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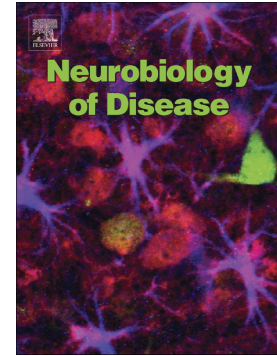
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Dopamine D2 receptor activation potently inhibits striatal glutamatergic transmission in a G2019S LRRK2 genetic model of Parkinson's disease

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Running head: Synaptic transmission in Lrrk2 mice

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Abstract

Among genetic abnormalities identified in Parkinson's disease (PD), mutations of the leucine-rich repeat kinase2 (*LRRK2*) gene, such as the G2019S missense mutation linked to enhanced kinase activity, are the most common. While the complex role of LRRK2 has not been fully elucidated, evidence that mutated kinase activity affects synaptic transmission has been reported. Thus, our aim was to explore possible early alterations of neurotransmission produced by the G2019S *LRRK2* mutation in PD. We performed electrophysiological patch-clamp recordings of striatal spiny projection neurons (SPNs) in the G2019S-*Lrrk2* knock-in (KI) mouse model of PD, in D1994S kinase-dead (KD), *Lrrk2* knock-out (KO) and wild-type (WT) mice. In G2019S *Lrrk2* KI mice, basal spontaneous glutamatergic transmission, synaptic facilitation, and NMDA/AMPA ratios were unchanged, whereas the stimulation of dopamine (DA) D2 receptor by quinpirole reduced the spontaneous and evoked excitatory postsynaptic currents (EPSC). Quinpirole reduced the EPSC amplitude of SPNs in KI but not in KD, KO and WT mice, suggesting that the enhanced LRRK2 kinase activity induced by the G2019S mutation is associated with the observed functional alteration of SPNs synaptic transmission. The effect of quinpirole was mediated by a phospholipase C (PLC)-dependent release of endocannabinoid, with subsequent activation of presynaptic cannabinoid receptor 1 and reduced release of glutamate. The key role of DA D2 receptor in reducing glutamatergic output in our LRRK2 genetic model of PD further supports the use of DA agonists in the treatment of early PD patients with LRRK2 mutations to counteract the disease progression.

Abbreviations

endocannabinoids (eCB); glycine to serine substitution at amino acid 2019 (G2019S); kinase-

dead (KD); leucine-rich repeat kinase2 (Lrrk2); L-sulpiride (Sulp); spiny projection neuron

(SPN); paired-pulse ratio (PPR); WIN55212-2 (WIN).

Introduction

Regulation of synaptic activity by different signaling systems is at the basis of network functioning in the central nervous system (CNS) and, within the basal ganglia, the glutamatergic and dopaminergic systems are mainly responsible of the modulation of spiny projection neurons (SPNs) and striatal functions (Calabresi et al., 2014; Calabresi et al., 1996; Kreitzer and Malenka, 2008; Lovinger, 2010).

Among pathogenic changes occurring in Parkinson's disease (PD) patients, reduced striatal dopamine (DA) levels and increased glutamatergic transmission into the nucleus striatum are major events linked to the motor impairment characterizing the disease (Calabresi et al., 2014; Obeso et al., 2017; Singh et al., 2016).

Several neurotoxic (Bezard and Przedborski, 2011; Blandini and Armentero, 2012; Gubellini et al., 2002) or genetic (Dawson et al., 2010) rodent models have been proposed to recapitulate synaptic modifications observed in PD.

Among genetic abnormalities identified in familial PD (Dawson et al., 2010; Lesage and Brice, 2009; Nuytemans et al., 2010), those located on the *leucine-rich repeat kinase2* gene (*LRRK2*) is found in autosomal dominant PD (Gao and Hong, 2011). Leucine-rich repeat kinase 2 (LRRK2) is a large protein, endowed with GTPase and kinase activity, that is particularly abundant in the nucleus striatum where it regulates striatal DA levels, synaptic vesicle trafficking, presynaptic glutamate release and DA receptor signal transduction (Beccano-Kelly et al., 2015; Piccoli et al., 2011). Among the most common *LRRK2* mutations found in PD patients the G2019S one is a missense mutation that confers enhanced kinase activity to LRRK2 (Dias et al., 2014; West et al., 2005; West et al., 2007; White et al., 2007; Zimprich et al., 2004). While to date the physiological and pathological role of LRRK2 has not been fully elucidated, evidence that a gain-of-function of mutated kinase activity affects synaptic transmission and neuronal viability has been reported (Beccano-Kelly et al., 2015; Tozzi et al., 2018).

Neuroprotective strategies that limit PD progression include D2 DA receptor agonists as a therapy for early PD (Cassarino et al., 1998; Ferrari-Toninelli et al., 2010; Herrero et al., 2011). For this reason, it might be relevant to explore possible effects of drugs acting on D2 receptors on striatal synaptic activity in Lrrk2 models of PD.

In the attempt to gain insights into possible alterations of glutamatergic neurotransmission produced by the G2019S LRRK2, we performed electrophysiological recordings from the nucleus striatum of G2019S *Lrrk2* knock-in (KI) mice to analyze glutamatergic synaptic currents. Testing the effect of the D2 DA receptor agonist quinpirole on this response, we found that this drug is effective in G2019S *Lrrk2* KI mice to reduce glutamatergic currents by an endocannabinoid-dependent mechanism of action.

Materials and Methods

Mice models and ethic statement on animal use

Mutant homozygous male mice carrying either the kinase-enhancing G2019S pathogenic mutation (KI) that genocopies the human G2019S PD-causing mutation or the kinase-inactivating point mutation D1994S (kinase-dead, KD) and mice lacking LRRK2 (knock-out, KO) backcrossed on a C57Bl/6J background were generated as previously reported (Herzig et al., 2011; Longo et al., 2014). Non-transgenic male wild-type (WT) mice were littermates obtained from the respective homozygous breeding. Mice were obtained from the University of Ferrara, they were kept under regular lighting conditions (12 h light/dark cycle) and given food and water ad libitum. All procedures were conducted in conformity with the European Communities Council Directive of November 1986 (86/609/ECC), in accordance with protocols approved by the Animal Care and Use Committee at the Universities of Perugia (Italy). All efforts were made to minimize the number of animals used and their suffering.

Electrophysiology

Six month-old G2019S KI, LRRK2 KO, KD and age-matched WT mice were sacrificed by cervical dislocation. The brain was rapidly removed and coronal corticostriatal slices (250 μm) were cut in Krebs' solution (in mmol/L: 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 10 glucose, 25 $NaHCO_3$) using a vibratome. The slices were maintained in Krebs' solution, bubbled with an O_2 95% and CO_2 5% gas mixture (pH = 7.4) at room temperature. Single coronal slices including the cortex and the striatum were transferred to a recording chamber and submerged in a continuously flowing Krebs' solution (33°C; 2.5–3ml/min) bubbled with a 95% O_2–5% CO_2 gas mixture. Whole-cell patch clamp recordings of SPNs of the dorsolateral striatum were performed with borosilicate electrodes (pipette resistance 4–7MΩ, access resistance 15–30MΩ; holding potential, $V_{hold} = -80$mV, membrane resistance 0.7-1.1 GΩ) containing (in mM): 145 K^+-gluconate, 0.1 $CaCl_2$, 2 $MgCl_2$, 0.1 EGTA, 10 HEPES, 0.3 Na-GTP and 2 Mg-ATP, adjusted to pH 7.3 with KOH. SPNs were identified by their hyperpolarized resting membrane potential (RMP, ~ -80mV), absence of spontaneous action potential discharge and presence of tonic firing activity during current-induced membrane depolarization. Signals were amplified with a Multiclamp 700B amplifier (Molecular Devices), recorded and stored on PC using pClamp10. A bipolar electrode, connected to a stimulation unit (Grass Telefactor), was located in the white matter between the cortex and the striatum to stimulate glutamatergic fibers (0.1Hz) and evoke excitatory post-synaptic currents (EPSCs). Spontaneous and evoked EPSCs were recorded in the continuous presence of 50 μM picrotoxin to block $GABA_A$ receptors. For NMDA/AMPA ratio the internal solution contained (in mM): 120 $CsMeSO_3$, 10 CsCl, 8 NaCl, 2 $MgCl_2$, 10 HEPES, 0.2 EGTA, 10 TEA, 5 QX314, 0.3 Na-GTP and 2 Mg-ATP, adjusted to pH 7.3 with CsOH. Ratios were calculated by dividing the NMDA-EPSC value, acquired at +40 mV holding 40 ms after the EPCS onset, by the EPSC negative peak amplitude acquired at -80 mV.

Drugs

Drugs were applied by dissolving them to the desired final concentration in the Kreb's solution and by switching the perfusion from control solution to drug-containing solution. AM-251, GSK2578215A, picrotoxin, quinpirole (Quin), L-sulpiride (Sulp), U73122, WIN55212-2 (WIN), were purchased from Tocris-Cookson (Bristol, UK). Drugs were dissolved into the Krebs' solution and applied by switching the perfusion from control solution to drug-containing solution.

Statistical analysis

Data analysis was performed off-line using Clampfit 10 (Molecular Devices) and GraphPad Prism 6 (GraphPad Software). Values in the text and figures are mean ± S.E.M, n representing the number of neurons. Statistical significance was determined by the Students' t-test or two-way ANOVA. The significance level was established at $p < 0.05$.

Results

G2019S knock-in (KI) mice show normal membrane electrical properties and glutamatergic synaptic transmission of striatal SPNs

Patch-clamp recordings of striatal SPNs were obtained from corticostriatal slices of six-month old G2019S KI mice and age-matched wild-type (WT) animals. Membrane electrical properties measured in the two groups of neurons by comparing the current-voltage relationship showed no difference for SPNs of KI and WT mice (Fig. 1A).

Spontaneous release of glutamate was subsequently evaluated by recording for 15 minutes spontaneous excitatory post-synaptic currents (sEPSCs) of SPNs of KI and WT mice, in the presence of 50 µM picrotoxin to block GABAA-mediated transmission. We found that both the amplitude and the frequency of sEPSCs were not significantly different in neurons of KI mice (amplitude = 12.9 ±0.8 pA, frequency = 2.1 ±0.1 Hz) and WT (amplitude = 14.0 ±1.2 pA,

frequency = 2.4 ±0.2 Hz) suggesting absence of any pre- or post-synaptic change of striatal glutamatergic transmission (Fig. 1B). Evoked EPSCs recordings allowed us to measure paired-pulse EPSC amplitudes and to calculate paired-pulse EPSCs ratios (PPRs) in KI (PPR = 1.38 ±0.08, n = 7) and WT (PPR = 1.36 ±0.07, n = 9) SPNs. We found that, in the two groups of SPNs, PPRs were not significantly different, further confirming no difference in presynaptic glutamate release (Fig. 1C). We also evaluated NMDA/AMPA ratios in SPNs of both groups of animals dividing the EPCS amplitude evoked at the holding potential of +40 mV by that at -80 mV. Results showed no significant differences between ratios calculated in SPNs of KI and WT mice confirming that post-synaptic glutamatergic transmission in KI mice is normal (KI, 0.39 ±0.14; WT, 0.41 ±0.06, Fig.1D).

Striatal SPNs of KI mice show reduced sEPSCs frequency by D2 receptor stimulation

Dopamine receptors are primarily involved in the modulation of striatal synaptic transmission and the D2 receptor represents an important pharmacological target for PD treatment (Connolly and Lang, 2014). In order to analyze the effect of DA D2 receptor stimulation on striatal SPNs we recorded sEPSCs of Lrrk2 KI and WT mice in the presence of 10 µM quinpirole bath applied for 10 minutes. We found that while in WT animals quinpirole did not significantly modify the sEPSC amplitude or frequency (Fig. 2A), in KI mice it significantly reduced the sEPSCs frequency by 31.3 ±5.2% with no effect on the amplitude, suggesting a presynaptic modulation of this drug on striatal neurotrasmission (Fig. 2A,B).

To further characterize the effect of the D2 receptor stimulation in KI mice, we recorded electrically-evoked EPSCs in the presence of different concentrations of quinpirole (0.1-10 µM) applied for 20 minutes. We calculated a dose-response curve for quinpirole on the EPSC amplitude that revealed a significant difference between groups, suggesting a possible increased sensitivity in neurons of KI mice to DA D2 receptor stimulation (Fig. 2C-F).

Accordingly, while the SPNs EPSCs were not affected by 10 μM quinpirole in WT mice (P>0.05), they were reduced by 38.47 ±1.76% in KI animals. We also found that in these mice the effect of quinpirole on the EPSCs amplitudes were prevented by 10 μM L-sulpiride, confirming the receptor specificity of the drug (Fig. 2F).

Pharmacological inhibition of Lrrk2 kinase activity does not prevent the quinpirole-induced SPNs EPSC amplitude reduction in G2019S KI mice

To confirm the hypothesis of an increased D2 receptor-mediated response to quinpirole in striatal slices ok KI mice we recorded EPSCs of striatal SPNs in KD and Lrrk2 KO mice, which carry a null kinase activity or lack the whole Lrrk2 protein, respectively. While 10 μM quinpirole reduced the SPNs EPSCs in KI mice (Fig. 2F and Fig. 3A), it had no effect on SPNs recorded from KD and KO suggesting the G2019S Lrrk2 mutation confers SPNs the sensitivity toward quinpirole (Fig. 3A).

Since the G2019S LRRK2 mutation is associated with gain of function activity, we attempted to prevent the quinpirole-induced SPNs EPSC reduction in KI mice by pharmacologically inhibiting the kinase site of LRRK2 with GSK2578215A. Therefore, slices were incubated with 1 μM GSK2578215A for 15 minutes and subsequently treated with this drug plus 10 μM quinpirole. GSK2578215A application *per se* did not change the EPSC amplitude. However, quinpirole, co-application for additional 10 minutes reduced the EPSC amplitude by 39.79 ±4.05%, similar to what observed in the SPNs of KI mice treated with quinpirole (Fig. 3B,C). These results suggest that, the synaptic modulation of SPNs by quinpirole, observed in the striatum of KI mice, does not likely depend on acute kinase LRRK2 activity.

Quinpirole reduces the SPNs EPSC amplitude in KI mice by CB1 receptor stimulation

DA is primarily involved in the induction of synaptic plasticity in the striatum, enabling long-term changes of SPNs synaptic transmission, such as long-term depression (LTD) (Calabresi et

al., 1992). DA exerts a modulatory effect on cortical glutamatergic inputs to SPNs through post-synaptic D2 receptors. The activation of these receptors, in fact, may lead to release of endocannabinoids (eCB) from the SPNs, that, as retrograde messengers activate presynaptic CB1 receptors on glutamatergic terminals (Kreitzer and Malenka, 2007) reducing glutamate release (Adermark and Lovinger, 2007; Gerdeman et al., 2002). To test for this possibility slices from KI mice were incubated for 30 minutes with 3 μM of the CB1 antagonist AM251. SPNs EPSCs were subsequently recorded for 10 minutes to obtain a stable baseline in the presence of AM251 and then recorded in the presence of AM251 plus 10 μM quinpirole for further 20 minutes. We found that in these pharmacological conditions quinpirole failed to reduce the EPSC amplitude (Fig. 4A). To test for a possible alteration of the CB1 receptor function in KI mice, SPNs EPSCs amplitudes were recorded in the presence of the CB1 receptor agonist WIN55212-2. We found that the EPSC amplitude of SPNs recorded from WT and KI animals was similarly reduced following the application of 3 μM WIN55212-2, suggesting no alteration of the CB1 receptor function in striatal SPNs of KI mice (Fig. 4B).

Dopamine D2 receptor stimulation triggers in striatal SPNs of KI mice phospholipase (PLC) activity

Endocannabinoids might be released by intracellular Ca^{2+} changes in SPNs following the stimulation of D2 receptors (Giuffrida et al., 1999; Piomelli, 2003; Tozzi et al., 2011; Tozzi et al., 2012). The observed enhanced inhibition of presynaptic neurotransmitter release by quinpirole in SPNs of KI mice might therefore reflect increased intracellular Ca^{2+} induced by the activation of a D2 receptors-triggered phospholipase C (PLC) (Rioult-Pedotti et al., 2015). To assess the role of PLC in this synaptic process we measured the effect of quinpirole in the presence of the PLC inhibitor U73122 in KI mice. SPNs EPSCs amplitude were recorded for 10 minutes in 10 μM U73122 to obtain a stable baseline and then for additional 20 minutes in the presence of U73122 plus 10 μM quinpirole. We found that PLC inhibition impeded

quinpirole effect preventing the reduction of the EPSC amplitude (Fig. 4D). Taken together these findings suggest that in KI mice striatal gluatamatergic release is reduced by D2 receptor stimulation that, in turn, promotes PLC activity and endocannabinoids release in SPNs (Fig. 5).

Discussion

The study of the genetic mutations associated to PD could unravel the pathogenic mechanisms involved in the early phases of the disease. The G2019S *LRRK2* mutation, the most common in both the familial and sporadic form of PD, accounting respectively for 3-19% and 1-6% of total cases (Gilks et al., 2005; Martin et al., 2014; Melrose, 2008; Puschmann, 2013), is found to be deeply implicated in the dysregulation of basal ganglia circuitry, even in non-symptomatic carriers (Helmich et al., 2015; Vilas et al., 2015).

We investigated the synaptic function of SPNs in the nucleus striatum, the main input station of the basal ganglia, using a genetic mouse model carrying the G2019S *Lrrk2* mutation (KI mice), in order to further elucidate the potential synaptic role of LRRK2.

We obtained three major novel findings: 1) adult KI mice present normal electrical membrane properties, spontaneous and evoked excitatory synaptic transmission of striatal SPNs; 2) pharmacological activation of the DA D2 receptor reduces glutamatergic synaptic transmission of striatal SPNs in KI mice; 3) the DA D2 receptor-dependent reduction of the glutamatergic activity observed in KI mice is mediated by PLC activation and post-synaptic release of eCBs, retrogradely activating presynaptic CB1Rs.

In the attempt to characterize the pathogenic mechanism underlying *LRRK2*-related PD, several animal models carrying different *Lrrk2* gene mutations have been studied (Volta and Melrose, 2017). These models expressing different genotypes, such as the R1441G or the G2019S *Lrrk2* mutations, also present different phenotypes, ranging from slight to more pronounced molecular and behavioral abnormalities which are largely age-dependent (Ramonet et al., 2011;

Tagliaferro et al., 2015; Tong et al., 2010; Xu et al., 2012). Interestingly, the subtle motor and non-motor behavioral alterations observed in *Lrrk2*-mutated mice reflect the early symptoms affecting PD patients during the earliest phases of the disease (Volta and Melrose, 2017). These models could therefore be instrumental to the understanding of early pathogenic neuronal and synaptic alterations characterizing PD. In fact, the G2019S *Lrrk2* KI mice used in this study are characterized by age-dependent changes in striatal DAT and VMAT2 function as well as α-synuclein inclusions (Longo et al., 2017), precocious susceptibility to synaptic dysfunction (Matikainen-Ankney et al., 2016) and hyper-kinetic phenotype resistant to age-related motor decline (Longo et al., 2014). Interestingly, patients carrying the same genetic mutation show abnormal DAT-SPECT reflecting the pre-motor phase early striatal synaptic abnormalities (Bergareche et al., 2016; Sierra et al., 2013).

Lrrk2 mutations might affect striatal function since this protein is known to be expressed both in striatal axons and dendrites (Belluzzi et al., 2012; Melrose et al., 2007) but while its role in synaptic transmission is not clear, a role of the protein in regulating pre-synaptic vesicles dynamics (Beccano-Kelly et al., 2014) and DA signaling at cortico-striatal synapses (Volta and Melrose, 2017) has been described. In the present work, we found that while in SPNs recorded from acute striatal slices of 6-month old KI mice electrical membrane properties and synaptic glutamatergic transmission are not affected, the pharmacological stimulation of DA D2 receptor reduced the glutamatergic transmission selectively in KI but not in WT mice, revealing the existence of subtle synaptic changes in KI animals. Our data are in line with previous findings showing in adult KI mice (P60) sEPSC frequencies similar to that recorded in non-transgenic animals (Matikainen-Ankney et al., 2016) and to what observed in adult mice overexpressing LRRK2-G2019S (Beccano-Kelly et al., 2015).

While motor impairment in PD is generally related to increased glutamatergic neurotransmitter signaling to the nucleus striatum (Calabresi et al., 2014; Obeso et al., 2017; Singh et al., 2016), the absence of clear changes of synaptic glutamatergic currents in our KI mice is suggestive of

a still preserved basal synaptic activity, resembling the most precocious phase of the disease. Moreover, while possible changes of cytosolic calcium levels might affect cell functions by altering neurotransmitter release, this seems not to be the case in SPNs of our KI mouse model since sEPSCs were not different from WT mice.

The reduction of spontaneous EPSC frequency by the D2 receptor agonist quinpirole, in SPNs of KI mice but not of KO, and KD mice, suggests a specific role of the enhanced kinase activity linked to the G2019S *Lrrk2* mutation in modulating the glutamatergic and/or dopaminergic striatal synaptic network. This effect appears to be dependent on the constitutive presence of G2019S LRRK2 in striatal SPNs of KI mice, since pharmacological inhibition LRRK2 kinase activity with GSK2578215A did not reverse the effects of quinpirole. The G2019S LRRK2 mutation might in fact trigger early age-dependent neuronal alterations, reflecting dysregulation of multiple enzymatic pathways that, in turn, might be responsible for the observed alteration of synaptic transmission. In fact, LRRK2 expression levels have been reported to rise in the dorsal striatum after birth, at a time when synapses are forming, suggesting that LRRK2 regulates development of striatal circuits early in the postnatal development by permanently shaping neural circuits (Matikainen-Ankney et al., 2016).

The causal link between the G2019S LRRK2 mutation and the changes of DA signaling affecting striatal glutamatergic transmission is still a matter of investigation. The G2019S LRRK2 mutation was observed to increase striatal membrane expression of DA D1 receptor (Migheli et al., 2013) coupled to impairment of the striatal stimulation-induced DA D1 receptor internalization (Rassu et al., 2017). Conversely, the effects of the different *Lrrk2* mutants on the DA D2 receptor is still under investigation, with experimental evidence showing that LRRK2 could modulate D2R expression influencing the intracellular receptor trafficking (Rassu et al., 2017).

Here, we report that the enhanced inhibitory effect of DA D2 receptor activation on striatal excitatory transmission observed in *Lrrk2* KI mice is mediated by the post-synaptic release of

eCBs, through activation of PLC, with a subsequent activation of pre-synaptic CB1 receptors. This evidence suggests increased sensitivity of the D2R/PLC/eCB pathway and subsequent marked reduction of glutamate synaptic activity release following the stimulation of the D2R in SPNs of mice carrying the G2019S LRRK2 mutation. Experiments testing the CB1R function in SPNs of both KI and WT mice ruled out any possible functional abnormalities of CB1Rs activation in our LRRK2 model.

Since D2Rs are expressed by different striatal neuronal subpopulations, including cholinergic interneurons (CINs) (Tozzi et al., 2011), we cannot rule-out that quinpirole may affect SPNs neurotransmission by acting on different striatal target neurons. Accordingly, our experiments performed in *Lrrk2* KI mice showed that quinpirole-mediated inhibition of EPSCs was present in virtually all the recorded SPNs, possibly suggesting that the observed response could be also mediated by striatal CINs and not only segregated in SPNs in a specific basal ganglia pathway. Collectively, these findings could help designing possible neuroprotective strategies for patients with early PD. The clinical use of DA agonists such as pramipexole, ropinirole and rotigotine, together with L-dopa, represents a well-established symptomatic therapy for patients with PD, especially in the earlier phases of the disease (Connolly and Lang, 2014). The key role of DA D2 receptor in reducing glutamatergic output in our *Lrrk2* genetic model of PD further support the use of DA agonists in the treatment of early PD patients that may carry *LRRK2* mutations. A further characterization of the complex molecular relationships existing between *Lrrk2* mutations and DA D2 receptor activation could help identifying specific pathogenic pathways and define new tools to assess potential neuroprotective pharmacological strategies aimed at delaying or preventing the classical motor and cognitive symptoms affecting PD patients carrying *LRRK2* mutations.

Acknowledgments

Conflicts of Interest

Nothing to report.

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Figure legends

Figure 1. Electrical membrane properties and synaptic response of striatal SPNs of WT and KI mice. A) Voltage traces acquired from striatal slices by patch clamp recordings of SPNs of wild-type (WT) and G2019S-*Lrrk2* knock-in (KI) mice following hyperpolarizing and depolarizing steps of currents. Current-voltage plots of SPNs recorded from WT and KI mice (right) (n=9 for each group, two-way ANOVA, KI vs WT, P>0.05). **B)** Histogram showing the amplitude and frequency of spontaneous EPSCs (sEPSC) recorded from SPNs of WT and KI mice (KI, n=16 *vs* WT, n=14, amplitude $t_{(28)}$=0.76; frequency unpaired Students' t-test, $t_{(28)}$=0.87, P>0.05). Representative traces showing sEPSCs events measured from SPNs of WT and KI mice. **C)** Histogram of the evoked EPSCs amplitude ratios (EPSC2/EPSC1) obtained by paired-pulse stimulation of SPNs recorded from WT and KI mice (KI, n=7 *vs* WT, n=9, unpaired Students' t-test, $t_{(14)}$=0.24, P>0.05). Paired EPSC traces, evoked by paired-pulse stimuli, recorded from SPNs of a WT and a KI mouse. **D)** Histogram of the NMDA/AMPA ratio and representative EPSC traces, acquired at +40 mV and -80 mV holding current from SPNs of WT and KI mice (KI, n=8 *vs* WT, n=4, unpaired Students' t-test, $t_{(10)}$=0.19, P>0.05).

Figure 2. Effect of quinpirole on spontaneous and evoked EPSCs of striatal SPNs of WT and KI mice. A-B) Histogram and representative traces showing the amplitude and frequency of sEPSC recorded from SPNs of WT (A) and KI mice (B) in control conditions and in the presence of quinpirole (Quin). (WT, control *vs* quinpirole, amplitude, n=5, paired Students' t-test, $t_{(4)}$=0.71, P>0.05; frequency, $t_{(4)}$=0.03, P>0.05). (KI, control *vs* quinpirole, paired Students' t-test, amplitude, n=7, $t_{(6)}$=0.14, P>0.05; frequency, n=7, $t_{(6)}$=5.98, P=0.001). *** p<0.001. **C)** Representative time-course plot of the evoked EPSC amplitude of a striatal SPN recorded from a KI mouse before, during and following the application for 10 minutes of quinpirole. **D)** Representative evoked EPSCs traces acquired in control conditions (left), after 20 minutes of quinpirole application (middle) and after washed-out of the drug in a SPN of a WT (top traces) and a KI mouse (bottom traces). **E)** Dose response curve of quinpirole effect on the EPSC

amplitude, expressed as percentage of reduction, of SPNs recorded from WT and KI mice (two-way ANOVA, group factor, $F_{(1,72)}=62.48$, P<0.0001) (KI mice, pre- vs post-quinpirole 10 µM, n=11, paired t-test $t_{(10)}=15.54$, P<0.0001). **F)** Time-course plots of the EPSC amplitudes acquired in control conditions and in the presence of quinpirole in SPNs of WT and KI mice and in SPNs of KI mice in the presence of L-sulpiride (KI, Sulp, pre- vs post-quinpirole, n=4, paired t-test $t_{(3)}=0.70$, P>0.05).

Figure 3. Quinpirole affects the SPNs EPSC amplitude of *Lrrk2* KI but not KD or KO mice A) Time-course plots of the EPSC amplitudes of striatal SPNs recorded from WT, KI, D1994S KD (KD) and *Lrrk2* knock-out (KO) mice in control conditions and after bath application of quinpirole for 20 minutes. (KO, EPSC reduction in Quin, 7.89% ±3.26, pre- vs post-quinpirole, paired t-test, n=8, $t_{(7)}=2.29$, P>0.05; KD, EPSC reduction in Quin, 4.68% ±3.10, pre- vs post-quinpirole, paired t-test n=6, $t_{(5)}=2.47$, P>0.05). **B)** Histogram of the EPSC amplitude of SPNs recorded in the presence of the LRRK2 inhibitor GSK2578215A (GSK) and in GSK plus quinpirole co-applied for 20 minutes (pre- vs post-quinpirole in GSK, paired t-test, n=6, $t_{(5)}=9.83$, P<0.001; quinpirole vs GSK plus quinpirole, unpaired Student's t-test, p>0.05). Representative superimposed traces recorded in absence (top) or presence (bottom) of GSK in KI mice showing the effect of the application of quinpirole. **C)** Representative time-course plot of the evoked EPSC amplitude of a striatal SPN recorded from a KI mouse in the continuous presence of GSK before, during and following the application for 10 minutes of quinpirole.

Figure 4. Quinpirole reduces the SPNs EPSC amplitude by a CB1 receptor- mediated mechanism. A) Time-course graph showing the effect of quinpirole on the EPSC amplitude of KI mice in control conditions and in the presence of 3µM of the CB1 receptor antagonist AM-251(paired t-test, n=7, $t_{(6)}=0.36$, P>0.05). **B)** Histogram and superimposed representative traces showing the effect of the CB1 receptor agonist WIN-55212-2 (3 µM) on the EPSCs amplitudes

in SPNs of WT and KI mice (WT, 51.1% ±3.19, paired t-test, n=4, $t_{(3)}$=15.94, P<0.001; KI, 53.9% ±7.5, paired, t-test, n=4, $t_{(3)}$=6.42, P<0.01; WT vs KI, unpaired Student's t-test, p>0.05). **C)** Time-course graph of EPSCs amplitudes and superimposed representative EPSCs traces acquired in SPNs of KI mice before and following the application of quinpirole in control conditions or in the presence of the PLC receptor agonist U73122 (10 µM) (EPSC reduction, 4.43% ±2.09, n=5, paired t-test $t_{(4)}$=1.63, P>0.05).

Figure 5. Scheme of the inhibition of D2 and CB1 receptor-dependent signaling by CB1 receptor antagonism and phospholipase C inhibition in striatal SPNs. The stimulation of DA D2 receptor by quinpirole activates the phospholipase C (PLC) and promotes the Ca^{2+}-dependent release of endocannabinoids (eCBs). ECBs diffuse to glutamatergic terminals and reduce neurotransmitter release by activating presynaptic CB1 receptors. These pathways are enhanced in striatal SPNs of Lrrk2 G2019S KI mice and inhibition of the PLC by U73122 or the CB1 receptor by AM-251 prevents in SPNs of these mice the effect of quinpirole. Red crosses represent inhibition of the enzymatic pathways.

Highlights

- G2019S-Lrrk2 mice present normal striatal spiny neurons glutamate transmission

- Dopamine D2R stimulation reduces striatal glutamatergic currents in Lrrk2 KI mice

- Quinpirole reduces glutamatergic EPSCs by PLC stimulation and endocannabinoid release
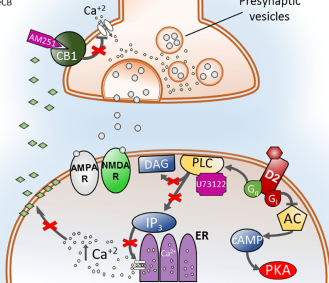
Graphics Abstract

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5