

ALTERED PROFILE AND D2-DOPAMINE RECEPTOR MODULATION OF HIGH VOLTAGE-ACTIVATED CALCIUM CURRENT IN STRIATAL MEDIUM SPINY NEURONS FROM ANIMAL MODELS OF PARKINSON'S DISEASE

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Abstract—In the present work we analyzed the profile of high voltage-activated (HVA) calcium (Ca^{2+}) currents in freshly isolated striatal medium spiny neurons (MSNs) from rodent models of both idiopathic and familial forms of Parkinson's disease (PD). MSNs were recorded from reserpine-treated and 6-hydroxydopamine (6-OHDA)-lesioned rats, and from DJ-1 and PINK1 (PTEN induced kinase 1) knockout ($^{-/-}$) mice. Our analysis showed no significant changes in total HVA Ca^{2+} current. However, we recorded a net increase in the L-type fraction of HVA Ca^{2+} current in dopamine-depleted rats, and of both N- and P-type components in DJ-1 $^{-/-}$ mice, whereas no significant change in Ca^{2+} current profile was observed in PINK1 $^{-/-}$ mice. Dopamine modulates HVA Ca^{2+} channels in MSNs, thus we also analyzed the effect of D1 and D2 receptor activation. The effect of the D1 receptor agonist SKF 83822 on Ca^{2+} current was not significantly different among MSNs from control animals or PD models. However, in both dopamine-depleted rats and DJ-1 $^{-/-}$ mice the D2 receptor agonist quinpirole inhibited a greater fraction of HVA Ca^{2+} current than in the respective controls. Conversely, in MSNs from PINK1 $^{-/-}$ mice we did not observe alterations in the effect of D2 receptor activation. Additionally, in both reserpine-treated and 6-OHDA-lesioned rats, the effect of quinpirole was occluded by the selective L-type Ca^{2+} channel blocker nifedipine, while in DJ-1 $^{-/-}$ mice it was mostly occluded by ω -conotoxin GVIA, blocker of N-type channels. These results demonstrate that both dopamine depletion and DJ-1 deletion induce a rearrangement in the HVA Ca^{2+} channel profile, specifically involving those channels that are selectively modulated by D2 receptors. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: HVA calcium channels, medium spiny neuron, 6-hydroxydopamine, reserpine, DJ-1, PINK1.

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Abbreviations: CN, cell capacitance; HBSS, Hank's balanced salt solution; HVA, high voltage-activated; MSNs, medium spiny neurons; PD, Parkinson's disease; PINK1, PTEN induced kinase 1; SNpc, substantia nigra pars compacta; 6-OHDA, 6-hydroxydopamine.

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Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by bradykinesia, rigidity, resting tremor, and postural instability. Although the occurrence of PD is largely sporadic, an increasing number of monogenic mutations in distinct genes—such as the large exonic deletions or frame-shift truncations, suggestive of a “loss of function” mechanism, found in DJ-1 and PINK1 (PTEN induced kinase 1) genes (Bonifati et al., 2003; Valente et al., 2004; Kitada et al., 2007)—has been linked to familial forms of parkinsonism, which clinically resemble idiopathic PD. The clinical features of idiopathic and familial PD are thought to result from a reduced dopaminergic input to the striatum, inducing a complex rearrangement in the functional anatomy of the basal ganglia. In particular, dopamine depletion has been shown to cause the loss of spines in medium spiny neurons (MSNs) from both animal models of PD and parkinsonian patients (for review see: Deutch et al., 2007; Smith and Villalba, 2008). This morphological alteration of MSNs has been recently ascribed to the disinhibition of Cav1.3-containing L-type Ca^{2+} channels, as a consequence of the reduced striatal dopaminergic tone (Day et al., 2006). In fact, chronic administration of an L-type channel antagonist completely prevented the spine loss induced in rats by acute DA depletion. Conversely, spine density was found to be significantly increased in *Cacna1d* $^{-/-}$ mice, lacking the Cav1.3 α 1 subunits. L-type Ca^{2+} channels have already been implicated in the selective degeneration of dopaminergic neurons in PD (Chan et al., 2009); these experimental data suggest that high voltage-activated (HVA) Ca^{2+} channels might play a direct role in striatal dysfunction, as well.

EXPERIMENTAL PROCEDURES

All experiments were performed in accordance with both the EC and Italian guidelines (86/609/EEC; D.Lvo 116/1992, respectively) and approved by the University of Rome “Tor Vergata” (n. 153/2001A).

Animal models

6-hydroxydopamine (6-OHDA)-lesioned rats. Dopamine denervation was obtained by injecting the neurotoxin 6-OHDA (1 mg/kg) into the substantia nigra pars compacta (SNpc) of adult male Wistar rats (150–170 g). Animals were anaesthetized with xylazine (7.5 mg/kg, i.m.) and ketamine (50 mg/kg, i.m.) and secured in a stereotaxic apparatus (Stoelting, Wooddale, IL, USA). The injection of 6-OHDA into the mesencephalon was performed according to the following coordinates (from bregma): AP, -4.4 ; L, $+1.2$; DV, -7.8 . After 15 days, dopamine denervation was as-

essed by apomorphine-driven test (1 mg/ml/kg). Animals showing ≥ 400 rotations/h ($n=17$) were used for the electrophysiological experiments. Fifteen rats were injected with saline solution in the same surgical sessions (sham-operated group).

Reserpine model. Adult Wistar rats (135–170 g) were injected i.p. with reserpine (3–4 mg/kg in 0.5% glacial acetic acid in PBS). Either 20–24 h ($n=13$) or 48 h ($n=4$) after reserpine injection rats displaying typical parkinsonian-like symptoms (bradykinesia, posture stiffness, and tremor) were used for electrophysiological recordings. Vehicle-treated controls were prepared in the same sessions ($n=15$).

Preparation of acutely dissociated striatal neurons

Coronal corticostriatal slices (450 μm thick) were cut as previously described (Martella et al., 2008) from brain tissue blocks of either male Wistar rat (125–150 g) or wild-type, DJ-1^{-/-}, and PINK1^{-/-} mice (aged 40–45 days) generated in J. Shen laboratories (Goldberg et al., 2005; Kitada et al., 2007). Data obtained from each animal group were compared to the respective controls. Vehicle-treated and sham-operated rats were utilized as controls for the reserpinized and 6-OHDA-lesioned animals, respectively, while wild-type littermates of the same mixed genetic background were utilized as controls for either DJ-1^{-/-} or PINK1^{-/-} mice. In brief, animals were killed under ether anaesthesia by cervical dislocation, the brains were rapidly removed, and coronal corticostriatal slices were cut with a vibratome in ice-cold Krebs' solution (in mM: 126 NaCl, 2.5 KCl, 1.3 MgCl₂, 1.2 NaH₂PO₄, 2.4 CaCl₂, 10 glucose, and 18 NaHCO₃). Afterward, as previously described (Martella et al., 2008) the striatum was dissected from slices and incubated first in HEPES-buffered Hank's balanced salt solution (HBSS), bubbled with 100% O₂ at 35 °C, and then in HBSS containing 0.5 mg/ml protease XIV for 30 min. After repeated wash-out in HBSS, the tissue was mechanically triturated with a graded series of fire-polished Pasteur pipettes. The cell suspension was placed in a Petri dish mounted on the stage of an inverted microscope (Nikon Diaphot, Japan). Healthy cells were allowed to settle for about 10–15 min.

Patch-clamp recordings

Freshly isolated MSNs were identified by their morphologic and electrophysiological properties (Martella et al., 2008). Patch-clamp recordings in the whole-cell configuration were performed by using glass pipettes (WPI PG52165-4, Germany) pulled with a Flaming-Brown puller (Sutter Instrument, Novato, CA, USA) and fire-polished before use. Pipette resistance ranged from 3 to 8 M Ω . The composition of the internal solution was (in mM): N-methyl-D-glucamine, 185; HEPES, 40; EGTA, 11; MgCl₂, 4; phosphocreatine, 20; ATP, 2 to 3; guanosine triphosphate (GTP), 0 to 0.2; leupeptin, 0.2; pH 7.36, 280 mOsm/L. After obtaining the cell access, cells were bathed in (mM): TEA-Cl, 155; CsCl₂, 5; HEPES, 10; and BaCl₂, 5, as the charge carrier; pH 7.35, 300 mOsm/L. Control and drug solutions were applied with a linear array of six gravity-fed capillaries positioned 500–600 μm close to the patched neuron. Recordings were made with an Axopatch 1D (Axon Instrument). Electrode resistances in bath were ~ 3 –6 M Ω . After formation of a G Ω seal and subsequent cell rupture, series resistance was compensated (75–85%) and periodically monitored. Data were low-pass filtered (corner frequency, 5 kHz). For data acquisition and analysis, pClamp 9.2 software (Axon Instruments) was used.

Total HVA Ca²⁺ current was examined by utilizing either ramp test (from -70 mV to $+40$ mV) or test pulse protocols (a single step from -60 mV to $+10$ mV; or incremental 10 mV steps from -70 to $+40$ mV, Fig. 1A). In order to pharmacologically identify the distinct components of HVA Ca²⁺ current, selective Ca²⁺ channel blockers were applied sequentially: L-type channel blocker nifedipine (NIFE, 1–5 μM), N-type channel blocker

ω -Conotoxin GVIA (Ctx-GVIA, 1 μM), P-type channels blocker ω -agatoxin IVA (Atx-IVA, 20 nM), and Q-type channel blocker ω -Conotoxin MVIIC (Ctx-MVIIC, 100 nM). The voltage dependence of activation was determined from measurements of tail current amplitude, which was expected to reflect the fraction of Ca²⁺ channels opened during the preceding depolarization.

Data analysis

Voltage-activated currents were leak subtracted. Cells exhibiting leak currents >10 pA were not included in this analysis. Cell capacitance (C_m) was monitored using the automated function of the Axopatch amplifier. A stable C_m -value over time was an important criterion for the evaluation of the quality of experiments. Statistical tests were performed using Microcal Origin (OriginLab, Northampton, MA, USA) and GraphPad Prism (GraphPad Software, San Diego, CA, USA) softwares. Values given in the text and in the figures are mean \pm SD of changes in the respective cell populations, unless otherwise stated. The *t*-test or Mann–Whitney test were used for assessing statistical significance where appropriate. Multiple groups were compared using one-way or two-way ANOVA. The Tukey HSD test was used for post hoc comparison of the ANOVA. Values were considered statistically significant when $P < 0.05$ with $\alpha = 0.001$.

Drug source

Nifedipine, ω -conotoxin GVIA, and ω -agatoxin IVA were from Tocris-Cookson, UK. All other compounds used were purchased from Sigma-Aldrich, Italy.

RESULTS

Increased L-type Ca²⁺ current fraction in striatal MSNs from dopamine-depleted rats

Two different experimental approaches were utilized to obtain striatal dopamine depletion modelling idiopathic PD (for review see: Bonsi et al., 2006; Gubellini et al., 2010). In a group of male Wistar rats ($n=17$) the neurotoxin 6-OHDA (1 mg/kg; Baunez et al., 1995) was delivered into the SNpc by stereotaxic injection. In the same surgical sessions, saline was injected into the SNpc of another group of 15 rats (sham-operated group). Furthermore, in a different group of animals endogenous amines were depleted by 3–4 mg/kg, i.p. reserpine treatment (Harrison et al., 2001) and after 24 ($n=13$) or 48 h ($n=4$) rats manifesting typical parkinsonian-like symptoms were utilized for the experiments (Baunez et al., 1995; Spadoni et al., 2004). Vehicle-treated controls were prepared in the same sessions ($n=15$).

Total HVA Ca²⁺ current was recorded from 173 MSNs acutely isolated from the striata of either vehicle- and reserpine-treated rats, or 6-OHDA-lesioned and sham-operated rats (Fig. 1A). Peak amplitude of the current was not statistically different either between vehicle- (625.7 ± 23.6 pA; $n=43$), and reserpine-treated rats (627.6 ± 33.2 pA; $n=45$; $P > 0.05$ Mann–Whitney test; not shown), or between sham-operated (671.8 ± 35.4 pA; $n=41$) and 6-OHDA-lesioned rats (611.2 ± 21.2 pA; $n=44$; $P > 0.05$ Mann–Whitney test; not shown). Moreover, the amplitude of total HVA Ca²⁺ current was not statistically different between the groups ($P > 0.05$ ANOVA followed by Tukey HSD test). In order to rule out changes in cell surface area

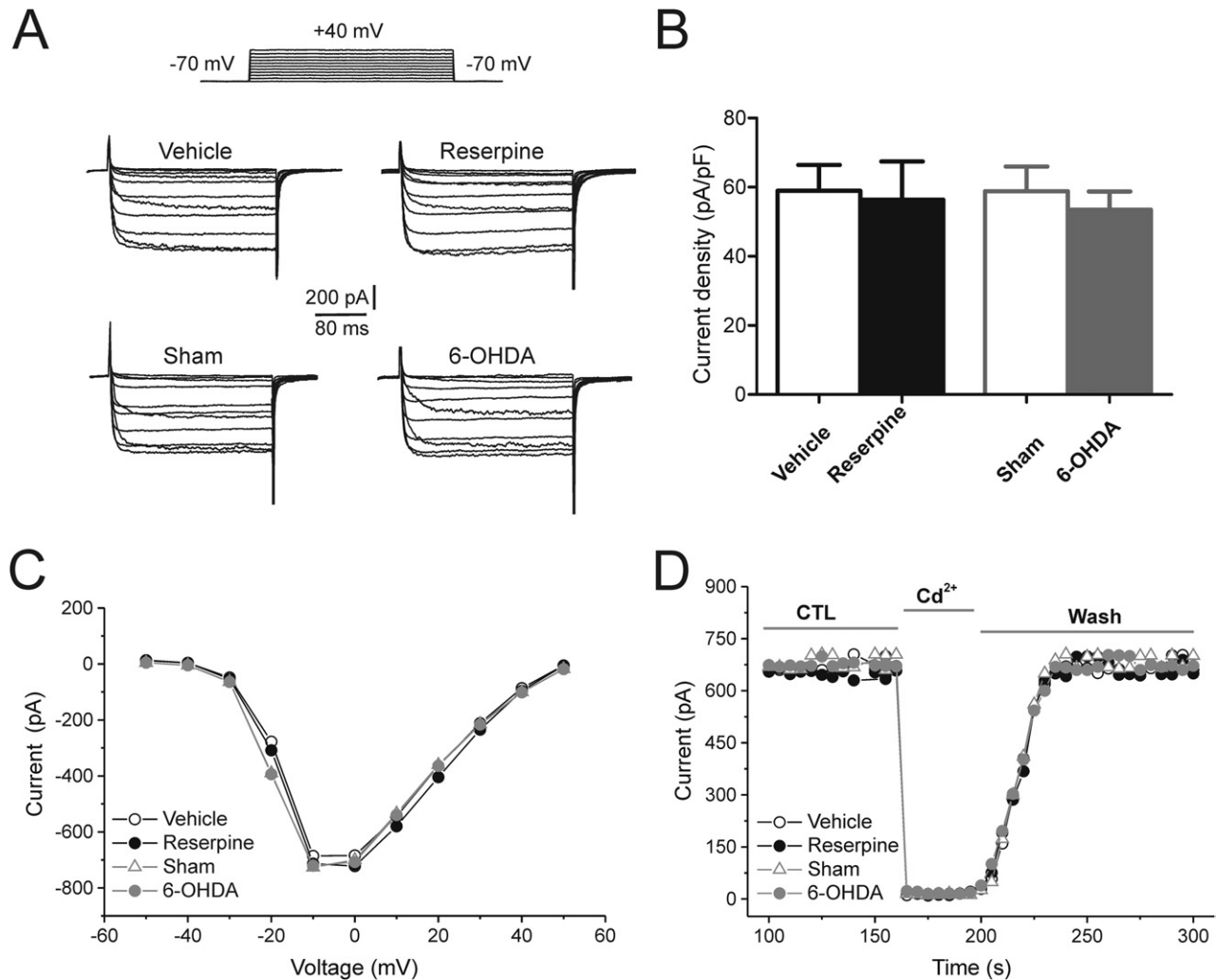


Fig. 1. Analysis of HVA Ca^{2+} currents recorded from MSNs in two different animal models of idiopathic PD. (A) Representative recordings of HVA Ca^{2+} currents from either vehicle- and reserpine-treated rats or 6-OHDA- and sham-lesioned animals. Inward currents were elicited with incremental step commands from -70 to $+40$ mV. (B) Plot representing the mean \pm SD of Ca^{2+} current density values (peak current amplitude / whole-cell capacitance). No significant differences were observed in the means among the groups. (C) Representative current–voltage relationship (I – V plot) showing HVA Ca^{2+} current half-maximal activation ($V_{1/2}$), obtained from measurements performed at steady state on the currents shown in (A). (D) Time-course of the effect of cadmium ($400 \mu\text{M}$) in representative recordings of MSNs from reserpine-treated, 6-OHDA-lesioned, and control rats.

that might affect whole cell current measurement, the whole cell capacitance (CN) was measured for each cell of every experimental group. The mean CN values did not differ among PD models and the respective controls (not shown; vehicle: 10.8 ± 1.3 pF, reserpine: 11.5 ± 2.1 pF, $P > 0.05$ Mann–Whitney test; sham: 11.6 ± 1.4 pF, 6-OHDA: 11.6 ± 1.2 pF, $P > 0.05$ Mann–Whitney test; $n = 10$ for each group; $P > 0.05$ ANOVA followed by Tukey HSD test), indicating that the somatic surface area was unchanged. In line with these data, the current/capacitance ratio, that is proportional to current density, did not show significant differences among the experimental groups (Fig. 1B; vehicle: 59.0 ± 7.5 pA/pF, reserpine: 56.4 ± 11.1 pA/pF, $P > 0.05$ Mann–Whitney test; sham: 58.8 ± 7.2 pA/pF, 6-OHDA: 53.3 ± 5.3 pA/pF, $P > 0.05$ Mann–Whitney test; $n = 10$ for each group; $P > 0.05$ ANOVA followed by Tukey HSD test).

Similarly, current activation and deactivation kinetics showed no differences among the groups (Fig. 1C; $n = 40$ for each group; $P > 0.05$ ANOVA, followed by Tukey HSD test). As expected, the recorded Ca^{2+} currents were fully and reversibly blocked by cadmium (Fig. 1D) in all recorded neurons ($n \geq 19$, $P < 0.05$ Mann–Whitney test).

The contribution of each Ca^{2+} channel subtype to total HVA current was pharmacologically addressed in the two rat PD models and their respective controls by sequentially applying the respective blockers (Fig. 2A). Bath-application of the selective blocker of N-type Ca^{2+} channels, ω -conotoxin GVIA (Ctx-GVIA, $1 \mu\text{M}$), produced an inhibitory effect on total HVA current that was not significantly different among the experimental groups (Fig. 2B; $P > 0.05$ ANOVA followed by Tukey HSD test). Indeed, $1 \mu\text{M}$ Ctx-GVIA inhibited Ca^{2+} current by $30.1 \pm 6.2\%$ in MSNs from vehicle-treated rats ($n = 7$) and by $33.1 \pm 5.6\%$ in reserpine-

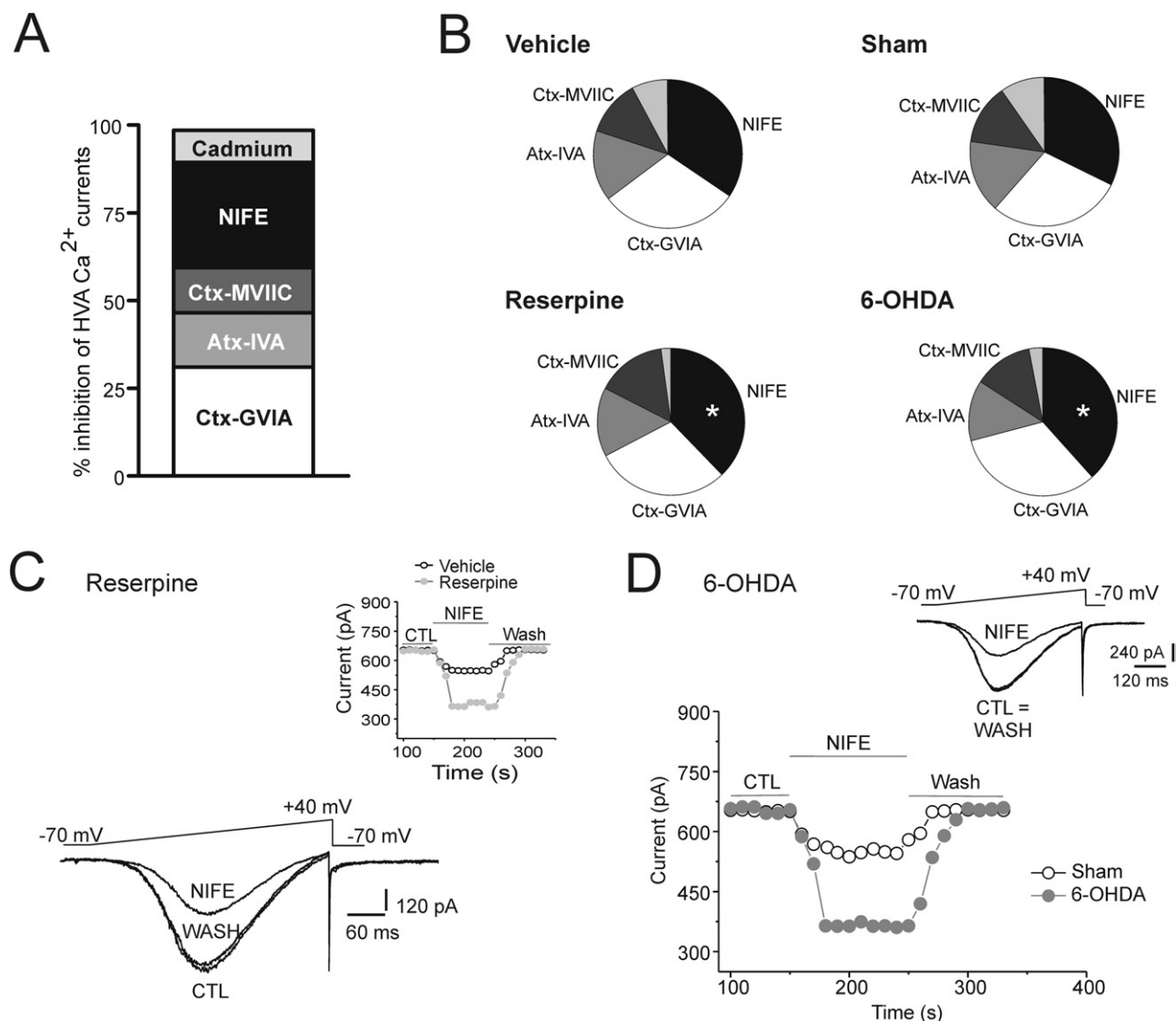


Fig. 2. The dihydropyridine sensitivity of HVA Ca^{2+} currents increases after either reserpine or 6-OHDA treatment. (A) Plot of a representative experiment on a control rat MSN where the single constituents of total HVA Ca^{2+} current were pharmacologically isolated by applying in sequence the selective blockers for L-, N-, Q-, and P-type Ca^{2+} channels (NIFE, Ctx-GVIA, Ctx-MVIIC, and Atx-IVA). Cadmium blocked the residual current. (B) Pie graphs reporting the mean inhibitory values of each channel subtype blocker in the different animal groups. The residual current that was blocked by cadmium is indicated in light grey. *Left.* The NIFE-sensitive component was significantly increased in reserpine-treated rats as compared to vehicle-treated rats. *Right.* A similar increase in the L-type Ca^{2+} current component is observed in 6-OHDA-lesioned rats. Asterisks indicate statistical significance. (C) Representative ramp recording from a reserpine-treated animal. *Inset:* Time-course showing the increased amount of current inhibited by the selective L-type channel blocker NIFE. (D) The time-course plot shows an enhanced NIFE-sensitive component of Ca^{2+} current in a recording from a 6-OHDA-lesioned rat. *Inset:* representative ramp recordings from a 6-OHDA-lesioned rat.

treated animals ($n=7$; Fig. 2B; $P>0.05$ Mann–Whitney test). Similarly, in the sham-operated group Ctx-GVIA caused an inhibition of $28.9\pm 5.3\%$ ($n=8$) vs. $32.1\pm 5.1\%$ in the 6-OHDA-lesioned rats ($n=8$; Fig. 2B; $P>0.05$ t -test). The analysis of both P- and Q-type components of HVA Ca^{2+} current in DA depleted animals did not show significant rearrangements in these channels (Fig. 2B). Indeed, the P-type blocker ω -agatoxin IVA (Atx-IVA, 20 nM) caused an inhibition of $15.2\pm 6.7\%$ of total Ca^{2+} current in vehicle-treated rats ($n=5$), vs. $17.0\pm 6.1\%$ in the reserpine-treated group ($n=5$; Fig. 2B; $P>0.05$ Mann–Whitney test). Similarly, no significant difference was observed in the

inhibitory effect of Atx-IVA between 6-OHDA- and sham-lesioned rats ($16.0\pm 6.1\%$ sham-operated group, $n=8$; $14.2\pm 3.3\%$ 6-OHDA-lesioned rats, $n=8$; Fig. 2B; $P>0.05$ t -test). Bath-applied ω -conotoxin MVIIC (Ctx-MIIC, 100 nM), selective Q-type channel blocker, reduced total HVA Ca^{2+} current by $12.8\pm 4.5\%$ and $16.8\pm 6.0\%$ in vehicle- and reserpine-treated rats, respectively, and by $13.0\pm 4.1\%$ and $13.4\pm 3.3\%$ in sham- and 6-OHDA-lesioned rats, respectively ($n=5$ for each group; Fig. 2B; $P>0.05$ Mann–Whitney test). Finally, we tested the effect of the dihydropyridine nifedipine (NIFE) at 1 and 5 μ M on total HVA Ca^{2+} currents of striatal MSNs. As the results obtained by using

the two doses of NIFE were not statistically different, data were pooled together. Reserpine treatment caused an increase of the inhibitory effect of NIFE to $42.2 \pm 5.0\%$ ($n=7$), with respect to $34.1 \pm 6.0\%$ in vehicle-treated rats ($n=9$; Fig. 2B, C; $P < 0.001$ Mann–Whitney test). A similar increase in the NIFE-sensitive component of total HVA current was observed after 6-OHDA-lesion, as application of the L-type blocker on MSNs from these animals caused an inhibition of $40.4 \pm 4.1\%$ in the recorded current, as compared to a reduction of $32.2 \pm 5.0\%$ in sham-operated rats ($n=6$ each group; Fig. 2B, D; $P < 0.05$ *t*-test).

HVA Ca²⁺ current profile in knockout mouse models of monogenic PD

Next, the channel profile of HVA Ca²⁺ current was analyzed in two knockout mouse models of PD, DJ-1^{-/-} and PINK1^{-/-} mice, and compared to their wild-type littermates (Fig. 3). The amplitude of total Ca²⁺ current recorded from MSNs (Martella et al., 2008) was not statistically different in the four mouse strains analyzed (not shown). Mean HVA Ca²⁺ current amplitude recorded from DJ-1^{-/-} mice was 451.2 ± 30.2 pA ($n=16$), and 413.9 ± 22.6 pA in their wild-type littermates ($n=16$; $P > 0.05$ *t*-test). Similarly, HVA Ca²⁺ current amplitude was 442.8 ± 30.0 pA in MSNs from PINK1^{-/-} mice ($n=13$) and 452.7 ± 33.2 pA in their wild-type littermates ($n=13$; $P > 0.05$ Mann–Whitney test).

The CN measurement indicated that the somatic surface area was unchanged among mice groups ($P > 0.05$ ANOVA followed by Tukey HSD test): DJ-1^{+/+} 11.6 ± 1.3 pF, DJ-1^{-/-} 11.7 ± 1.5 pF (not shown; $n=10$ each; $P > 0.05$ Mann–Whitney test); PINK1^{+/+}: 11.5 ± 1.4 pF, PINK1^{-/-} 11.7 ± 0.9 pF (not shown; $n=10$ each; $P > 0.05$ Mann–Whitney test). Accordingly, the current density, as indicated by the current/capacitance ratio, did not show significant differences among the experimental groups (Fig. 3B; DJ-1^{+/+} 44.5 ± 4.8 pA/pF, DJ-1^{-/-} 49.5 ± 6.1 pA/pF, $P > 0.05$ Mann–Whitney test; PINK1^{+/+} 45.2 ± 6.9 pA/pF, PINK1^{-/-} 43.2 ± 3.7 pA/pF, $P > 0.05$ Mann–Whitney test; $n=10$ for each group; $P > 0.05$ ANOVA followed by Tukey HSD test). No significant differences in current deactivation or activation were observed among the mice strains, as well (Fig. 3C, D; $P > 0.05$ ANOVA, followed by Tukey HSD test). The recorded currents were fully blocked by 400 μM cadmium (Fig. 3E, F).

The profile of HVA Ca²⁺ current in knockout mouse models of PD was pharmacologically analyzed by applying sequentially the subtype selective channel blockers (Fig. 4). Bath application of 5 μM NIFE reduced the Ca²⁺ current amplitude by $23.0 \pm 4.4\%$ in DJ-1^{+/+} mice ($n=7$), and by $17.1 \pm 3.6\%$ in DJ-1^{-/-} mice ($n=7$; Fig. 4A; $P > 0.05$ Mann–Whitney test). Likewise, NIFE reduced total Ca²⁺ current by a similar extent in PINK1^{-/-} ($28.6 \pm 5.5\%$; $n=10$), and PINK1^{+/+} mice ($30.2 \pm 6.0\%$; $n=10$; Fig. 4C, D; $P > 0.05$ *t*-test). Interestingly, analysis of N- and P-type HVA Ca²⁺ current fractions gave different results in the two genetic models of PD. In DJ-1^{-/-} mice HVA Ca²⁺ current profiling showed an increased N-type channel-mediated component, with respect to control wild-type lit-

termates (DJ-1^{-/-}: $50.0 \pm 6.4\%$; $n=5$; DJ-1^{+/+}: $32.6 \pm 5.2\%$; $n=5$; Fig. 4A, B; $P < 0.001$ Mann–Whitney test). Conversely, the N-type fraction of Ca²⁺ current was similar in PINK1^{-/-} and PINK1^{+/+} mice (PINK1^{+/+}: $24.6 \pm 4.1\%$; $n=11$; PINK1^{-/-}: $20.4 \pm 5.0\%$; $n=11$; Fig. 4C, D; $P > 0.05$ Mann–Whitney). Along with the rearrangement in the contribution of N-type channels to HVA Ca²⁺ current, an increased P-type-mediated component was observed in DJ-1^{-/-} mice. Indeed, Atx-IVA (20 nM) caused an inhibitory effect of $16.3 \pm 4.2\%$ in DJ-1^{+/+} ($n=5$) vs. $30.1 \pm 5.7\%$ in DJ-1^{-/-} mice ($n=5$; Fig. 4A, B; $P < 0.001$ Mann–Whitney test). Again, in PINK1^{-/-} no significant rearrangement of this channel subtype was observed (PINK1^{+/+}: $15.2 \pm 2.4\%$; $n=6$; PINK1^{-/-}: $13.4 \pm 4.0\%$; $n=6$; Fig. 4C; $P > 0.05$ *t*-test). The inhibitory effect of the Q-type Ca²⁺ channel blocker Ctx-MVIIC (100 nM) on total HVA Ca²⁺ current was not significantly altered either in DJ-1^{-/-} or in PINK1^{-/-} mice (DJ-1^{+/+}: $19.2 \pm 3.1\%$; $n=6$; DJ-1^{-/-}: $15.7 \pm 4.9\%$; $n=7$; Fig. 4A; $P > 0.05$ Mann–Whitney test; and PINK1^{+/+}: $14.3 \pm 3.4\%$; $n=6$; PINK1^{-/-}: $16.0 \pm 4.5\%$; $n=9$; Fig. 4C; $P > 0.05$ Mann–Whitney).

Analysis of dopamine-dependent modulation of HVA Ca²⁺ channels in rat and mouse models of PD

Striatal D1 or D2 receptor activation modulates HVA Ca²⁺ channels (Surmeier et al., 1995; Hernandez-Lopez et al., 2000; Pisani et al., 2000). Therefore the altered profile of HVA Ca²⁺ current observed in reserpine-treated and 6-OHDA-lesioned rats, as well as in DJ-1^{-/-} mice, might deeply impact the response to dopamine receptor activation in MSNs. We addressed this issue by analyzing the effect of D1 and D2 receptor activation on HVA Ca²⁺ current in MSNs isolated from animal models of PD. The D1 receptor agonist SKF 83822 (10 μM) caused an inhibition of Ca²⁺ current in most MSNs from control animals (vehicle-treated group: $17.6 \pm 4.7\%$; $n=11$ out of 13; sham-operated group: $15.6 \pm 2.7\%$; $n=11$ out of 13; not shown) that was not significantly altered in dopamine-depleted animals (reserpine-treated rats: $15.7 \pm 3.2\%$; $n=16$ out of 19; 6-OHDA-lesioned rats: $13.1 \pm 7.6\%$; $n=13$ out of 16; not shown; $P > 0.05$ Mann–Whitney test and ANOVA). Similarly, in both knockout mouse strains, DJ-1^{-/-} and PINK1^{-/-}, the effect of D1-like receptor activation was not statistically different from the effect observed in the respective wild-type littermates. Indeed, 10 μM SKF 83822 reduced HVA Ca²⁺ current by $11.7 \pm 4.1\%$ ($n=8$ out of 9) and $13.0 \pm 5.5\%$ ($n=7$ out of 8) in MSNs from DJ-1^{+/+} and DJ-1^{-/-}, respectively, and by $16.8 \pm 3.9\%$ in 9 out of 11 PINK1^{+/+} cells and $15.3 \pm 3.3\%$ in 10 out of 13 PINK1^{-/-} neurons, respectively (not shown; $P > 0.05$ Mann–Whitney test). Bath application of the D1 receptor antagonist SCH23390 (10 μM) prevented the inhibitory effect of SKF 83822 on HVA Ca²⁺ currents in all the animal groups.

Interestingly, we observed a substantial alteration of the quinpirole-mediated modulation of HVA Ca²⁺ currents. Bath application of the D2 receptor agonist quinpirole (10 μM) caused an inhibition of total Ca²⁺ current recorded either from reserpine-treated or 6-OHDA-lesioned rats, that was significantly increased with respect to control

Fig. 3. Analysis of HVA Ca^{2+} currents in two genetic mouse models of PD. (A) Representative recordings showing HVA Ca^{2+} currents evoked from MSNs of DJ1^{-/-} and PINK1^{-/-} mice and their wild-type littermates by means of step commands (from -70 to +40 mV). (B) Plot of HVA Ca^{2+} current density measured from MSNs of the different mouse strains. Data are reported as mean \pm SD. (C) *I-V* plot of the representative step-evoked Ca^{2+} currents shown in (A). (D) Plot of tail current amplitudes, obtained from the same recordings shown in (A). No significant differences were observed in the properties of HVA Ca^{2+} currents between DJ1^{-/-}, PINK1^{-/-} mice, and the respective controls. (E) Representative ramp recording of HVA Ca^{2+} current from a DJ1^{-/-} mouse. Cadmium (400 μM) fully and reversibly blocked the recorded current. (F) Time-course of the effect of cadmium in representative recordings of MSNs from DJ1^{-/-}, PINK1^{-/-} mice and the respective controls.

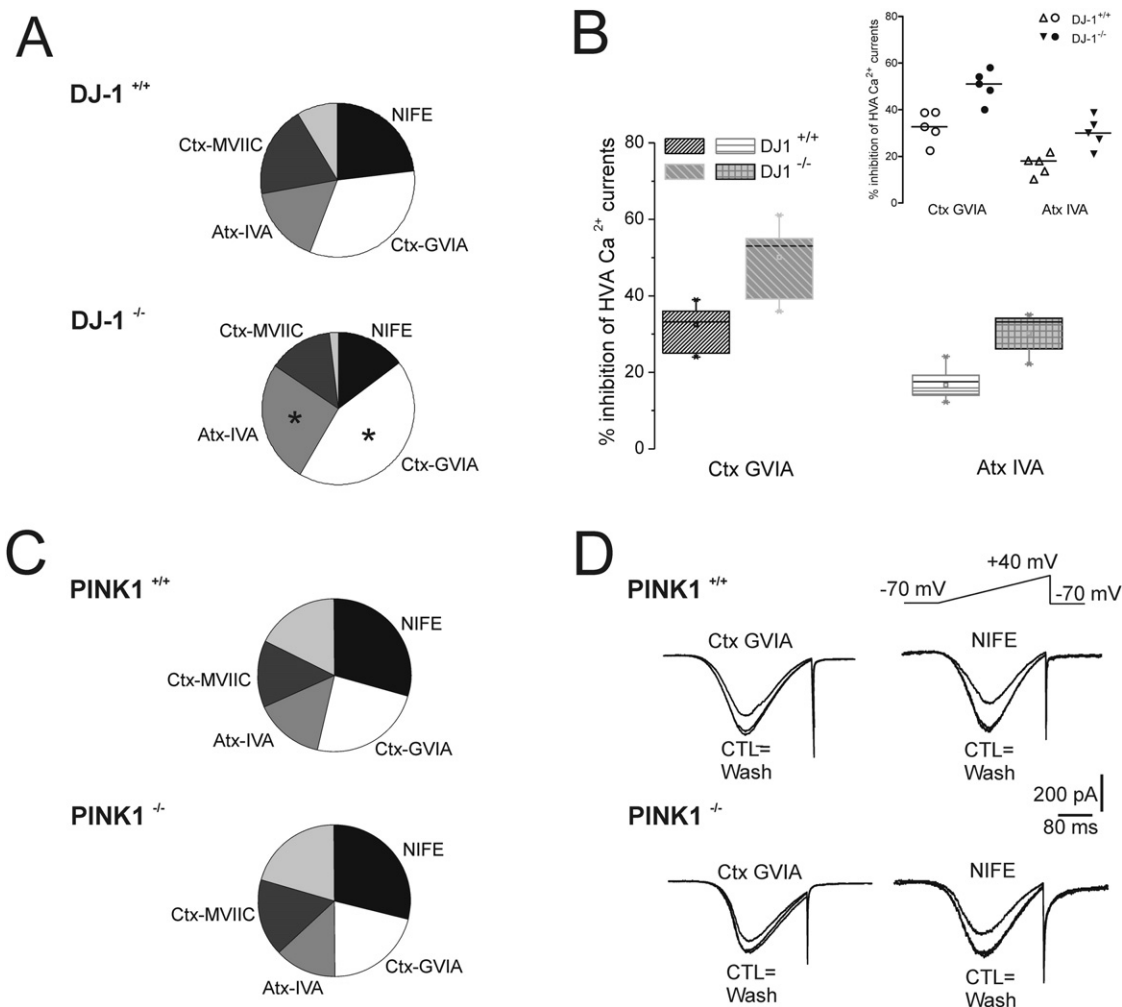


Fig. 4. DJ-1^{-/-} mice show an increase in the Ctx GVIA- and Atx IVA-sensitive components of HVA Ca²⁺ current. (A) Pie plots summarizing the mean portion of HVA Ca²⁺ current inhibited by the selective blockers of L-, N-, Q-, and P-type Ca²⁺ channels, applied in sequence during recordings from DJ-1^{+/+} and DJ-1^{-/-} mice. The residual current that was blocked by cadmium is indicated in light grey. Both the NIFE- and Atx IVA-sensitive components of HVA Ca²⁺ currents were significantly (*) increased in MSNs from DJ-1^{-/-} mice as compared to DJ-1^{+/+} MSNs. (B) Whisker Chart and scatter dot (inset) plots of the inhibitory effect of Ctx-GVIA and Atx-IVA shown in (A). The median value for each experimental group is shown. (C) The mean contribution of each Ca²⁺ channel type to total HVA current is not significantly different in PINK1^{+/+} and PINK1^{-/-} MSNs. (D) Representative recordings of HVA Ca²⁺ current from PINK1^{+/+} and PINK1^{-/-} in the presence of either NIFE or Ctx GVIA, showing no significant difference between the groups.

animals (vehicle-treated: $28.0 \pm 4.7\%$; $n=13$ out of 15; reserpine-treated: $42.3 \pm 7.1\%$; $n=16$ out of 19; sham-operated: $27.0 \pm 5.4\%$; $n=19$ out of 21; 6-OHDA-lesioned: $40.0 \pm 7.1\%$; $n=19$ out of 23; Fig. 5; $P < 0.001$ Mann–Whitney). Similarly, quinpirole caused an increased inhibition of total HVA Ca²⁺ current in MSNs from DJ-1^{-/-} mice ($34.7 \pm 6.1\%$; $n=5$), with respect to the DJ-1^{+/+} group ($24.0 \pm 7.1\%$; $n=5$; Fig. 6A, B; $P < 0.001$ Mann–Whitney test). In contrast, we did not observe significant changes in the inhibitory effect of quinpirole on HVA Ca²⁺ current in PINK1^{-/-} mice ($24.6 \pm 7.1\%$; $n=6$ out of 7), compared to PINK1^{+/+} mice ($26.4 \pm 7.8\%$; $n=6$ out of 8; Fig. 6A; $P > 0.05$ *t*-test).

Occlusion experiments with all the selective Ca²⁺ channel blockers were then performed to investigate the channel subtype involved in the altered inhibitory effect of D2 receptor on HVA Ca²⁺ current (not shown). Only NIFE

was able to occlude the effect of quinpirole in control rats (vehicle-treated: NIFE $32.1 \pm 4.9\%$; NIFE plus quinpirole $32.1 \pm 5.0\%$; $n=5$; Fig. 5C; $P > 0.05$ Mann–Whitney test; sham-lesioned: NIFE $28.2 \pm 3.0\%$; NIFE plus quinpirole $28.6 \pm 4.2\%$; $n=7$; Fig. 5C; Mann–Whitney test) and in both groups of DA-depleted animals (reserpine-treated: NIFE $41.2 \pm 5.1\%$; NIFE plus quinpirole $40.0 \pm 6.6\%$; $n=7$; Fig. 5C; Mann–Whitney test; 6-OHDA-lesioned: NIFE $38.4 \pm 4.1\%$; NIFE plus quinpirole $37.6 \pm 5.6\%$; $n=9$; Fig. 5C, D; Mann–Whitney test). In PINK1^{-/-} mice, as well as in their wild-type littermates, the quinpirole-mediated inhibitory effect on HVA Ca²⁺ current was fully occluded by the selective N-type channel blocker Ctx-GVIA (PINK1^{-/-} mice: Ctx-GVIA $24.4 \pm 4.5\%$; Ctx-GVIA plus quinpirole $23.9 \pm 3.2\%$; $n=6$; PINK1^{+/+} mice: Ctx-GVIA $25.2 \pm 3.7\%$; Ctx-GVIA plus quinpirole $24.8 \pm 3.3\%$; $n=6$; data not shown; $P > 0.05$ *t*-test), while the other Ca²⁺ channel

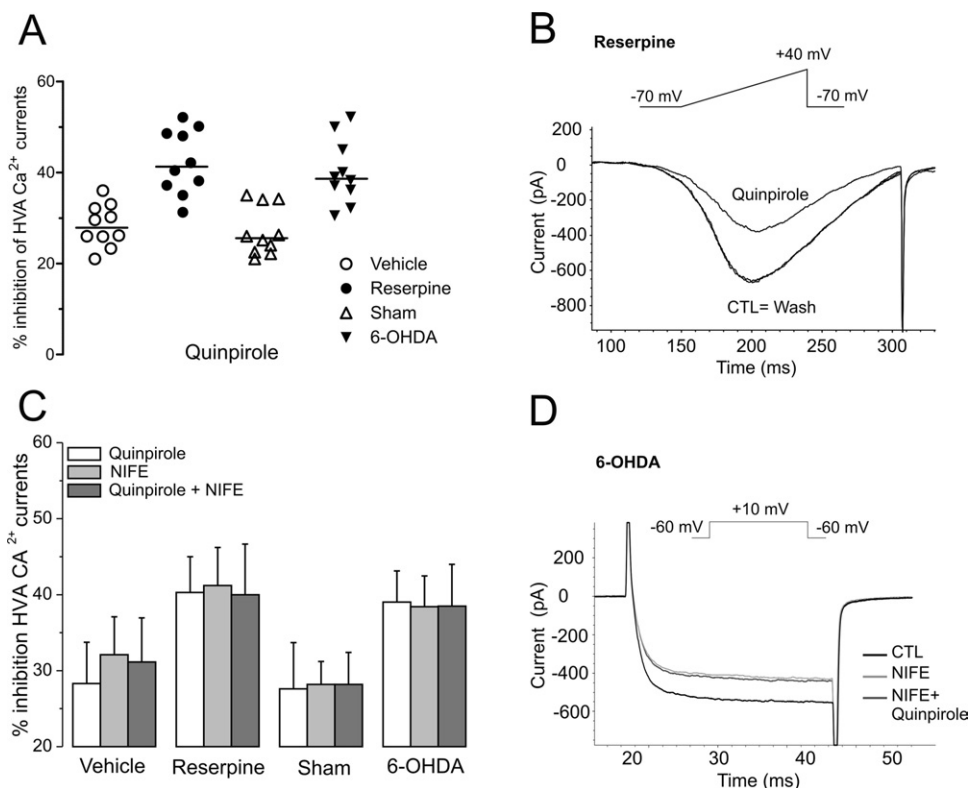


Fig. 5. Increased modulation of L-type channels by D2 receptor in reserpine- and 6-OHDA-treated rats. (A) Scatter dot plot summarizing the inhibitory effect of the D2 receptor agonist quinpirole on reserpine-treated and 6-OHDA-lesioned rats. The graph shows a significantly increased inhibitory effect of quinpirole on both reserpine and 6-OHDA MSNs as compared to the respective controls. To avoid confusion, only 10 values per group have been plotted. The median value for each experimental group is shown. (B) Representative ramp recording (from -70 to 40 mV, 0.3 – 0.6 mV/ms) of MSN total HVA Ca^{2+} current from a reserpine-treated rat, showing the inhibitory effect of the D2 receptor agonist quinpirole ($10 \mu\text{M}$). (C) The inhibitory effect of D2 receptor activation is mimicked and occluded by blockade of L-type Ca^{2+} channels in each animal group. (D) Representative recording showing the occlusion by NIFE of the inhibitory effect of quinpirole on step-induced HVA Ca^{2+} current in an MSN from a 6-OHDA-lesioned rat.

blockers tested were ineffective in preventing the inhibitory effect of quinpirole (not shown). Similarly, both in DJ-1^{-/-} and DJ-1^{+/+} mice Ctx-GVIA fully occluded the effect of quinpirole (DJ-1^{-/-} mice: Ctx-GVIA 35.2 ± 4.2 ; Ctx-GVIA plus quinpirole $35.0 \pm 5.9\%$; $n=5$; Fig. 6B, C; $P>0.05$ Mann–Whitney test; DJ-1^{+/+} mice: Ctx-GVIA 26.2 ± 5.9 ; Ctx-GVIA plus quinpirole $25.0 \pm 4.5\%$; $n=6$; Fig. 6B; $P>0.05$ t -test).

DISCUSSION

Acute dopamine depletion induces the upregulation of the D2 receptor-modulated Ca^{2+} currents in rats

The present data show that dopamine depletion following reserpine treatment or 6-OHDA-induced lesion of dopaminergic fibers induces in rat MSNs a rearrangement in the profile of HVA Ca^{2+} channels, with an increase in the L-type component, as well as in their modulation by D2 receptors. While the coupling of D2 receptor to L-type Ca^{2+} channels is not altered by dopamine depletion, the inhibitory effect of its activation on this current component is increased. Indeed, the D2 receptor agonist quinpirole inhibits a larger fraction of total Ca^{2+} current in these animal models than in controls, but its inhibitory effect is occluded by NIFE in both dopamine-depleted rats and

controls. This observation is in line with reports suggesting the development of supersensitivity of postsynaptic receptors to dopamine in PD (LaHoste and Marshall, 1993; Bezard and Gross, 1998; Blandini et al., 2000; Zhen et al., 2002; Gubellini et al., 2010). At present it is controversial whether this might be due to an increase in the number of D2 receptors or to an altered D2 receptor-mediated intracellular signaling. Experimental evidence has demonstrated an increased D2 receptor-G_i protein coupling (Rubinstein et al., 1990; Radja et al., 1993; Butkerait et al., 1994; Zhen et al., 2002). The observation that the L-type component of total Ca^{2+} current is increased in MSNs from reserpine and 6-OHDA rats without any change in cell surface area, is in favour of a direct effect of DA depletion on the number/permeability of L-type channels. In fact, overall the present results are consistent with a previous report suggesting the existence of a positive feedback between L-type Ca^{2+} channel activity and the expression of *Cacna1d* gene, coding for the Cav1.3 α 1 subunit that is selectively targeted by the D2 receptor (Fass et al., 1999; Olson et al., 2005). Acute dopamine depletion following reserpine or 6-OHDA treatment might disinhibit L-type channels and their positive feedback to mRNA expression in MSNs and, in turn, cause the increase in L-type Ca^{2+} current fraction that we observe in our experiments. Most

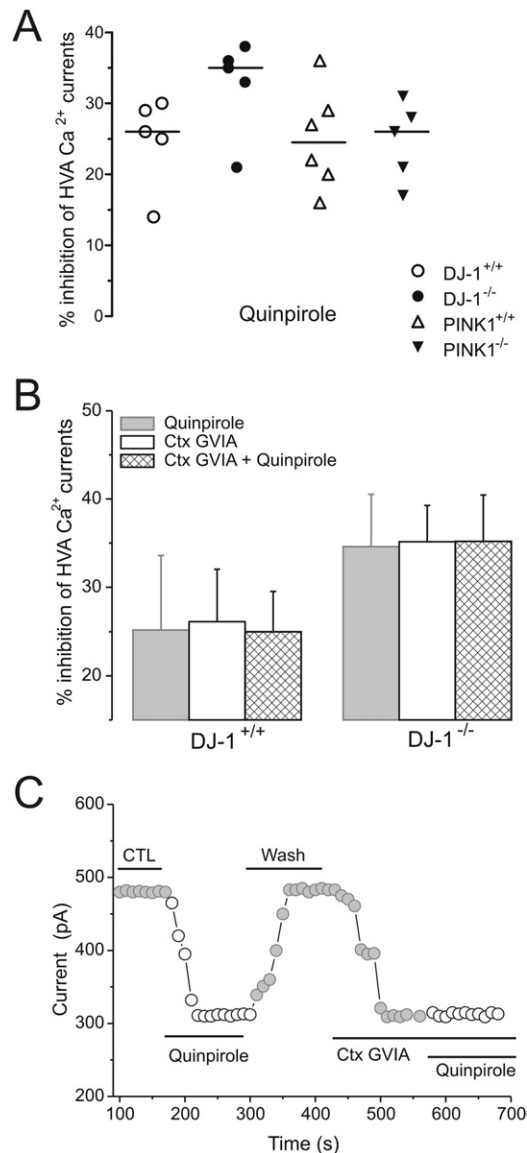


Fig. 6. Increased inhibitory effect of D2 dopamine receptor activation on N-type channels in DJ-1^{-/-} mice. (A) Scatter dot plot of the inhibitory effect of D2 receptor agonist quinpirole on DJ-1^{-/-} and PINK1^{-/-} mice and the respective controls. An increased inhibitory effect of quinpirole on HVA Ca^{2+} current can be observed in MSNs from DJ-1^{-/-} mice, but not in PINK1^{-/-} mice, as compared to the respective wild-type littermates. The median value for each experimental group is shown. (B) Histogram showing that the inhibitory effect of D2 receptor activation is mimicked and occluded by blockade of N-type Ca^{2+} channels in DJ-1^{-/-} mice. (C) Time-course of a representative occlusion experiment in an MSN from DJ-1^{-/-} mice.

recently, Prieto and coworkers (Prieto et al., 2009) performed an analysis of HVA Ca^{2+} current in 6-OHDA lesioned rats. These authors observed a small, non-significant increase in the L-type component recorded from MSNs after 6-OHDA lesion. Though this discrepancy might be due to some methodological differences (6-OHDA dose and age of animals; Salgado et al., 2005; Martella et al., 2008) it is worth noting that we confirmed our findings in a further acute model, reserpine-depleted rats, that is free

from adaptive phenomena that might take place after 6-OHDA lesion (Fuentes et al., 2009; Galati et al., 2009).

Striatal MSNs strongly express L-type Ca^{2+} channels containing the Cav1.3 α 1 subunit at the glutamatergic postsynaptic density in the head of dendritic spines, where they act as negative regulators of spine stability, since deletion of Cav1.3 α 1 subunits induces spine proliferation and disinhibition induces spine loss (Day et al., 2006). Interestingly, chronic administration of an L-type channel antagonist completely prevented the spine loss induced in MSNs by acute dopamine depletion, suggesting that a reduced dopamine tone might cause the disinhibition of L-type channels, a sustained elevation in intraspine Ca^{2+} , and, in turn, the disassembly of cytoskeleton in spines (Day et al., 2006). Of interest, our data indeed provide direct evidence of an increased L-type component of HVA Ca^{2+} currents, at least at the soma of MSNs from dopamine depleted rats, and further suggest an increased expression of these channels in these models of PD (Fass et al., 1999; Olson et al., 2005).

DJ-1 deletion causes the upregulation of the Ca^{2+} channels modulated by D2 receptor in mice

In MSNs from two genetic mouse models of monogenic PD we observed different profiles of HVA Ca^{2+} currents. Deletion of PINK1 gene did not cause significant rearrangements in Ca^{2+} channels. Conversely, in DJ-1^{-/-} mice we observed an increase in both N- and P-type current fractions. Moreover, in DJ-1^{-/-} mice, similarly to what observed after acute dopamine depletion in rats, D2 receptor activation by quinpirole caused an increased inhibitory effect on Ca^{2+} current.

P/Q-type channels exert a major role in the initiation of action-potential-evoked neurotransmitter release at central nervous system synapses (Pietrobon, 2010), such as striatal GABA release (Arias-Montano et al., 2007). It is therefore likely, given that the increase in P-type current we recorded at the somatic level was found also at MSNs terminals, that the striatal GABAergic output might be affected in DJ-1^{-/-} mice.

Our data show that the D2 receptor-mediated inhibition of Ca^{2+} current is occluded by Ctx-GVIA in all mice strains, suggesting a preferential action of this receptor on N-type channels in mouse MSNs. A modulatory effect of D2 receptor agonists on N-type channels has been previously described in striatal cholinergic interneurons from both rat and mouse (Yan et al., 1997; Cabrera-Vera et al., 2004; Pisani et al., 2006). Moreover, presynaptic D2 receptors on the terminals of GABAergic afferents to cholinergic interneurons, reported to derive predominantly from striatal MSNs (Bolam et al., 1986; Bennett and Wilson, 1998, 1999), down-regulate GABA release and inhibit GABA_A receptor-mediated postsynaptic potentials by selectively blocking N-type Ca^{2+} channels (Pisani et al., 2000; Momiyama and Koga, 2001). Interestingly, in a heterologous expression system, D2 receptors have been shown to physically interact with N-type channels and regulate their trafficking to the plasma membrane (Kisilevsky and Zamponi, 2008). Indeed, co-expression of the N-type channel

with the D2 receptor produced, in the absence of agonist, a significant increase in channel surface expression. This observation is in line with our data suggesting an increased N-type channel expression in MSNs from DJ-1^{-/-} mice, where a reduced dopamine overflow in the striatum has been reported (Goldberg et al., 2005), and strikingly recalls the modulatory role of D2 receptors on L-type channel expression in MSNs from rats described above, suggesting that this might be a common physiological activity of the D2 receptor displaying specie- and possibly cell-specific features. The apparent discrepancy with other reports showing a modulatory effect of D2 receptors on L-type Ca²⁺ channels in mice (Olson et al., 2005; Day et al., 2006) might be due to a different type of preparation preserving the neuronal processes and/or the age of the animals utilized, as Ca²⁺ currents undergo a profound rearrangement during postnatal development in MSNs (Martella et al., 2008). The observation that the inhibitory effect of D2 receptor activation is occluded by Ctx-GVIA in both DJ-1^{-/-} and wild-type mice demonstrates that the coupling of D2 receptor to N-type Ca²⁺ channels in MSNs from mice is not altered by adaptive mechanisms following DJ-1 deletion, whereas the effect of its activation is increased, thus suggesting the development of supersensitivity of postsynaptic D2 receptors also in this model of monogenic PD, similarly to what is believed to occur in dopamine-depleted animals. Although DJ-1^{-/-} mice have normal numbers of dopaminergic neurons in the SNpc, evoked dopamine overflow in the striatum is markedly reduced, primarily as a result of increased reuptake (Goldberg et al., 2005). It might be speculated that the deletion of PINK1 gene triggers different adaptive mechanisms in MSNs, as in PINK1^{-/-} mice neither the profile of HVA Ca²⁺ channels nor the D2 receptor-mediated modulatory effect are significantly altered. Indeed, though the numbers of dopaminergic neurons and dopamine receptors are unchanged, and evoked dopamine release is reduced similarly to DJ-1^{-/-} mice, however, contrary to DJ-1^{-/-} mice (Goldberg et al., 2005), D2 receptor activation is not sufficient to rescue corticostriatal synaptic plasticity deficits (Kitada et al., 2007). On the other hand, recent experimental evidence has suggested that PINK-1 and DJ-1 might define parallel pathways, partially overlapping downstream, as DJ-1 can partially rescue PINK1 loss in *Drosophila* (Hao et al., 2010).

CONCLUSION

KO mice are regarded as models of the preclinical changes that might occur due to DJ-1 or PINK1 deficiency in early-onset PD forms (Dawson et al., 2010), while acute dopamine depletion in rats models the more common idiopathic PD. The evidence we reported here of an increased activity of the HVA Ca²⁺ channels selectively modulated by D2 dopamine receptor in MSNs from both dopamine depleted rats and DJ-1^{-/-} mice strongly suggests the convergence of different pathophysiological mechanisms characterized by either acute or inborn striatal dopamine transmission deficits on a common pathway.

These data are in accordance with the hypothesis that striatal dopamine depletion could lead to disinhibition of specific high-voltage-activated calcium channels and, in turn, to striatal spine loss in the parkinsonian condition (Day et al., 2006). Moreover, they might provide a molecular mechanism for the supersensitivity of dopamine D2 receptor reported in animal models and PD patients (LaHoste and Marshall, 1993; Bezard and Gross, 1998; Blandini et al., 2000; Zhen et al., 2002; Gubellini et al., 2010).

Significant alterations in corticostriatal synaptic plasticity have been described in animal models of acute dopamine depletion (Calabresi et al., 1992, 2007; Pisani et al., 2005; Kreitzer and Malenka, 2007). Also, in DJ-1^{-/-} mice long-term depression (LTD) of corticostriatal synapses is selectively impaired (Goldberg et al., 2005). The expression of LTD requires spine integrity, D1 and D2 receptor activation and a highly regulated Ca²⁺ entry from HVA channels (Calabresi et al., 1992; Bonsi et al., 2003). Therefore, the alteration of the D2-dependent modulation of Ca²⁺ channels observed in both dopamine-depleted and DJ-1 null mice might underlie the absence of LTD.

Accordingly, the hypothesis that Ca²⁺ channel blockers could be effective in the treatment of PD and L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesias (Rodnitzky, 1999; Chan et al., 2009; Surmeier et al., 2010) is supported by experimental evidence obtained from PD animal models (Day et al., 2006; Meredith et al., 2008; Schuster et al., 2009). Indeed, L-type channel antagonism in 6-OHDA-lesioned rats prevents striatal spine loss and the development of abnormal involuntary movements upon L-DOPA treatment, and attenuates nigrostriatal degeneration in chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated mice.

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