly inactivating delayed rectifier currents regulate MSN firing. **A.** Outward currents evoked by +10 mV incremental voltage steps from a holding potential of -90 mV in absence (control) and presence of low concentrations of 4-AP (0.5 mM) and TEA (10 mM). Note the robust reduction of the slow inactivating delayed rectifiers. Symbols at the end of current traces indicate where amplitudes were measured. **B.** Plot of averaged current amplitude expressed as I/I_{max} as a function of voltage from 6 MSNs. **C.** Representative example of one MSN firing rate evoked by a depolarizing current pulse (+230 pA) before and during exposure to 4AP and TEA. Note the sharp increase of firing in presence of the drugs. **D.** RMP, Rin and number of action potentials before (control) and during drug exposure. **E.**

Averaged RMP, Rin and action potential numbers over 7 MSNs before and during drug exposure.

Figure 7.

Dopamine receptor enhances delayed rectifier currents in a subpopulation of MSNs. **A.** Representative outward currents evoked by +10 mV incremental voltage steps from a holding potential of -90 mV in presence of 0.5 μ M TTX illustrate the stability of current amplitudes over time. **B.** Current-voltage relationships of currents shown in **A**. **C.** Averaged current amplitude of outward currents evoked every 20 sec for 10 min with a +100 mV voltage step from a holding potential of -90 mV. Inset shows superimposed currents from a representative MSN, in response to the same single voltage step from a holding of -90 mV

to +20 mV. **D.** Averaged current amplitude monitored every 20 sec for 25 min before (control) and during A-77636 exposure. From MSN used in same graph, insert shows overlaid current traces taken in control and 10 min after beginning of A-77636 exposure. **E.** In conditions similar to D, A-77636 enhances current amplitudes in a different MSN. **F.** Averaged effects (white columns, $n = 4$), or lack thereof (grey columns, $n = 8$), of A-77636 on I_A and slowly inactivating (I_{dr}) current amplitudes. **G to E** panels show, as with A-77636, that quinpirole has no effects or increases current amplitude in a subset of MSNs.

Figure 8.

D1R and D2R agonists fail to inhibit MSNs firing in presence of I_{dr} blockers. **A.** Action potentials evoked by +230 pA current steps at different time points before and during exposure to A-77636 with or without TEA/4-AP. No firing inhibition was observed in presence of the D1R agonist. **B.** Action potentials evoked by +210 pA current steps at different time points before and during exposure to quinpirole with or without TEA/4-AP. Presence of quinpirole failed to decrease firing. **C.** Averaged number of action potentials from a group of 8 MSNs shows a marked increase of firing rate in all MSNs in presence of 4-AP, but no inhibition when A-77636 was added. **D.** Similarly, quinpirole failed to alter firing patterns in all (n = 8) MSNs tested.

Figure 9.

BK channels control the inhibition of MSNs firing by D1 receptors. **A.** Outward currents evoked by a series of 10 mV voltage steps from a holding potentials of -40 to $+30$ mV. Blockade of BK channels with 1 mM TEA decreased current amplitude, an effect amplified with a larger (10 mM) TEA concentration. Symbols at the end of current traces indicate where amplitude was measured for graphs shown in **B.** **B.** Averaged effects of 1 mM and 10 mM TEA on outward current amplitude measure between -40 and $+30$ mV in 9 MSNs. **C.** Effects of 1 mM TEA on evoked firing of a representative MSN. Note the slight increase of

the number of action potentials in presence of 1 mM TEA, and a small decrease after adding A-77636. **D.** Graphs showing the RMP (left panel), Rin (middle panel), and number of action potentials monitored every 20 sec from neuron shown in C. **E.** Averaged RMP, Rin, and number of action potentials recorded every 20 sec for up to 14 min in absence (Control), or presence of 1 mM TEA or 1 mM TEA + A-77636 in 14 MSNs.

Figure 10.

$\alpha\beta$ 4 BK channels mediate inhibition of MSNs firing by D1 receptors. **A** Action potentials evoked by a depolarizing current pulse during exposure to 0.5 μ M Ibtx and Ibtx + A-77636. DAR1 agonist inhibited firing in 3 MSNs (dark filled circles). **B** Train of action potentials evoked in presence of Ibtx alone (black traces) and Ibtx + A-77636 (red traces). Note the robust reduction of the number of action potentials and the lack of effects on the MSN RMP and Rin. **C** Graphs show averaged RMP and Rin of all MSNs (n = 9) monitored every 20 sec for the duration of recordings. **D** Number of action potentials during exposure to 10 μ M paxilline (Pax) and Pax + A-77636. DAR1 failed to significantly inhibit MSNs firing in all MSNs tested (dark filled circles). **E** Train of action potentials evoked in presence of Pax alone (black traces) and Pax + A-77636 (red traces). **F** Graphs averaged RMP and Rin of all 10 recorded MSNs before and during exposure to A-77636.