Check for updates

Received: 8 June 2020 | Revised: 4 November 2020 | Accepted: 9 November 2020

DOI: 10.1111/jnc.15240

REVIEW

Journal of Neurochemistry JNC The Official Journal of the International Society for Neurochemistry WILEY

LRRK2 at the pre-synaptic site: A 16-years perspective

Francesca Pischedda | Giovanni Piccoli

CIBIO, Università degli Studi di Trento, Italy & Dulbecco Telethon Institute, Trento, Italy

Correspondence
Giovanni Piccoli, CIBIO, Università degli Studi di Trento, Italy & Dulbecco Telethon Institute, Trento, Italy.
Email: giovanni.piccoli@unitn.it

Funding information
Fondazione Cassa Di Risparmio Di Trento E Rovereto, Grant/Award Number: 2019.0230; Ministero dell'Istruzione, dell'Università e della Ricerca, Grant/Award Number: PRIN-2017ENN4FY; Fondazione Telethon, Grant/Award Number: TDPG00514TA; Fondazione Cariplo, Grant/Award Number: 2019-3415

This is a Review for the special issue "Presynaptic Dysfunction and Disease".

Abstract

Parkinson's disease is a common neurodegenerative disorder and is clinically characterized by bradykinesia, rigidity, and resting tremor. Missense mutations in the leucine-rich repeat protein kinase-2 gene (LRRK2) are a recognized cause of inherited Parkinson's disease. The physiological and pathological impact of LRRK2 is still obscure, but accumulating evidence indicates that LRRK2 orchestrates diverse aspects of membrane trafficking, such as membrane fusion and vesicle formation and transport along actin and tubulin tracks. In the present review, we focus on the special relation between LRRK2 and synaptic vesicles. LRRK2 binds and phosphorylates key actors within the synaptic vesicle cycle. Accordingly, alterations in dopamine and glutamate transmission have been described upon LRRK2 manipulations. However, the different modeling strategies and phenotypes observed require a critical approach to decipher the outcome of LRRK2 at the pre-synaptic site.

KEYWORDS

LRRK2, Parkinson's disease, presynaptic bouton, synaptic vesicle

1 | BACKGROUND

Parkinson's disease (PD) affects 2% of the population above 65 years. It is characterized by the death of dopaminergic neurons in the substantia nigra and its most common clinical traits are bradykinesia, rigidity, and resting tremor (Moore et al., 2005; Obeso et al., 2017; Schulz et al., 2016). Mutations in the Leucine-rich repeat kinase 2 (LRRK2) gene have been unequivocally related to familial late-onset PD (Singleton et al., 2017). The first evidence of the genetic link between LRRK2 and PD dates back to 2002, when a new PD locus, PARK8 (OMIM 609007), was identified in a large Japanese family (Funayama et al., 2002). Soon after, three papers appeared describing that PARK8 codes for LRRK2 and that LRRK2 mutations cause autosomal dominant PD (Funayama et al., 2005; Paisan-Ruiz et al., 2004; Zimprich et al., 2004). Since then, eight pathological missense mutations (i.e., N1437H, R1441C/G/H/S, Y1699C, G2019S, and I2020T) have been characterized (Hui et al., 2018; Schulz et al., 2016). In the Taiwanese populations a relatively common LRRK2 variant, G2385R, that moderately increases PD risk has been identified (Tan, 2006; Tan et al., 2009).

Recent epidemiological studies attribute LRRK2 mutations to 13% of familial PD cases, with the most common G2019S mutation accounting for 4% of familial and 1% of sporadic PD [reviewed in (Tolosa et al., 2020)].

2 | THE BASICS OF LRRK2

Leucine-rich repeat kinase 2 (LRRK2) is a very large protein consisting of 2,527 amino acids, encompassing multiple functional domains: from N- to C-terminus armadillo ankyrin, the namesake leucine-rich repeats, a ROC GTPase, a COR dimerization domain, a kinase domain, and WD40 repeats (Bosgraaf & Van Haastert, 2003; Gilsbach et al., 2018; Mills et al., 2012).

Abbreviations: AP, action potential; BAC, bacterial artificial chromosomes; DA, dopamine; EPSC, excitatory post-synaptic current; FSCV, fast-scan cyclic voltammetry; KI, knock-in; LOF, loss-of-function; MSN, striatal medium spiny neuron; PD, Parkinson's disease; RRP, ready releasable pool; SV, synaptic vesicles.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2020 The Authors. *Journal of Neurochemistry* published by John Wiley & Sons Ltd on behalf of International Society for Neurochemistry

LRRK2 exists both as monomer and dimer, being the dimer likely the active species (Berger et al., 2010; Sen et al., 2009). A double enzymatic activity characterizes LRRK2: it behaves as a serine–threonine kinase and possesses a GTPase activity, mediated by the ROC-COR domains.

Besides the well-characterized catalytic domains, the N-terminal Armadillo, the LRR, and Ankyrin repeats as well as the C-terminal WD40 domain host multiple protein interaction sites and allow LRRK2 dimerization. Accumulating evidence indeed describes LRRK2 as a molecular hub orchestrating numerous protein networks (Porras et al., 2015). LRRK2 is phosphorylated by various kinases on an N-terminal stretch of serine residues (Nichols, 2017) and de-phosphorylated by PP1 (Lobbestael et al., 2013). 14-3-3s bind phosphorylated Ser910/Ser935 and maintain LRRK2 in a latent hypoactive state (Mamais et al., 2014; Muda et al., 2014). One striking feature of the majority of LRRK2 pathogenic mutations—except for G2019S—is that they all display moderate to severe loss of S910/S935 phosphorylation (Nichols et al., 2010). Thus, cellular phosphorylation is a key event for controlling the LRRK2 function (Athanasopoulos et al., 2018). The mutations within the LRRK2 kinase domain (G2019S and I2020T) increase LRRK2 activity in vitro (Gloeckner et al., 2006; Greggio et al., 2006) and in vivo (Steger et al., 2016), possibly by disrupting the inhibited kinase domain conformation (Schmidt et al., 2019). The mutations falling within the ROC and Cor domain (N1437H, R1441G/C/H, Y1699C) suppress GTPase activity, promote GTP binding (Nguyen & Moore, 2017), and eventually increase LRRK2 kinase activity (Sheng et al., 2012). Finally, the G2385R mutation modifies WD40 domain folding and interaction (Carrion et al., 2017; Piccoli et al., 2014) and enhances LRRK2 GTPase (Ho et al., 2016) and kinase activity by a yet unknown mechanism (Zhang et al., 2019). It is well accepted that the primary pathogenic mechanism arises from kinase hyper-activation (Greggio et al., 2006). LRRK2 phosphorylates its targets at a conserved Thr/Ser motif (Gloeckner et al., 2010; Pungaliya et al., 2010). The most robust substrates for LRRK2 kinase activity identified so far are a panel of Rab proteins, including Rab8A and Rab10 (Steger et al., 2016). All PD-linked LRRK2 mutants show increased kinase activity on Rabs (Jeong et al., 2018; Steger et al., 2016). Other LRRK2 targets have been suggested, such as endophilin A1 (Matta et al., 2012), ribosomal protein s15 (Martin et al., 2014), N-ethylmaleimide sensitive fusion protein (NSF) (Belluzzi et al., 2016), synaptojanin-1 (Islam et al., 2016), P62/SQSTM1 (Kalogeropulou et al., 2018), auxillin (Nguyen & Krainc, 2018), and synapsin I (Marte et al., 2019).

LRRK2 can be detected in different tissues, with the highest value reached in the brain, kidney, and lung (Giasson et al., 2006). Of note, LRRK2 expression is quite relevant also in immune cells, including B lymphocytes, monocytes, and neutrophils [reviewed in (Cabezudo et al., 2020; Dzamko, 2017; Wallings et al., 2020)]. Recently, the role of LRRK2 in astrocytes has been gaining interest (di Domenico et al., 2019; Sanyal et al., 2020; Zhao et al., 2018). In mouse and rat brain, strong LRRK2 mRNA and protein expression have been observed in the cerebral cortex, striatum, hippocampus, and amygdala nuclei. Moderate mRNA and protein expression were detected in the thalamus and hypothalamus. Notably, LRRK2 protein expression is relatively low in the substantia nigra and in the ventral tegmental area of the midbrain where dopaminergic neurons are particularly abundant (Higashi et al., 2007; Taymans et al., 2006). KO validated monoclonal antibodies detected LRRK2 at high level in the cortex and striatum in rat and mouse brain. Moreover, the highest level is detected in cortical pyramidal neurons of layer V and in striatal medium spiny neurons (West et al., 2014). In particular, expression of Lrrk2 has been detected in Dopamine receptor-1 and −2–positive striatal medium spiny neurons (Giesert et al., 2013).

Single-cell RNA seq has allowed an unprecedented mapping of gene expression at the cellular level. LRRK2 mRNA has been positively identified in cortical neurons (Zeisel et al., 2015) but not in P7 murine dopaminergic neurons (Hook et al., 2018). However, West and colleagues found that in adult mice (but not in rats), LRRK2 protein is expressed in the substantia nigra pars compacta (West et al., 2014), suggesting that its expression in the dopaminergic system may start at a later developmental stage.

LRRK2 is not observed at the subcellular level in the nucleus but distributed throughout the cytoplasm, where it binds the cytoskeletal system and localizes to organelle membranes (Biskup et al., 2006; Gandhi et al., 2008; Hatano et al., 2007; Meixner et al., 2011). In particular, LRRK2 co-precipitates and interacts with synaptic vesicles (SV) (Biskup et al., 2006; Piccoli et al., 2014).

3 | LRRK2 FUNCTION AT THE PRE-SYNAPTIC SITE

A vast effort has been dedicated to revealing LRRK2 physiological and pathological function. Several Lrrk2 KO rodent models exist and have been instrumental in gaining insights into the putative role of LRRK2 [reviewed in (Volta & Melrose, 2017; Xiong et al., 2017)]. It is established that Lrrk2 KO animals are viable, fertile, with average lifespan and do not show any overt motor phenotypes. Instead, they share peripheral traits, such as morphological abnormalities in the lungs and kidneys (Herzig et al., 2011). Interestingly, large-scale analyses demonstrated that reduced LRRK2 protein levels are not associated with human-specific phenotype (Blauwendraat et al., 2018; Whiffin et al., 2020). Along with this 16-year long investigation, different and sometimes unexpected functions have been attributed to LRRK2: microtubules and microfilaments dynamics, protein synthesis, cellular signaling, mitochondrial homeostasis, ubiquitin-proteasome system and lysosomal protein clearance, and eventually synaptic transmission [reviewed in (Berwick et al., 2019; Taylor & Alessi, 2020)].

The discovery that Rab proteins are the principal substrate for LRRK2 (Steger et al., 2016) strongly links LRRK2 to the vesicular system. Rabs orchestrate membrane trafficking, including

the processes of vesicle formation, vesicle movement along actin and tubulin tracks, membrane fusion, and regulate SV trafficking (Wandinger-Ness & Zerial, 2014). In particular, Rab5 is a marker of the early endosome and plays an important role in clathrin-mediated endocytosis (He et al., 2017). Rab5-driven SV fusion with early endosome has been proposed to be crucial for the highly recycling ready releasable pool (RRP) (Hoopmann et al., 2010). Finally, Rab5 manipulations result in defective SV recycling (Fischer von Mollard et al., 1994; Shimizu et al., 2003; Wucherpfennig et al., 2003).

In vitro evidence suggests that LRRK2 binds or phosphorylates key proteins involved in SV cycle other than Rab5, such as endophilinA, NSF, dynamin, synaptojanin1, auxilin, snapin, synapsin I, AP2 complex, and CaV2.1 channel (Arranz et al., 2015; Bedford et al., 2016; Belluzzi et al., 2016; Heaton et al., 2020; Islam et al., 2016; Matta et al., 2012; Shin et al., 2008; Stafa et al., 2014; Yun et al., 2013). Clearly, LRRK2 is potentially well-positioned to govern different steps along with both SV endo- and exocytosis (see Box 1).

In 2008, Shin and colleagues linked LRRK2 to SV endocytosis (Shin et al., 2008). Since that initial study, several other papers have contributed to describing LRRK2 as a modulator of SV dynamics in neurons. We discuss 32 original contributions focusing on LRRK2 at the pre-synaptic site (listed in table 1) and try to solve some controversies.

4 | THE IMPACT OF LRRK2 LOSS-OF-FUNCTION AT THE PRE-SYNAPTIC SITE

Loss-of-function (LOF) models have helped to clarify the role of LRRK2 within the pre-synaptic bouton. Shin and colleagues took advantage of sypHy and FM-dye assay to monitor SV exo- and exocytosis in rat hippocampal neurons upon siRNA-mediated LRRK2 down-regulation (Shin et al., 2008). In particular, they applied a robust electrical stimulation to mobilize the entire recycling pool. Interestingly, both LRRK2 silencing and over-expression slowed

Box 1 The pre-synaptic bouton.

SV pool: within the pre-synaptic terminal, three major SV pools are classically recognized: a readily releasable pool (RRP), a recycling pool, and a reserve pool. The RRP consists of SV docked and primed for release. These SV can be rapidly exocytosed and support transmission during sporadic action potential (AP) firing. The recycling pool consists of SV recruited during sustained activity stimulation and accounts for typically about 10%–20% of all vesicles. Finally, the reserve pool hosts vesicles that are incapable of AP-evoked exocytosis. The resting pool encompasses more than 50% of the entire SV content. Such a large non-releasable pool could serve as a reservoir of SV that can be mobilized after chronic synaptic depression; it might be involved upon pre-synaptic potentiation; it could allow TTX-insensitive spontaneous vesicle release; it could serve as a local reservoir for proteins instrumental for SV cycling [reviewed in (Denker & Rizzoli, 2010; Rizzoli & Betz, 2005). Other additional SV pools have been proposed: the spontaneously releasing pool, responsible for the spontaneous release (Sara et al., 2005); the stranded vesicle pool (Wienisch & Klingauf, 2006), containing merged synaptic vesicles; and the "super-pool," composed of SV shuffling among nearby active synapses (Darcy et al., 2006). While readily releasable pool vesicles are docked at the active zone membrane, the other SV pools are mixed and disperse within the pre-synaptic terminal. This finding suggests that each SV pool is unequivocally identified by molecular tags, such as an interacting soluble protein. Synapsins dynamically organize the resting pool by reversibly tethering SV to each other and to actin filaments in a phosphorylation-dependent manner (Cesca et al., 2010; Fdez & Hilfiker, 2006). Synapsins, in particular the isoform I, have been suggested as the molecular flag of the resting pool. Tomosyn1 inhibits SV priming by interacting with the SNARE proteins syntaxin and SNAP25 and regulates the balance between the total recycling pool and the resting pool (Cazares et al., 2016).
Exocytosis: as action potential in the form of a Na+-driven depolarization wave reaches the pre-synaptic bouton, voltage-gated calcium channels open and allow inward Ca^{2+} current. Local increase of [Ca^{2+}] is the final trigger of SV fusion. The three SNAREs proteins Syntaxin-1, Synaptobrevin, and SNAP-25, have a key role in membrane fusion. In the so-called primed state, the SNARE complex is partially assembled, but synaptotagmin-1 and complexins still inhibit SV fusion. Upon the influx of Ca^{2+}, Synaptotagmin-1 inhibition is relieved, the SNARE complex forces together the vesicle and plasma membranes and eventually drives SV fusion and NT release.
Endocytosis: upon fusion, SV must be recycled. The ATPase NSF in complex with SNAP catalyzes the release of SNARE complexes, thus allowing the first step of SV endocytosis and recycling. Vesicle recycling can happen via clathrin-mediated endocytosis (Saheki & De Camilli, 2012), kiss-and-run, that is, a transient SV fusion without the full collapse of the SV membrane (Chanaday et al., 2019), bulk endocytosis, that is, the massive removal of SV membranes within a short time, usually following a very intense stimulation, and finally clathrin-independent fast (Cousin, 2009) or ultrafast endocytosis (Watanabe & Boucrot, 2017). In an alternative pathway, SV retrieved via clathrin-dependent or independent mechanisms fuse with an endosome from which they bud in a fusion-competent state (Hoopmann et al., 2010).

TABLE 1 The table lists the papers discussed in this review. It provides first author name and year of publication (paper), kind of genetic or pharmacological manipulation of LRRK2 (LRRK2 status), neuronal population or tissue investigated (neuron/tissue), model organism (organism), primary experimental approach (technique), and sum-up of the results (outcome)

Paper	LRRK2 status	Neuron/tissue	Organism	Technique	Outcome
Arranz et al., 2015	KO	striatal culture	rat Long Evans	SypHy; TEM	impairment of endocytosis
Arranz et al., 2015	KO	hippocampal cultures	mouse C57BL/6J	electrophysiology	impairment of endocytosis
Beccano-Kelly, Kuhlmann, et al., 2014	KI G2019S	cortical culture	mouse C57BL/6J	electrophysiology	increased mEPSC frequency
Beccano-Kelly, Kuhlmann, et al., 2014	KO	cortical culture	mouse C57BL/6J	electrophysiology	no alteration
Beccano-Kelly, Kuhlmann, et al., 2014	OE human wild-type (BAC, endogenous promoter)	cortical culture	mouse C57BL/6J	electrophysiology	no alteration
Beccano-Kelly, Volta, et al., 2014	KO	Cortico-striatal slices (SPN)	mouse C57BL/6J	electrophysiology	no alteration
Beccano-Kelly, Volta, et al., 2014	OE human wild-type (BAC, endogenous promoter)	Cortico-striatal slices (MSN)	mouse C57BL/6J	electrophysiology	no alteration in sEPSC
Beccano-Kelly, Volta, et al., 2014	KO	Cortico-striatal slices (glutamatergic neurons)	mouse C57BL/6J	electrophysiology	no alteration
Beccano-Kelly, Volta, et al., 2014	OE human wild-type (BAC, endogenous promoter)	Cortico-striatal slices (glutamatergic neurons)	mouse C57BL/6J	electrophysiology	no alteration in PPR
Beccano-Kelly, Volta, et al., 2014	OE human wild-type (BAC, endogenous promoter)	dopaminergic system	mouse C57BL/6J	microdialysis	decreased basal DA release; increased presynaptic D2R activity
Belluzzi et al., 2016	OE human G2019S (BAC)	cortical culture	mouse C57BL/6J	sypHy	increased SV exo/endocytosis
Belluzzi et al., 2016	pharmacological inhibition (GSK)	cortical culture	mouse C57BL/6J	sypHy	decreased SV cycle
Chou et al., 2014	OE human G2019S (CMV)	striatal slices	mouse FVB/*N*	fast scan cyclic voltammetry; electrophysiology	decreased evoked DA release; no high-frequency LTD in MSN
Chou et al., 2014	OE human G2019S (CMV)	hippocampal slices	mouse FVB/*N*	electrophysiology	no alteration in STP
Chou et al., 2014	OE human G2019S (CMV)	cerebellar slices	mouse FVB/*N*	electrophysiology	no alteration in STP
Cirnaru et al., 2014	pharmacological inhibition (IN−1)	cortical culture	mouse C57BL/6J	Anti-synaptotagmin uptake assay; electrophysiology	decreased SV endocytosis; reduced mEPSC
Creed et al., 2019	KO	Dopaminergic, glutamatergic, cholinergic system	rat Long Evans	In vivo microdialysis	no alteration
Giesert et al., 2017	Knock-down (constitutive)	cortical culture	mouse C57BL/6J	Anti-synaptotagmin uptake assay	no alteration
Giesert et al., 2017	KI R1441C	cortical culture	mouse C57BL/6J	Anti-synaptotagmin uptake assay	no alteration
Hinkle et al., 2012	KO	dopaminergic system	mouse C57BL/6J	In vivo microdialysis	no alteration
Li et al., 2010	OE murine wild-type (BAC, endogenous promoter)	striatal slices	mouse C57BL/6J	fast scan cyclic voltammetry	increased evoked DA release
Li et al., 2010	OE murine G2019S (BAC)	striatal slices	mouse C57BL/6J	fast scan cyclic voltammetry	decreased evoked DA release

(Continues)

TABLE 1 (Continued)

Paper	LRRK2 status	Neuron/tissue	Organism	Technique	Outcome
Liu et al., 2015	OE human wild-type (only in DA neurons)	dopaminergic system	mouse C57BL/6J	Microdialysis; fast scan cyclic voltammetry	increased evoked DA release
Liu et al., 2015	OE human G2019S (only in DA neurons)	dopaminergic system	mouse C57BL/6J	Microdialysis; fast scan cyclic voltammetry	decreased evoked DA release
Maas et al., 2017	KO	hippocampal cultures	mouse C57BL/6J	SypHy; FM-dye; electrophysiology	no alteration
Maas et al., 2017	KO	Cortico-striatal cultures	mouse C57BL/6J	sypHy	increased SV endocytosis
Marte et al., 2019	OE human G2019S (BAC)	cortical culture	mouse C57BL/6J	sypHy	increased SV exo/endocytosis
Marte et al., 2019	pharmacological inhibition (IN−1; PF)	glutamatergic system	mouse C57BL/6J	SypHy; microdyalisis; electrophysiology	decreased SV exocytosis
Matikainen-Ankney et al., 2016	KI G2019S	corticostriatal slices	mouse C57BL/6NTac	electrophysiology	increased sEPSC frequency due to glutamatergic afferents
Matikainen-Ankney et al., 2016	KI kinase dead	corticostriatal slices	mouse C57BL/6NTac	electrophysiology	no alteration in sEPSC
Matta et al., 2012	KO	NMJs	drosophila	FM-dye; electrophysiology;TEM	decreased SV endocytosis
Melrose et al., 2010	OE human wild-type (BAC, endogenous promoter)	dopaminergic system	Mouse FVB/*N*	microdialysis	decreased basal DA
Melrose et al., 2010	OE human G2019S (BAC)	dopaminergic system	Mouse FVB/*N*	microdialysis	decreased basal DA
Mercatelli et al., 2019	KO	striatal synaptosome	mouse C57BL/6J	microdialysis	increased evoked DA release
Mercatelli et al., 2019	KI G2019S	striatal synaptosome	mouse C57BL/6J	microdialysis	no alteration in DA release
Mercatelli et al., 2019	KI Kinase dead	striatal synaptosome	mouse C57BL/6J	microdialysis	increased evoked DA release
Mercatelli et al., 2019	pharmacological inhibition (GSK)	striatal synaptosome	mouse C57BL/6J	microdialysis	decreased evoked DA release; increased evoked Glut release;
Mercatelli et al., 2019	pharmacological inhibition (GSK)	cortical synaptosome	mouse C57BL/6J	microdialysis	decreased evoked Glut release
Nguyen & Krainc, 2018	KI R1441C	iDA	human	TEM	reduced SV number
Pan et al., 2017	OE murine wild-type (BAC, endogenous promoter)	midbrain culture	mouse C57BL/6J	sypHy	no alteration in SV exo-endo
Pan et al., 2017	OE murine G2019S (BAC)	midbrain culture	mouse C57BL/6J	sypHy	reduced SV endocytosis
Pan et al., 2017	OE murine wild-type (BAC, endogenous promoter)	cortical culture	mouse C57BL/6J	sypHy	no alteration in SV exo-endo
Pan et al., 2017	OE murine G2019S (BAC)	cortical culture	mouse C57BL/6J	sypHy	increased SV exocytosis
Parisiadou et al., 2014	KO	Cortico-striatal slices (SPN)	mouse C57BL/6J	electrophysiology	reduced mEPSC frequency; no PPF
Piccoli et al., 2011	Knock-down (acute)	cortical culture	mouse C57BL/6J	Anti-synaptotagmin uptake assay; electrophysiology;TEM	Increased SV fusion; paired-pulse depression

(Continues)

TABLE 1 (Continued)

Paper	LRRK2 status	Neuron/tissue	Organism	Technique	Outcome
Plowey et al., 2014	OE human wild-type (CMV promoter)	cortical culture	rat Sprague–Dawley	electrophysiology	no alteration in mEPSC
Plowey et al., 2014	OE R1441C	cortical culture	rat Sprague–Dawley	electrophysiology	increased mEPSC frequency
Plowey et al., 2014	OE human G2019S (CMV)	cortical culture	rat Sprague–Dawley	electrophysiology	increased mEPSC frequency
Qin et al., 2017	KO	striatal slices	Mouse FVB/*N*	fast scan cyclic voltammetry; electrophysiology	no alteration in DA release
Qin et al., 2017	pharmacological inhibition (GSK; GNE; IN–1)	striatal slices	Mouse FVB/*N*	fast scan cyclic voltammetry; electrophysiology	no alteration in DA release
Sanchez et al., 2014	OE R1441G (BAC)	striatal slices	Mouse FVB/*N*	fast scan cyclic voltammetry	no alteration in DA release
Shin et al., 2008	OE human G2019S (CMV)	hippocampal cultures	rat Sprague–Dawley	SypHy; FM-dye	decreased SV endocytosis
Shin et al., 2008	Knock-down (acute)	hippocampal cultures	rat	SypHy; FM-dye	impairment of endocytosis
Sloan et al., 2016	OE human wild-type (BAC, endogenous promoter)	striatum	rat Sprague–Dawley	fast scan cyclic voltammetry	decreased evoked DA release
Sloan et al., 2016	OE human G2019S (BAC, endogenous promoter)	striatum	rat Sprague–Dawley	fast scan cyclic voltammetry	decreased evoked DA release
Sloan et al., 2016	OE human R1441C (BAC, endogenous promoter)	striatum	rat Sprague–Dawley	fast scan cyclic voltammetry	decreased evoked DA release
Tong et al., 2009	KI R1441C	chromaffin cell	mouse B6/129	electrophysiology	decreased evoked DA release; increased evoked Glut release;
Tozzi et al., 2018	KI G2019S	corticostriatal slices	mouse C57BL/6J	electrophysiology	decreased D2 mediated sEPSC
Tozzi et al., 2018	KI Kinase dead	corticostriatal slices	mouse C57BL/6J	electrophysiology	no alteration in EPSC
Tozzi et al., 2018	KO	corticostriatal slices	mouse C57BL/6J	electrophysiology	no alteration in amplitude
Volta et al., 2015	OE human G2019S (BAC)	striatum	mouse C57BL/6J	microdialysis	no alteration in basal or evoked DA release; reduced response to D2 mediated inhibition
Volta et al., 2017	KI G2019S	striatal slices	mouse C57BL/6J	fast scan cyclic voltammetry; electrophysiology	increased sEPSC frequency; no alteration in DA release
Xiong et al., 2018	OE human G2019S (only in DA neurons)	DA neuron	mouse C57BL/6J	TEM	impairment of endocytosis
Yue et al., 2015	KI G2019S	striatum	mouse C57BL/6J	In vivo microdialysis	decreased evoked DA release
Yun et al., 2013	OE human G2019S (CMV)	hippocampal cultures	rat Sprague–Dawley	syphy	Decreased RRP size

Abbreviations: BAC, bacterial artificial chromosome; LTD, long-term depression; mEPSC, miniature excitatory post-synaptic current; PPR, paired-pulse ratio; sEPSC, spontaneous excitatory post-synaptic current; sypHy, synaptopHluorin assay; TEM, transmission electron microscopy.

down the rate of synaptic vesicle endocytosis and eventually caused an increase in FM-dye loading. However, different outcomes have been reported for glutamatergic terminals in other LOF models.

In detail, SV dynamics is normal in hippocampal neurons prepared from KO mice (Maas et al., 2017) or in cortical cultures obtained from mice expressing constitutively a LRRK2 shRNA (Giesert et al., 2017). Similarly, another pre-synaptic controlled parameter, namely the frequency of post-synaptic currents, is not affected in LRRK2 KO murine cortical culture (Beccano-Kelly, Kuhlmann, et al., 2014) or cortico-striatal slice (Beccano-Kelly, Volta, et al., 2014). It may well be that the impact of LRRK2 LOF gets compensated along with embryonic development while it becomes evident upon acute LRRK2 manipulation in post-natal models. Lastly, the possibility of off-target effects of shRNA strategies has to be taken into account.

Studies focusing on other neuronal populations appear to provide a more robust outcome. LRRK2 down-regulation correlates with abnormal endocytic vesicles, such as clathrin-coated endocytic intermediates in rat striatum (Arranz et al., 2015). SypHy assay revealed that LRRK2 KO delayed the endocytosis of recycling pool SV in striatal cultures prepared from rats (Arranz et al., 2015). Excitatory post-synaptic current (EPSC) frequency resulted in impaired striatal cultures prepared from LRRK2 KO mice (Parisiadou et al., 2014). Similarly, imaging studies showed that SV endocytosis is deficient in striatal culture prepared from LRRK2 KO mice (Maas et al., 2017) or rats (Arranz et al., 2015). GABAergic cells encompass about 90% of striatal neurons. These findings open the possibility that LRRK2 sustains SV recycling in a synapse-specific manner.

However, neurotransmitter release does not seem to be particularly sensitive to LRRK2 LOF.

LRRK2 KO does not affect basal and evoked dopamine (DA) release as judged by in vivo microdialysis (Creed et al., 2019; Hinkle et al., 2012) or fast-scan cyclic voltammetry (FSCV) (Qin et al., 2017).

Similarly, basal and evoked glutamate release resulted being normal in isolated synaptosome prepared from LRRK2 KO mice (Mercatelli et al., 2019).

It has to be mentioned that the very same experimental setting revealed an increased DA release in LRRK2 KO mice (Mercatelli et al., 2019). Isolated synaptosome constitutes indeed a powerful tool to dissect potential synapse-specific effect. However, DA transmission in basal ganglia is finely tuned by a complex inter-neuronal cross-talk: approaches such as in vivo microdialysis or FSCV may catch the final outcome of the entire circuitry.

We can conclude that LRRK2 down-regulation does not have a dramatic impact on the amount of neurotransmitter released.

Still, some reports claim that LRRK2 LOF may affect SV exocytosis at the glutamatergic terminal. In particular, a functional link between LRRK2 and RRP SV has been proposed. Paired-pulse protocol and bath-sucrose pulse are convenient methods to engage SV belonging to the RRP. siRNA-mediated LRRK2 ablation mimics sucrose application in terms of recruitment of SV and affects paired-pulse plasticity in murine cortical cultures (Piccoli et al., 2011). Accordingly, sucrose fails to further increase post-synaptic current frequency in LRRK2 KO murine hippocampal cultures (Arranz et al., 2015). Finally, LRRK2 silencing increased the fusion of docked SV in a heterologous cellular system (Carrion et al., 2017). Thus, reduced LRRK2 protein level may stimulate exocytosis limited to the RRP. Such a subtle event may escape the detection limit of many experimental approaches.

5 | THE IMPACT OF LRRK2 OVER-EXPRESSION AT THE PRE-SYNAPTIC SITE

Among the first models generated to investigate LRRK2 function, bacterial artificial chromosome (BAC) over-expressing animals have been the object of many studies. However, BAC models have a considerable caveat. BAC strategy implies the non-physiological expression of human/murine mutant protein on the top of endogenously expressed rodent LRRK2.

BAC-mediated over-expression (OE) of human (Beccano-Kelly, Volta, et al., 2014) or murine (Pan et al., 2017) wild-type LRRK2 did not alter synaptic activity in striatal medium spiny (MSN) or cortical neurons. A similar outcome was reported in rat cortical neurons upon LRRK2 acute OE (Plowey et al., 2014).

Instead, the dopaminergic terminal seems to suffer LRRK2 OE. BAC-mediated OE of human wild-type LRRK2 resulted in decreased basal DA release in mice (Beccano-Kelly, Volta, et al., 2014; Melrose et al., 2010) and decrease evoked DA release in rats (Sloan et al., 2016). Conversely, the introduction of murine LRRK2 locus via BAC (Li et al., 2010) or the selective expression of human LRRK2 in DA neurons (Liu et al., 2015) brought to an elevated evoked release of dopamine in mice.

It has been noted that the expression of LRRK2 protein from mouse BAC constructs closely mimics endogenous LRRK2 distribution in the mouse brain. Instead, human BAC constructs drive LRRK2 expression in additional tissues, such as the hippocampus (Melrose et al., 2010). The different tissue expression may account for the divergent outcome on DA release reported in murine versus human BAC rodent models.

6 | THE HYPO- AND HYPER-ACTIVATION OF LRRK2 AT THE SYNAPTIC SITE

We contributed to describing LRRK2 as a critical scaffolding protein at the pre-synaptic site, modulating SV dynamics via protein interaction. The ongoing development of (ever more) specific LRRK2 kinase inhibitors has helped to dissect the impact of its enzymatic activity at the pre-synaptic site. Acute treatment with IN-1 (Cirnaru et al., 2014), PF-06447475 (Marte et al., 2019) severely impairs pre-synaptic activity in murine cortical cultures or hippocampal slices in terms of EPSC frequency. Accordingly, GSK2578215A (Mercatelli et al., 2019), IN-1, and PF-06447475 (Marte et al., 2019), reduce evoked glutamate release from isolated cortical synaptosomes. This outcome has been reported also in a G2019S transgenic mouse line: LRRK2 pharmacological inhibition

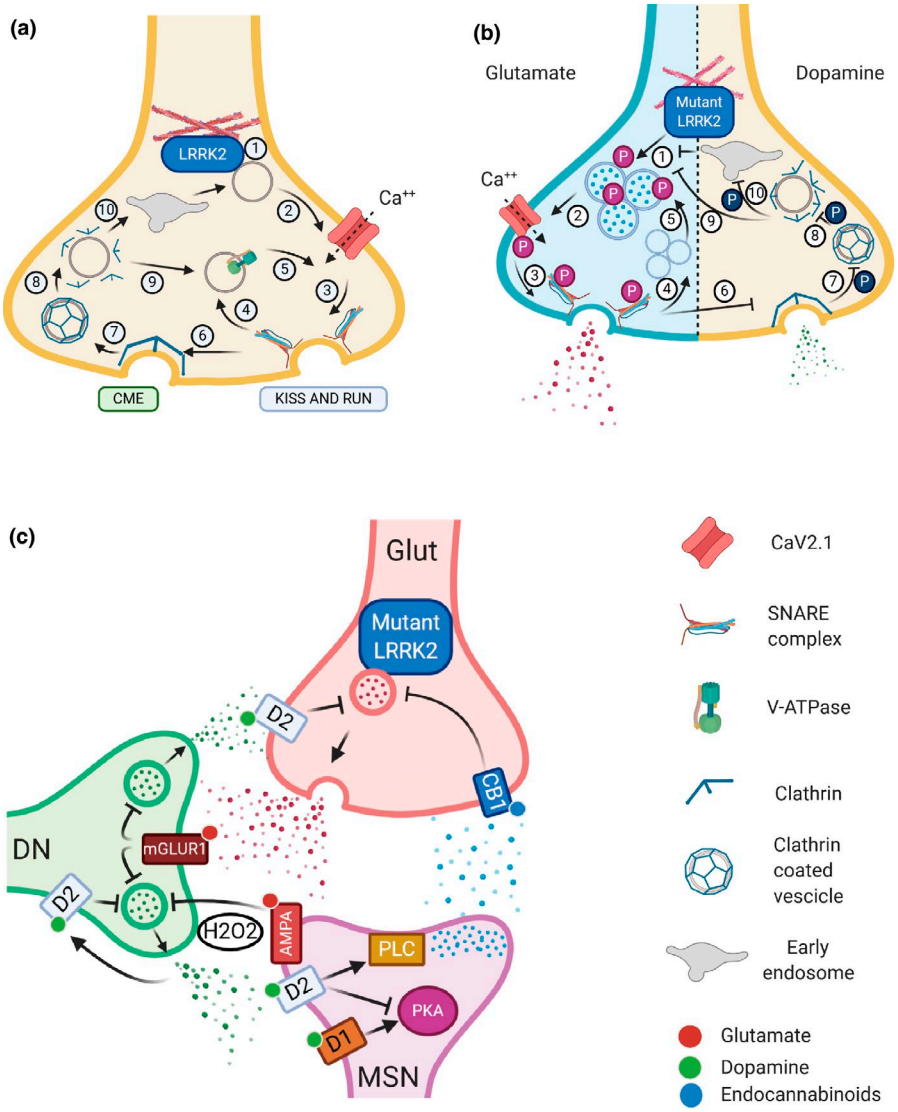

FIGURE 1 (a) Physiological role at the pre-synaptic bouton. LRRK2 is well-positioned to influence the entire SV cycle. (1) LRRK2 interacts with actin and synapsin I, which tether SV belonging to the reserve pool. (2) LRRK2 facilitates inward Ca^{2+} current via CaV2.1. (3) Local Ca^{2+} increase drives the assembly of the SNARE complex that eventually triggers SV fusion. LRRK2 binds SNAP25, syntaxin, and the associated proteins synaptotagmin and snapin. (4) Upon SV fusion, NSF, a well-characterized LRRK2 interactor, allows SNARE complex disassembly. (5) In the kiss-and-run model, SV do not entirely fuse with pre-synaptic membrane, detach from the membrane, and are acidified by the V-ATPase proton pump before re-entering the cycle. (6) In case SV completely fuse to the active zone membrane, clathrin-mediated endocytosis (CME) intervenes to recycle SV lipids and proteins. (7) Upon clathrin deposition, endophilinA promotes membrane invagination. The GTPase dynamin is required for the fission of endocytic membrane vesicle. (8) Auxillin and synaptojanin facilitate clathrin uncoating. LRRK2 interacts with endophilin, dynamin, auxillin, and synaptojanin. (9) SV are now ready to re-enter the cycle. (10) Rab5 drives SV fusion with early endosome, in a pathway suggested being crucial to maintaining RRP. LRRK2 binds and phosphorylates Rab5. (b) Impact of LRRK2 mutation. (1) LRRK2 phosphorylates synapsin I, thus promoting SV mobilization from the recycling pool. (2) LRRK2 G2019S stimulates inward Ca^{2+} current in a kinase-dependent manner. (3) Increased local Ca^{2+} facilitates SV fusion. (4) Upon LRRK2 phosphorylation, NSF catalyzes more efficiently SNARE disassembly. (5) SV recycle to be ready for the next round of fusion. At the glutamatergic terminal, LRRK2 mutations increase exocytosis. (6) LRRK2 kinase activity impacts on CME. Upon clathrin deposition, LRRK2 phosphorylation perturbs (7) vesicle budding acting on endophilinA and (8) uncoating acting on auxillin and synaptojanin. (9) SV uncoating is mandatory for re-entering the cycle. (10) Rab5 favors the fusion with the endosome of SV retrieved via clathrin-dependent or -independent mechanisms. LRRK2 phosphorylation prevents Rabs binding to the membrane. In the DA bouton, LRRK2 hyper-activation hampers SV clathrin-mediated endocytosis. (c) LRRK2 within basal ganglia. Striatal GABAergic MSN neurons receive glutamatergic stimulation from layer V cortical pyramidal neurons and modulatory dopaminergic input from substantia nigra (SN) pars compacta DA neurons. MSN neurons express either D1 (stimulatory, direct pathway) or D2 (inhibitory, indirect pathway) DA receptor. D2 receptors are also present at glutamatergic pre-synaptic terminals and dampen glutamate release. MSN within the indirect pathway produce and release endocannabinoids via PLC. Endocannabinoids inhibit pre-synaptically glutamatergic afferents via the CB1 receptor. An increased striatal glutamatergic release characterizes G2019S models. Such glutamatergic tone may inhibit DA release directly via mGluR1 localized on DA terminals or indirectly via AMPAR-mediated production of H_2O_2 in MSN. However, G2019S mice show a strong endocannabinoids-driven glutamatergic LTD. These findings open the possibility that G2019S mutation strikes the direct and indirect pathway differently

hampered exocytosis only in cortical neurons (Pan et al., 2017). Acute LRRK2 inhibition increased (Mercatelli et al., 2019) or left unaltered (Qin et al., 2017) DA release. Therefore, the suppression of neurotransmitter release appears to be quite selective for the glutamatergic synapses. The cell-specific expression of LRRK2 may explain this difference.

Similarly, the genetic ablation of LRRK2 kinase activity resulted in equal (Tozzi et al., 2018) or even increased (Mercatelli et al., 2019) evoked DA release. However, it has to be mentioned that in the models presented above, MSN electrophysiological properties were almost normal, suggesting no major defects in the excitatory cortical afferent upon LRRK2 genetic inactivation (Matikainen-Ankney et al., 2016; Tozzi et al., 2018). The different outcomes upon acute versus constitutive LRRK2 kinase inhibition may be because of potential compensatory phenomena or off-target effects upon prolonged incubation with kinase inhibitors.

Given the pathological relevance of LRRK2 mutations, a colossal effort has been spent to investigate their functional consequence at the synaptic site. Again, different outcomes have been reported in each specific neuron. BAC-driven expression of human LRRK2 G2019S boosts glutamatergic neurons, as witnessed by increased SV dynamics in murine cortical culture (Belluzzi et al., 2016; Marte et al., 2019), higher frequency of post-synaptic currents in rat cortical cultures (Plowey et al., 2014) and increased glutamate release in the rat striatum (Sloan et al., 2016). Conversely, human LRRK2 G2019S OE reduces SV number (Xiong et al., 2018) and basal (Melrose et al., 2010) or evoked (Chou et al., 2014; Liu et al., 2015; Sloan et al., 2016) DA release in the dopaminergic system. Similarly, murine LRRK2 G2019S over-expression via BAC increased SV exocytosis in cortical cultures while it reduced SV endocytosis in DA neurons (Pan et al., 2017) as well as DA release (Li et al., 2010). These reports strongly suggest that BAC-driven LRRK2 G2019S expression increases glutamatergic neuron activity while it depresses DA release.

As mentioned above, the OE of human wild-type LRRK2 per se was sufficient to impair basal DA release in mice (Beccano-Kelly, Volta, et al., 2014; Melrose et al., 2010) and decreased evoked DA release in rats (Sloan et al., 2016). LRRK2 is barely detectable in the substantia nigra in rodents (Giesert et al., 2013; West et al., 2014). Upon over-expression, it might instead reach the critical level necessary to interfere with DA release. These observations impair the physiological interpretation of results coming from BAC models.

A knock-in (KI) model represents the ultimate resource to gain insights into the synaptic impact of LRRK2 G2019S mutation. Complementary approaches confirmed that G2019S mutation stimulates glutamatergic neurons, as witnessed by increased EPSC frequency (Beccano-Kelly, Kuhlmann, et al., 2014) or enhanced exocytosis (Pan et al., 2017) in cortical cultures and increased glutamatergic transmission in striatal slices (Matikainen-Ankney et al., 2016; Volta et al., 2017). Instead, no major alteration has been reported in DA release (Mercatelli et al., 2019; Volta et al., 2017). Therefore, the DA phenotype described in BAC LRRK2 G2019S models may depend more on the excessive LRRK2 protein level than the G2019S mutation itself. Still, Pan et al. described a specific impairment of endocytosis in G2019S KI midbrain neuronal cultures (Pan et al., 2017). Interestingly, rodent KI models of R1441C mutation demonstrate a decreased evoked DA release and an increased evoked glutamate release (Tong et al., 2009) as well as increased frequency of cortical EPSC (Plowey et al., 2014). Finally, Yue et al. found an impairment of DA release in 12- but not in 6-month-old G2019S KI mice, despite a normal TH^-positive neuron count (Yue et al., 2015), suggesting the relevance of aging.

7 | A MODEL OF LRRK2 AT THE SYNAPTIC SITE

Altogether the reports discussed so far depict an integrated and synapse-specific role for LRRK2 at the pre-synaptic bouton.

Identifying LRRK2 substrates may help to decipher the connection between its kinase activity and the bouton-specific SV-dynamics. LRRK2-mediated phosphorylation of synapsin I abolishes its binding with actin (Marte et al., 2019). Furthermore, the heterologous expression of G2019S LRRK2 increases Ca^{2+} inward current via pre-synaptic CaV2.1 channel (Bedford et al., 2016). Eventually, biochemical evidence report that LRRK2 phosphorylates NSF on T645, increasing its ATPase activity and, eventually, its capability to dissociate the SNARE complex (Belluzzi et al., 2016), that is, the first step of SV recycling. Altogether, these in vitro data may link LRRK2 kinase activity with the increase of exocytosis.

Recent evidence attributes a crucial role to LRRK2 in clathrin-dependent mechanisms (Heaton et al., 2020). Auxilin, endophilin, and synaptojanin 1 have been proposed as LRRK2 targets and might provide a mechanistic link between LRRK2 and endocytosis. LRRK2 phosphorylation of auxilin at Ser627 abolishes auxilin association with clathrin and eventually disrupts SVE (Nguyen & Krainc, 2018). Recruitment of endophilin to clathrin coated pit is crucial for SV uncoating. Phosphorylation of the Endophilin A BAR domain by LRRK2 hampers its ability to dissociate from membranes and impairs SV endocytosis (Matta et al., 2012). In vitro, LRRK2 phosphorylates synaptojanin 1 in two sites, T1131, and S1142 within the C-terminal proline-rich domain, crucial for the interaction with endophilin A (Islam et al., 2016). Therefore, LRRK2-dependent phosphorylation of synaptojanin-1 may interfere with the binding to endophilin, and thus negatively influences endophilin-dependent endocytosis. Finally, upon phosphorylation, Rab proteins lose their ability to bind upstream and downstream proteins, get trapped on intracellular membranes, and hamper vesicle endocytosis [reviewed in (Pfeffer, 2018)].

Thus, an evident paradox exists: while in vitro data show that LRRK2 phosphorylation stimulates SNARE disassembly via NSF, other experimental evidence reports that it impairs the subsequent clathrin-mediated endocytosis. The mechanistic description of endocytosis may help to solve this issue.

After the necessary disassembly of SNARE complex catalyzed by NSF, SV recycling can occur through clathrin-dependent

and independent mechanisms [reviewed in (Mayor et al., 2014; Milosevic, 2018)]. Stimuli that are just sufficient to deplete the RRP typically imply a fast clathrin-independent retrieval, while stronger stimulation recruits the larger recycling pool and requires the slow clathrin-dependent mechanism. If this holds, LRRK2 may have a different impact depending on the activity taking place at the given terminal and, consequently, on the specific SV pool mobilized: it boosts basal release while undermining any high-frequency events that require clathrin-mediated endocytosis.

Still, it is difficult to understand why LRRK2 kinase activity plays opposing roles at glutamatergic versus dopaminergic terminal. Mutations in auxilin (Edvardson et al., 2012; Köroğlu et al., 2013) and synaptojanin 1 (Quadri et al., 2013) have been recognized as causative in a rare form of familial PD. These findings enlighten the physiological relevance of clathrin-mediated endocytosis in the DA system. Indeed, DA neurons are spontaneously active with firing patterns that range from regular pacemaker rhythm to high-frequency burst firing (Bunney et al., 1991). Therefore, it is tempting to speculate that DA neurons rely on clathrin-mediated endocytosis to sustain their activity. Furthermore, glutamatergic and dopaminergic pre-synaptic machinery differs at the molecular level (Liu et al., 2018). For example, synapsin I, II, and III are differentially expressed in each terminal, with each of them playing a specific role in glutamate or GABA and DA release, respectively (Gitler et al., 2004; Kile et al., 2010).

Therefore, one intriguing hypothesis is that LRRK2 supports exocytosis at the glutamatergic terminal. If confirmed, the LRRK2-driven phosphorylation of NSF, synapsin I, and CaV2.1 may sustain this phenomenon. Instead, at DA terminal, LRRK2 might impair clathrin-mediated endocytosis acting on Rabs or on other suggested targets such as endophilinA, auxilin, and synaptojanin 1.

Finally, locating LRRK2 within the circuitry acting in basal ganglia may provide further hints. The striatum is composed essentially by GABAergic neurons decorated by spines (the MSN) that receive modulatory dopaminergic input from the SN pars compacta and a massive excitatory signal from cortical pyramidal neurons. Striatal MSN neurons express either D1 (stimulatory, direct pathway) or D2 (inhibitory, indirect pathway) DA receptor. Anatomical evidence indicates that D2Rs are present at glutamatergic pre-synaptic terminals in the striatum (Wang & Pickel, 2002), where they dampen glutamate release (Hsu et al., 1995). In turn, glutamate inhibits DA release indirectly via AMPAR-mediated production of H_2O_2 in MSN and directly via mGluR1 localized on DA terminals [reviewed in (Zhang & Sulzer, 2012). Finally, MSN within the indirect pathway produce and release endocannabinoids, such as anandamide, in response to simultaneous depolarization and D2 receptor activation. Endocannabinoids activate CB1 receptors on the glutamatergic pre-synaptic terminal and eventually result in a long-lasting decrease in glutamate release [reviewed in (Lovinger & Mathur, 2012)].

G2019S models demonstrate an increased glutamatergic activity together with an impaired DA release. Indeed, glutamate and dopamine release are tightly interconnected: G2019S mutation may dampen DA release acting from the glutamatergic afferents. Evoking a role for LRRK2 in cortico-striatal plasticity may also conciliate the inconsistent results regarding LRRK2 expression at the DA terminal; LRRK2 G2019S could reduce DA release even if not expressed in DA neurons themselves.

Intriguingly, in G2019S KI mice, Tozzi et al. found a strong D2 receptor-dependent reduction of the glutamatergic activity via pre-synaptic CB1Rs (Tozzi et al., 2018). In particular, their work indicates that the G2019S LRRK2 mutation increases the sensitivity of the D2R/endocannabinoid pathway in MSNs and triggers glutamatergic long-term depression. Furthermore, preliminary experimental evidence shows that LRRK2 G2019S modulates D2R membrane expression influencing its intracellular trafficking (Rassu et al., 2017).

These findings open the possibility that G2019S mutation has a different impact on the direct and indirect pathway. In particular, the retrograde D2R/endocannabinoids pathway may buffer the glutamatergic action on DA release in the indirect pathway. A recent work has enlightened the impact of LRRK2 R1441C and, to a lesser extent, G2019S mutations on the structure and function of the striatal spiny projection neurons synapses belonging to the direct pathway (Chen et al., 2020): clearly, a pathway-specific investigation of LRRK2 physiological and pathological action is needed.

8 | CONCLUDING REMARKS

Notwithstanding some experimental incongruence, there is full agreement that LRRK2 plays a key role at the pre-synaptic site and that LRRK2 pathological kinase activity may detrimentally affect basal ganglia functionality. Brain imaging studies showed alterations of the nigrostriatal system in asymptomatic G2019S carriers, such as an increased striatal dopamine turnover (Sossi et al., 2010), a reduced F-DOPA uptake (Gersel Stokholm *et al.* 2020), and a reorganization of corticostriatal circuits (Helmich et al., 2015; Vilas et al., 2015, 2016) in the absence of any overt degeneration. These observations suggest that LRRK2 affects cortico-striatal plasticity early in the pathological progression and a recent study in a LRRK2 G2019S KI mouse model supports this hypothesis (Guevara et al., 2020). DAT imaging indicates a milder phenotype in G2019 LRRK2-PD patients (Simuni et al., 2020). Indeed, further studies are needed to elucidate how LRRK2 mutations eventually bring to PD. But the strong impact of the G2019S mutation on the corticostriatal afferents leaves open the possibility that LRRK2 causes PD starting from the glutamatergic site.

ACKNOWLEDGEMENTS

The authors thank Dr. Samine Jessica Isaac and Martina Sevegnani for their careful reading of the manuscript. G.P. received support by Fondazione Telethon (grant TDPG00514TA), MIUR (PRIN-2017ENN4FY), and Fondazione Cariplo (project 2019-3415). F.P. received support by Fondazione Caritro (project 2019.0230). The authors declare that there is no conflict of interest.

ORCID

Giovanni Piccoli https://orcid.org/0000-0001-5262-7903

REFERENCES

Arranz, A. M., Delbroek, L., Van Kolen, K., Guimarães, M. R., Mandemakers, W., Daneels, G., Matta, S., Calafate, S., Shaban, H., Baatsen, P., & De Bock, P. J. (2015). LRRK2 functions in synaptic vesicle endocytosis through a kinase-dependent mechanism. *Journal of Cell Science*, *128*, 541–552. https://doi.org/10.1242/jcs.158196

Athanasopoulos, P. S., Heumann, R., & Kortholt, A. (2018). The role of (auto)-phosphorylation in the complex activation mechanism of LRRK2. *Biological Chemistry*, *399*, 643–647.

Beccano-Kelly, D. A., Kuhlmann, N., Tatarnikov, I., Volta, M., Munsie, L. N., Chou, P., Cao, L.-P., Han, H., Tapia, L., Farrer, M. J., & Milnerwood, A. J. (2014). Synaptic function is modulated by LRRK2 and glutamate release is increased in cortical neurons of G2019S LRRK2 knock-in mice. *Frontiers in Cellular Neuroscience*, *8*, 301.

Beccano-Kelly, D. A., Volta, M., Munsie, L. N., Paschall, S. A., Tatarnikov, I., Co, K., Chou, P., Cao, L. P., Bergeron, S., Mitchell, E., & Han, H. (2014). LRRK2 overexpression alters glutamatergic presynaptic plasticity, striatal dopamine tone, postsynaptic signal transduction, motor activity and memory. *Human Molecular Genetics*, *24*, 1336–1349.

Bedford, C., Sears, C., Perez-Carrion, M., Piccoli, G., & Condliffe, S. B. (2016). LRRK2 regulates voltage-gated calcium channel function. *Frontiers in Molecular Neuroscience*, *9*, 35.

Belluzzi, E., Gonnelli, A., Cirnaru, M.-D., Marte, A., Plotegher, N., Russo, I., Civiero, L., Cogo, S., Carrion, M. P., Franchin, C., & Arrigoni, G. (2016). LRRK2 phosphorylates pre-synaptic N-ethylmaleimide sensitive fusion (NSF) protein enhancing its ATPase activity and SNARE complex disassembling rate. *Molecular Neurodegeneration*, *11*, 1.

Berger, Z., Smith, K. A., & Lavoie, M. J. (2010). Membrane localization of LRRK2 is associated with increased formation of the highly active LRRK2 dimer and changes in its phosphorylation. *Biochemistry*, *49*, 5511–5523.

Berwick, D. C., Heaton, G. R., Azeggagh, S., & Harvey, K. (2019). LRRK2 Biology from structure to dysfunction: Research progresses, but the themes remain the same. *Molecular Neurodegeneration*, *14*, 49.

Biskup, S., Moore, D. J., Celsi, F., Higashi, S., West, A. B., Andrabi, S. A., Kurkinen, K., Yu, S. W., Savitt, J. M., Waldvogel, H. J., & Faull, R. L. (2006). Localization of LRRK2 to membranous and vesicular structures in mammalian brain. *Annals of Neurology*, *60*, 557–569.

Blauwendraat, C., Reed, X., Kia, D. A., Gan-Or, Z., Lesage, S., Pihlstrøm, L., Guerreiro, R., Gibbs, J. R., Sabir, M., Ahmed, S., & Ding, J. (2018). Frequency of Loss of function variants in LRRK2 in Parkinson disease. *JAMA Neurology*, *75*, 1416–1422.

Bosgraaf, L., & Van Haastert, P. J. M. (2003). Roc, a Ras/GTPase domain in complex proteins. *Biochimica Et Biophysica Acta*, *1643*, 5–10.

Bunney, B. S., Chiodo, L. A., & Grace, A. A. (1991). Midbrain dopamine system electrophysiological functioning: A review and new hypothesis. *Synapse (New York, N. Y.)*, *9*, 79–94. https://doi.org/10.1002/syn.890090202

Cabezudo, D., Baekelandt, V., & Lobbestael, E. (2020). Multiple-hit hypothesis in Parkinson's disease: LRRK2 and inflammation. *Frontiers in Neuroscience*, *14*, 376. https://doi.org/10.3389/fnins.2020.00376

Carrion, M. D. P., Marsicano, S., Daniele, F., Marte, A., Pischedda, F., Di Cairano, E., Piovesana, E., von Zweydorf, F., Kremmer, E., Gloeckner, C. J., Onofri, F., Perego, C., & Piccoli, G. (2017). The LRRK2 G2385R variant is a partial loss-of-function mutation that affects synaptic vesicle trafficking through altered protein interactions. *Scientific Reports*, *7*, 5377. https://doi.org/10.1038/s41598-017-05760-9

Cazares, V. A., Njus, M. M., Manly, A., Saldate, J. J., Subramani, A., Ben-Simon, Y., Sutton, M. A., Ashery, U., & Stuenkel, E. L. (2016). Dynamic partitioning of synaptic vesicle pools by the SNARE-binding protein tomosyn. *Journal of Neuroscience*, *36*, 11208–11222. https://doi.org/10.1523/JNEUROSCI.1297-16.2016

Cesca, F., Baldelli, P., Valtorta, F., & Benfenati, F. (2010). The synapsins: Key actors of synapse function and plasticity. *Progress in Neurobiology*, *91*, 313–348. https://doi.org/10.1016/j.pneurobio.2010.04.006

Chanaday, N. L., Cousin, M. A., Milosevic, I., Watanabe, S., & Morgan, J. R. (2019). The synaptic vesicle cycle revisited: new insights into the modes and mechanisms. *Journal of Neuroscience*, *39*, 8209–8216. https://doi.org/10.1523/JNEUROSCI.1158-19.2019

Chen, C., Soto, G., Dumrongprechachan, V., Bannon, N., Kang, S., Kozorovitskiy, Y., & Parisiadou, L. (2020). Pathway-specific dysregulation of striatal excitatory synapses by LRRK2 mutations. *Elife*, *9*, e58997. https://doi.org/10.7554/eLife.58997

Chou, J.-S., Chen, C.-Y., Chen, Y.-L., Weng, Y.-H., Yeh, T.-H., Lu, C.-S., Chang, Y.-M., & Wang, H.-L. (2014). (G2019S) LRRK2 causes early-phase dysfunction of SNpc dopaminergic neurons and impairment of corticostriatal long-term depression in the PD transgenic mouse. *Neurobiology of Diseases*, *68*, 190–199.

Cirnaru, M. D., Marte, A., Belluzzi, E., Russo, I., Gabrielli, M., Longo, F., Arcuri, L., Murru, L., Bubacco, L., Matteoli, M., Fedele, E., Sala, C., Passafaro, M., Morari, M., Greggio, E., Onofri, F., & Piccoli, G. (2014). LRRK2 kinase activity regulates synaptic vesicle trafficking and neurotransmitter release through modulation of LRRK2 macro-molecular complex. *Frontiers in Molecular Neuroscience*, *7*, 49. https://doi.org/10.3389/fnmol.2014.00049

Cousin, M. A. (2009). Activity-dependent bulk synaptic vesicle endocytosis–a fast, high capacity membrane retrieval mechanism. *Molecular Neurobiology*, *39*, 185–189.

Creed, R. B., Menalled, L., Casey, B., Dave, K. D., Janssens, H. B., Veinbergs, I., van der Hart, M., Rassoulpour, A., & Goldberg, M. S. (2019). Basal and evoked neurotransmitter levels in Parkin, DJ-1, PINK1 and LRRK2 knockout rat striatum. *Neuroscience*, *409*, 169–179. https://doi.org/10.1016/j.neuroscience.2019.04.033

Darcy, K. J., Staras, K., Collinson, L. M., & Goda, Y. (2006). Constitutive sharing of recycling synaptic vesicles between presynaptic boutons. *Nature Neuroscience*, *9*, 315–321.

Denker, A., & Rizzoli, S. O. (2010). Synaptic vesicle pools: An update. *Frontiers in Synaptic Neuroscience*, *2*, 135. https://doi.org/10.3389/fnsyn.2010.00135

di Domenico, A., Carola, G., Calatayud, C., Pons-Espinal, M., Muñoz, J. P., Richaud-Patin, Y., Fernandez-Carasa, I., Gut, M., Faella, A., Parameswaran, J., Soriano, J., Ferrer, I., Tolosa, E., Zorzano, A., Cuervo, A. M., Raya, A., & Consiglio, A. (2019). Patient-specific iPSC-derived astrocytes contribute to non-cell-autonomous neurodegeneration in Parkinson's disease. *Stem Cell Reports*, *12*, 213–229. https://doi.org/10.1016/j.stemcr.2018.12.011

Dzamko, N. L. (2017). LRRK2 and the immune system. *Advances in Neurobiology*, *14*, 123–143.

Edvardson, S., Cinnamon, Y., Ta-Shma, A., Shaag, A., Yim, Y.-I., Zenvirt, S., Jalas, C., Lesage, S., Brice, A., Taraboulos, A., Kaestner, K. H., Greene, L. E., & Elpeleg, O. (2012). A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. *PLoS One*, *7*, e36458. https://doi.org/10.1371/journal.pone.0036458

Fdez, E., & Hilfiker, S. (2006). Vesicle pools and synapsins: New insights into old enigmas. *Brain Cell Biology*, *35*, 107–115. https://doi.org/10.1007/s11068-007-9013-4

Fischer von Mollard, G., Stahl, B., Walch-Solimena, C., Takei, K., Daniels, L., Khoklatchev, A., De Camilli, P., Südhof, T. C., & Jahn, R. (1994). Localization of Rab5 to synaptic vesicles identifies endosomal intermediate in synaptic vesicle recycling pathway. *European Journal of Cell Biology*, *65*, 319–326.

Funayama, M., Hasegawa, K., Kowa, H., Saito, M., Tsuji, S., & Obata, F. (2002). A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1. *Annals of Neurology*, *51*, 296–301.

Funayama, M., Hasegawa, K., Ohta, E., Kawashima, N., Komiyama, M., Kowa, H., Tsuji, S., & Obata, F. (2005). An LRRK2 mutation as a cause for the parkinsonism in the original PARK8 family. *Annals of Neurology*, *57*, 918–921.

Gandhi, P. N., Wang, X., Zhu, X., Chen, S. G., & Wilson-Delfosse, A. L. (2008). The Roc domain of leucine-rich repeat kinase 2 is sufficient for interaction with microtubules. *Journal of Neuroscience Research*, *86*, 1711–1720.

Gersel Stokholm, S. M., Garrido, A., Tolosa, E., Serradell, M., Iranzo, A., Østergaard, K., Borghammer, P., Moller, A., Parbo, P., Staer, K., & Brooks, D. J. (2020). Imaging dopamine function and microglia in asymptomatic LRRK2 mutation carriers. *Journal of Neurology.267*(8), 2296–2300. https://doi.org/10.1007/s00415-020-09830-3

Giasson, B. I., Covy, J. P., Bonini, N. M., Hurtig, H. I., Farrer, M. J., Trojanowski, J. Q., & Van Deerlin, V. M. (2006). Biochemical and pathological characterization of Lrrk2. *Annals of Neurology*, *59*, 315–322.

Giesert, F., Glasl, L., Zimprich, A., Ernst, L., Piccoli, G., Stautner, C., Zerle, J., Hölter, S. M., Vogt Weisenhorn, D., & Wurst, W. (2017). The pathogenic LRRK2 R1441C mutation induces specific deficits modeling the prodromal phase of Parkinson's disease in the mouse. *Neurobiology of Diseases*, *105*, 179–193.

Giesert, F., Hofmann, A., Bürger, A., Zerle, J., Kloos, K., Hafen, U., Ernst, L., Zhang, J., Vogt-Weisenhorn, D. M., & Wurst, W. (2013). Expression analysis of Lrrk1, Lrrk2 and Lrrk2 splice variants in mice. *PLoS One*, *8*, e63778.

Gilsbach, B. K., Eckert, M., & Gloeckner, C. J. (2018). Regulation of LRRK2: Insights from structural and biochemical analysis. *Biological Chemistry*, *399*, 637–642.

Gitler, D., Takagishi, Y., Feng, J., Ren, Y., Rodriguiz, R. M., Wetsel, W. C., Greengard, P., & Augustine, G. J. (2004). Different presynaptic roles of synapsins at excitatory and inhibitory synapses. *Journal of Neuroscience*, *24*, 11368–11380.

Gloeckner, C. J., Boldt, K., von Zweydorf, F., Helm, S., Wiesent, L., Sarioglu, H., & Ueffing, M. (2010). Phosphopeptide analysis reveals two discrete clusters of phosphorylation in the N-terminus and the Roc domain of the Parkinson-disease associated protein kinase LRRK2. *Journal of Proteome Research*, *9*, 1738–1745.

Gloeckner, C. J., Kinkl, N., Schumacher, A., Braun, R. J., O'Neill, E., Meitinger, T., Kolch, W., Prokisch, H., & Ueffing, M. (2006). The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity. *Human Molecular Genetics*, *15*, 223–232.

Greggio, E., Jain, S., Kingsbury, A., Bandopadhyay, R., Lewis, P., Kaganovich, A., van der Brug, M. P., Beilina, A., Blackinton, J., Thomas, K. J., Ahmad, R., Miller, D. W., Kesavapany, S., Singleton, A., Lees, A., Harvey, R. J., Harvey, K., & Cookson, M. R. (2006). Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiology of Diseases*, *23*, 329–341. https://doi.org/10.1016/j.nbd.2006.04.001

Guevara, C. A., Matikainen-Ankney, B. A., Kezunovic, N., LeClair, K., Conway, A. P., Menard, C., Flanigan, M. E., Pfau, M., Russo, S. J., Benson, D. L., & Huntley, G. W. (2020). LRRK2 mutation alters behavioral, synaptic, and nonsynaptic adaptations to acute social stress. *Journal of Neurophysiology*, *123*, 2382–2389. https://doi.org/10.1152/jn.00137.2020

Hatano, T., Kubo, S.-I., Imai, S., Maeda, M., Ishikawa, K., Mizuno, Y., & Hattori, N. (2007). Leucine-rich repeat kinase 2 associates with lipid rafts. *Human Molecular Genetics*, *16*, 678–690.

He, K., Marsland, R., Upadhyayula, S., Song, E., Dang, S., Capraro, B. R., Wang, W., Skillern, W., Gaudin, R., Ma, M., & Kirchhausen, T. (2017). Dynamics of phosphoinositide conversion in clathrin-mediated endocytic traffic. *Nature*, *552*, 410–414. https://doi.org/10.1038/nature25146

Heaton, G. R., Landeck, N., Mamais, A., Nalls, M. A., Nixon-Abell, J., Kumaran, R., Beilina, A. et al (2020). Sequential screening nominates the Parkinson's disease associated kinase LRRK2 as a regulator of Clathrin-mediated endocytosis. *Neurobiology of Diseases*, *141*, 104948.

Helmich, R. C., Thaler, A., van Nuenen, B. F. L., Gurevich, T., Mirelman, A., Marder, K. S., Bressman, S., Orr-Urtreger, A., Giladi, N., Bloem, B. R., & Toni, I. (2015). Reorganization of corticostriatal circuits in healthy G2019S LRRK2 carriers. *Neurology*, *84*, 399–406. https://doi.org/10.1212/WNL.0000000000001189

Herzig, M. C., Kolly, C., Persohn, E., Theil, D., Schweizer, T., Hafner, T., Stemmelen, C., Troxler, T. J., Schmid, P., Danner, S., & Schnell, C. R. (2011). LRRK2 protein levels are determined by kinase function and are crucial for kidney and lung homeostasis in mice. *Human Molecular Genetics*, *20*, 4209–4223.

Higashi, S., Moore, D. J., Colebrooke, R. E., Biskup, S., Dawson, V. L., Arai, H., Dawson, T. M., & Emson, P. C. (2007). Expression and localization of Parkinson's disease-associated leucine-rich repeat kinase 2 in the mouse brain. *Journal of Neurochemistry*, *100*, 368–381. https://doi.org/10.1111/j.1471-4159.2006.04246.x

Hinkle, K. M., Yue, M., Behrouz, B., Dächsel, J. C., Lincoln, S. J., Bowles, E. E., Beevers, J. E., Dugger, B., Winner, B., Prots, I., Kent, C. B., Nishioka, K., Lin, W.-L., Dickson, D. W., Janus, C. J., Farrer, M. J., & Melrose, H. L. (2012). LRRK2 knockout mice have an intact dopaminergic system but display alterations in exploratory and motor co-ordination behaviors. *Molecular Neurodegeneration*, *7*, 25. https://doi.org/10.1186/1750-1326-7-25

Ho, D. H., Jang, J., Joe, E.-H., Son, I., Seo, H., & Seol, W. (2016). G2385R and I2020T mutations increase LRRK2 GTPase activity. *BioMed Research International*, *2016*, 7917128.

Hook, P. W., McClymont, S. A., Cannon, G. H., Law, W. D., Morton, A. J., Goff, L. A., & McCallion, A. S. (2018). Single-cell RNA-Seq of mouse dopaminergic neurons informs candidate gene selection for sporadic Parkinson disease. *American Journal of Human Genetics*, *102*, 427–446.

Hoopmann, P., Punge, A., Barysch, S. V., Westphal, V., Bückers, J., Opazo, F., Bethani, I., Lauterbach, M. A., Hell, S. W., & Rizzoli, S. O. (2010). Endosomal sorting of readily releasable synaptic vesicles. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 19055–19060.

Hsu, K. S., Huang, C. C., Yang, C. H., & Gean, P. W. (1995). Presynaptic D2 dopaminergic receptors mediate inhibition of excitatory synaptic transmission in rat neostriatum. *Brain Research*, *690*, 264–268. https://doi.org/10.1016/0006-8993(95)00734-8

Hui, K. Y., Fernandez-Hernandez, H., Hu, J., Schaffner, A., Pankratz, N., Hsu, N.-Y., Chuang, L.-S., Chuang, L. S., Carmi, S., Villaverde, N., Li, X., & Rivas, M. (2018). Functional variants in the LRRK2 gene confer shared effects on risk for Crohn's disease and Parkinson's disease. *Science Translational Medicine*, *10*(423), eaai7795. https://doi.org/10.1126/scitranslmed.aai7795

Islam, M. S., Nolte, H., Jacob, W., Ziegler, A. B., Pütz, S., Grosjean, Y., Szczepanowska, K. et al (2016). Human R1441C LRRK2 regulates the synaptic vesicle proteome and phosphoproteome in a Drosophila model of Parkinson's disease. *Human Molecular Genetics*, *25*, 5365–5382.

Jeong, G. R., Jang, E.-H., Bae, J. R., Jun, S., Kang, H. C., Park, C.-H., Shin, J.-H., Yamamoto, Y., Tanaka-Yamamoto, K., Dawson, V. L., Dawson, T. M., Hur, E.-M., & Lee, B. D. (2018). Dysregulated phosphorylation of Rab GTPases by LRRK2 induces neurodegeneration. *Molecular Neurodegeneration*, *13*, 8. https://doi.org/10.1186/s13024-018-0240-1

Kalogeropulou, A. F., Zhao, J., Bolliger, M. F., Memou, A., Narasimha, S., Molitor, T. P., Wilson, W. H., Rideout, H. J., & Nichols, R. J. (2018). P62/SQSTM1 is a novel leucine-rich repeat kinase 2 (LRRK2) substrate that enhances neuronal toxicity. *The Biochemical Journal*, *475*, 1271–1293.

Kile, B. M., Guillot, T. S., Venton, B. J., Wetsel, W. C., Augustine, G. J., & Wightman, R. M. (2010). Synapsins differentially control dopamine and serotonin release. *Journal of Neuroscience*, *30*, 9762–9770. https://doi.org/10.1523/JNEUROSCI.2071-09.2010

Köroğlu, Ç., Baysal, L., Cetinkaya, M., Karasoy, H., & Tolun, A. (2013). DNAJC6 is responsible for juvenile Parkinsonism with phenotypic variability. *Parkinsonism & Related Disorders*, *19*, 320–324.

Li, X., Patel, J. C., Wang, J., Avshalumov, M. V., Nicholson, C., Buxbaum, J. D., Elder, G. A., Rice, M. E., & Yue, Z. (2010). Enhanced striatal dopamine transmission and motor performance with LRRK2 overexpression in mice is eliminated by familial Parkinson's disease mutation G2019S. *Journal of Neuroscience*, *30*, 1788–1797. https://doi.org/10.1523/JNEUROSCI.5604-09.2010

Liu, C., Kershberg, L., Wang, J., Schneeberger, S., & Kaeser, P. S. (2018). Dopamine secretion is mediated by sparse active zone-like release sites. *Cell*, *172*, 706–718.e15.

Liu, G., Sgobio, C., Gu, X., Sun, L., Lin, X., Yu, J., Parisiadou, L. et al (2015). Selective expression of Parkinson's disease-related Leucine-rich repeat kinase 2 G2019S missense mutation in midbrain dopaminergic neurons impairs dopamine release and dopaminergic gene expression. *Human Molecular Genetics*, *24*, 5299–5312.

Lobbestael, E., Zhao, J., Rudenko, I. N., Beylina, A., Gao, F., Wetter, J., Beullens, M. et al (2013). Identification of protein phosphatase 1 as a regulator of the LRRK2 phosphorylation cycle. *The Biochemical Journal*, *456*, 119–128.

Lovinger, D. M., & Mathur, B. N. (2012). Endocannabinoids in striatal plasticity. *Parkinsonism & Related Disorders*, *18*(Suppl 1), S132–134.

Maas, J. W. J., Yang, J., & Edwards, R. H. (2017). Endogenous leucine-rich repeat kinase 2 slows synaptic vesicle recycling in striatal neurons. *Frontiers in Synaptic Neuroscience*, *9*, 5.

Mamais, A., Chia, R., Beilina, A., Hauser, D. N., Hall, C., Lewis, P. A., Cookson, M. R., & Bandopadhyay, R. (2014). Arsenite stress down-regulates phosphorylation and 14-3-3 binding of leucine-rich repeat kinase 2 (LRRK2), promoting self-association and cellular redistribution. *Journal of Biological Chemistry*, *289*, 21386–21400.

Marte, A., Russo, I., Rebosio, C., Valente, P., Belluzzi, E., Pischedda, F., Montani, C., Lavarello, C., Petretto, A., Fedele, E., & Baldelli, P. (2019). LRRK2 phosphorylation on synapsin I regulates glutamate release at presynaptic sites. *Journal of Neurochemistry*, *150*, 264–281.

Martin, I., Kim, J. W., Lee, B. D., Kang, H. C., Xu, J.-C., Jia, H., Stankowski, J., Kim, M. S., Zhong, J., Kumar, M., & Andrabi, S. A. (2014). Ribosomal protein s15 phosphorylation mediates LRRK2 neurodegeneration in Parkinson's disease. *Cell*, *157*, 472–485.

Matikainen-Ankney, B. A., Kezunovic, N., Mesias, R. E., Tian, Y., Williams, F. M., Huntley, G. W., & Benson, D. L. (2016). Altered development of synapse structure and function in striatum caused by Parkinson's disease-linked LRRK2-G2019S mutation. *Journal of Neuroscience*, *36*, 7128–7141.

Matta, S., Van Kolen, K., da Cunha, R., van den Bogaart, G., Mandemakers, W., Miskiewicz, K., De Bock, P.-J., Morais, V. A., Vilain, S., Haddad, D., & Delbroek, L. (2012). LRRK2 controls an EndoA phosphorylation cycle in synaptic endocytosis. *Neuron*, *75*, 1008–1021.

Mayor, S., Parton, R. G., & Donaldson, J. G. (2014). Clathrin-independent pathways of endocytosis. *Cold Spring Harb Perspect Biol*, *6*. https://doi.org/10.1101/cshperspect.a016758

Meixner, A., Boldt, K., Van Troys, M., Askenazi, M., Gloeckner, C. J., Bauer, M., Marto, J. A., Ampe, C., Kinkl, N., & Ueffing, M. (2011). A QUICK screen for Lrrk2 interaction partners–leucine-rich repeat kinase 2 is involved in actin cytoskeleton dynamics. *Molecular & Cellular Proteomics: MCP*, *10*, M110.001172. https://doi.org/10.1074/mcp.M110.001172

Melrose, H. L., Dächsel, J. C., Behrouz, B., Lincoln, S. J., Yue, M., Hinkle, K. M., Kent, C. B., Korvatska, E., Taylor, J. P., Witten, L., & Liang, Y. Q. (2010). Impaired dopaminergic neurotransmission and microtubule-associated protein tau alterations in human LRRK2 transgenic mice. *Neurobiology of Diseases*, *40*, 503–517.

Mercatelli, D., Bolognesi, P., Frassineti, M., Pisanò, C. A., Longo, F., Shimshek, D. R., & Morari, M. (2019). Leucine-rich repeat kinase 2 (LRRK2) inhibitors differentially modulate glutamate release and Serine935 LRRK2 phosphorylation in striatal and cerebrocortical synaptosomes. *Pharmacol Res Perspect*, *7*, e00484.

Mills, R. D., Mulhern, T. D., Cheng, H.-C., & Culvenor, J. G. (2012). Analysis of LRRK2 accessory repeat domains: Prediction of repeat length, number and sites of Parkinson's disease mutations. *Biochemical Society Transactions*, *40*, 1086–1089. https://doi.org/10.1042/BST20120088

Milosevic, I. (2018). Revisiting the role of clathrin-mediated endoytosis in synaptic vesicle recycling. *Frontiers in Cellular Neuroscience*, *12*, 27. https://doi.org/10.3389/fncel.2018.00027

Moore, D. J., West, A. B., Dawson, V. L., & Dawson, T. M. (2005). Molecular pathophysiology of Parkinson's disease. *Annual Review of Neuroscience*, *28*, 57–87.

Muda, K., Bertinetti, D., Gesellchen, F., Hermann, J. S., von Zweydorf, F., Geerlof, A., Jacob, A., Ueffing, M., Gloeckner, C. J., & Herberg, F. W. (2014). Parkinson-related LRRK2 mutation R1441C/G/H impairs PKA phosphorylation of LRRK2 and disrupts its interaction with 14-3-3. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, E34–43. https://doi.org/10.1073/pnas.1312701111

Nguyen, A. P. T., & Moore, D. J. (2017). Understanding the GTPase Activity of LRRK2: Regulation, function, and neurotoxicity. *Advances in Neurobiology*, *14*, 71–88.

Nguyen, M., & Krainc, D. (2018). LRRK2 phosphorylation of auxilin mediates synaptic defects in dopaminergic neurons from patients with Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 5576–5581.

Nichols, R. J. (2017). LRRK2 phosphorylation. *Advances in Neurobiology*, *14*, 51–70.

Nichols, R. J., Dzamko, N., Morrice, N. A., Campbell, D. G., Deak, M., Ordureau, A., Macartney, T., Tong, Y., Shen, J., Prescott, A. R., & Alessi, D. R. (2010). 14-3-3 binding to LRRK2 is disrupted by multiple Parkinson's disease-associated mutations and regulates cytoplasmic localization. *Biochemical Journal*, *430*, 393–404. https://doi.org/10.1042/BJ20100483

Obeso, J. A., Stamelou, M., Goetz, C. G., Poewe, W., Lang, A. E., Weintraub, D., Burn, D., Halliday, G. M., Bezard, E., Przedborski, S., Lehericy, S., Brooks, D. J., Rothwell, J. C., Hallett, M., DeLong, M. R., Marras, C., Tanner, C. M., Ross, G. W., Langston, J. W., … Stoessl, A. J. (2017). Past, present, and future of Parkinson's disease: A special essay on the 200th Anniversary of the Shaking Palsy. *Movement Disorders*, *32*, 1264–1310. https://doi.org/10.1002/mds.27115

Paisán-Ruíz, C., Jain, S., Evans, E. W., Gilks, W. P., Simón, J., van der Brug, M., de Munain, A. L., Aparicio, S., Gil, A. M., Khan, N., Johnson, J., Martinez, J. R., Nicholl, D., Carrera, I. M., Peña, A. S., de Silva, R., Lees, A., Martí-Massó, J. F., Pérez-Tur, J., … Singleton, A. B. (2004). Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron*, *44*, 595–600. https://doi.org/10.1016/j.neuron.2004.10.023

Pan, P.-Y., Li, X., Wang, J., Powell, J., Wang, Q., Zhang, Y., Chen, Z., Wicinski, B., Hof, P., Ryan, T. A., & Yue, Z. (2017). Parkinson's disease-associated LRRK2 hyperactive kinase mutant disrupts synaptic vesicle trafficking in ventral midbrain neurons. *Journal of Neuroscience*, *37*, 11366–11376. https://doi.org/10.1523/JNEUROSCI.0964-17.2017

Parisiadou, L., Yu, J., Sgobio, C., Xie, C., Liu, G., Sun, L., Gu, X.-L., Lin, X., Crowley, N. A., Lovinger, D. M., & Cai, H. (2014). LRRK2 regulates synaptogenesis and dopamine receptor activation through modulation of PKA activity. *Nature Neuroscience*, *17*, 367–376.

Pfeffer, S. R. (2018). LRRK2 and Rab GTPases. *Biochemical Society Transactions*, *46*, 1707–1712.

Piccoli, G., Condliffe, S. B., Bauer, M., Giesert, F., Boldt, K., De Astis, S., Meixner, A., Sarioglu, H., Vogt-Weisenhorn, D. M., Wurst, W., Gloeckner, C. J., Matteoli, M., Sala, C., & Ueffing, M. (2011). LRRK2

controls synaptic vesicle storage and mobilization within the recycling pool. *Journal of Neuroscience*, *31*, 2225–2237. https://doi.org/10.1523/JNEUROSCI.3730-10.2011

Piccoli, G., Onofri, F., Cirnaru, M. D., Kaiser, C. J. O., Jagtap, P., Kastenmüller, A., Pischedda, F., Marte, A., Von Zweydorf, F., Vogt, A., & Giesert, F. (2014). LRRK2 binds to neuronal vesicles through protein interactions mediated by its C-terminal WD40 domain. *Molecular and Cellular Biology*, *34*, 2147–2161.

Plowey, E. D., Johnson, J. W., Steer, E., Zhu, W., Eisenberg, D. A., Valentino, N. M., Liu, Y.-J., & Chu, C. T. (2014). Mutant LRRK2 enhances glutamatergic synapse activity and evokes excitotoxic dendrite degeneration. *Biochimica Et Biophysica Acta*, *1842*, 1596–1603.

Porras, P., Duesbury, M., Fabregat, A., Ueffing, M., Orchard, S., Gloeckner, C. J., & Hermjakob, H. (2015). A visual review of the interactome of LRRK2: Using deep-curated molecular interaction data to represent biology. *Proteomics*, *15*, 1390–1404. https://doi.org/10.1002/pmic.201400390

Pungaliya, P. P., Bai, Y., Lipinski, K., Anand, V. S., Sen, S., Brown, E. L., Bates, B., Reinhart, P. H., West, A. B., Hirst, W. D., & Braithwaite, S. P. (2010). Identification and characterization of a leucine-rich repeat kinase 2 (LRRK2) consensus phosphorylation motif. *PLoS One*, *5*, e13672. https://doi.org/10.1371/journal.pone.0013672

Qin, Q., Zhi, L.-T., Li, X.-T., Yue, Z.-Y., Li, G.-Z., & Zhang, H. (2017). Effects of LRRK2 inhibitors on nigrostriatal dopaminergic neurotransmission. *CNS Neuroscience & Therapeutics*, *23*, 162–173. https://doi.org/10.1111/cns.12660

Quadri, M., Fang, M., Picillo, M., Olgiati, S., Breedveld, G. J., Graafland, J., Wu, B., Xu, F., Erro, R., Amboni, M., & Pappatà, S. (2013). Mutation in the SYNJ1 gene associated with autosomal recessive, early-onset Parkinsonism. *Human Mutation*, *34*, 1208–1215.

Rassu, M., Del Giudice, M. G., Sanna, S., Taymans, J. M., Morari, M., Brugnoli, A., Frassineti, M., Masala, A., Esposito, S., Galioto, M., Valle, C., Carri, M. T., Biosa, A., Greggio, E., Crosio, C., & Iaccarino, C. (2017). Role of LRRK2 in the regulation of dopamine receptor trafficking. *PLoS One*, *12*, e0179082. https://doi.org/10.1371/journal.pone.0179082

Rizzoli, S. O., & Betz, W. J. (2005). Synaptic vesicle pools. *Nature Reviews Neuroscience*, *6*, 57–69.

Saheki, Y., & De Camilli, P. (2012). Synaptic vesicle endocytosis. *Cold Spring Harbor Perspectives in Biology*, *4*, a005645. https://doi.org/10.1101/cshperspect.a005645

Sanchez G., Varaschin R. K., Büeler H., Marcogliese P. C., Park D. S. and Trudeau L.-E. (2014) Unaltered striatal dopamine release levels in young Parkin knockout, Pink1 knockout, DJ-1 knockout and LRRK2 R1441G transgenic mice. *PLoS One 9*, e94826. https://doi.org/10.1371/journal.pone.0094826

Sanyal, A., DeAndrade, M. P., Novis, H. S., Lin, S., Chang, J., Lengacher, N., Tomlinson, J. J., Tansey, M. G., & LaVoie, M. J. (2020). Lysosome and inflammatory defects in GBA1-mutant astrocytes are normalized by LRRK2 inhibition. *Movement Disorders*, *35*, 760–773.

Sara, Y., Virmani, T., Deák, F., Liu, X., & Kavalali, E. T. (2005). An isolated pool of vesicles recycles at rest and drives spontaneous neurotransmission. *Neuron*, *45*, 563–573. https://doi.org/10.1016/j.neuron.2004.12.056

Schmidt, S. H., Knape, M. J., Boassa, D., Mumdey, N., Kornev, A. P., Ellisman, M. H., Taylor, S. S., & Herberg, F. W. (2019). The dynamic switch mechanism that leads to activation of LRRK2 is embedded in the DFGψ motif in the kinase domain. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 14979–14988.

Schulz, J. B., Hausmann, L., & Hardy, J. (2016). 199 years of Parkinson disease – what have we learned and what is the path to the future? *Journal of Neurochemistry*, *139*, 3–7.

Sen, S., Webber, P. J., & West, A. B. (2009). Dependence of leucine-rich repeat kinase 2 (LRRK2) kinase activity on dimerization. *Journal of Biological Chemistry*, *284*, 36346–36356.

Sheng, Z., Zhang, S., Bustos, D., Kleinheinz, T., Le Pichon, C. E., Dominguez, S. L., Solanoy, H. O. et al (2012). Ser1292 autophosphorylation is an indicator of LRRK2 kinase activity and contributes to the cellular effects of PD mutations. *Science Translational Medicine*, *4*, 164ra161.

Shimizu, H., Kawamura, S., & Ozaki, K. (2003). An essential role of Rab5 in uniformity of synaptic vesicle size. *Journal of Cell Science*, *116*, 3583–3590.

Shin, N., Jeong, H., Kwon, J., Heo, H. Y., Kwon, J. J., Yun, H. J., Kim, C.-H., Han, B. S., Tong, Y., Shen, J., & Hatano, T. (2008). LRRK2 regulates synaptic vesicle endocytosis. *Experimental Cell Research*, *314*, 2055–2065.

Simuni, T., Brumm, M. C., Uribe, L., Caspell-Garcia, C., Coffey, C. S., Siderowf, A., Alcalay, R. N. et al (2020). Clinical and dopamine transporter imaging characteristics of leucine rich repeat kinase 2 (LRRK2) and glucosylceramidase beta (GBA) Parkinson's disease Participants in the Parkinson's progression markers initiative: A cross-sectional study. *Movement Disorders*, *35*, 833–844.

Singleton, A. B., Hardy, J. A., & Gasser, T. (2017). The birth of the modern era of Parkinson's disease genetics. *Journal of Parkinson's Disease*, *7*, S87–S93.

Sloan, M., Alegre-Abarrategui, J., Potgieter, D., Kaufmann, A.-K., Exley, R., Deltheil, T., Threlfell, S., Connor-Robson, N., Brimblecombe, K., Wallings, R., & Cioroch, M. (2016). LRRK2 BAC transgenic rats develop progressive, L-DOPA-responsive motor impairment, and deficits in dopamine circuit function. *Human Molecular Genetics*, *25*, 951–963.

Sossi, V., de la Fuente-Fernández, R., Nandhagopal, R., Schulzer, M., McKenzie, J., Ruth, T. J., Aasly, J. O., Farrer, M. J., Wszolek, Z. K., & Stoessl, J. A. (2010). Dopamine turnover increases in asymptomatic LRRK2 mutations carriers. *Movement Disorders*, *25*, 2717–2723.

Stafa, K., Tsika, E., Moser, R., Musso, A., Glauser, L., Jones, A., Biskup, S., Xiong, Y., Bandopadhyay, R., Dawson, V. L., & Dawson, T. M. (2014). Functional interaction of Parkinson's disease-associated LRRK2 with members of the dynamin GTPase superfamily. *Human Molecular Genetics*, *23*, 2055–2077.

Steger, M., Tonelli, F., Ito, G., Davies, P., Trost, M., Vetter, M., Wachter, S., Lorentzen, E., Duddy, G., Wilson, S., & Baptista, M. A. (2016). Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. *Elife*, *5*, e12813.

Tan, E.-K. (2006). Identification of a common genetic risk variant (LRRK2 Gly2385Arg) in Parkinson's disease. *Annals of the Academy of Medicine of Singapore*, *35*, 840–842.

Tan, E. K., Peng, R., Wu, Y. R., Wu, R. M., Wu-Chou, Y. H., Tan, L. C., An, X. K., Chen, C. M., Fook-Chong, S., & Lu, C. S. (2009). LRRK2 G2385R modulates age at onset in Parkinson's disease: A multi-center pooled analysis. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics.*, *150B*, 1022–1023.

Taylor, M., & Alessi, D. R. (2020). Advances in elucidating the function of leucine-rich repeat protein kinase-2 in normal cells and Parkinson's disease. *Current Opinion in Cell Biology*, *63*, 102–113.

Taymans, J. M., Van den Haute, C., & Baekelandt, V. (2006). Distribution of PINK1 and LRRK2 in rat and mouse brain. *Journal of Neurochemistry*, *98*, 951–961.

Tolosa, E., Vila, M., Klein, C., & Rascol, O. (2020). LRRK2 in Parkinson disease: Challenges of clinical trials. *Nature Reviews. Neurology*, *16*, 97–107.

Tong, Y., Pisani, A., Martella, G., Karouani, M., Yamaguchi, H., Pothos, E. N., & Shen, J. (2009). R1441C mutation in LRRK2 impairs dopaminergic neurotransmission in mice. *Proceedings of the National Academy of Sciences*, *106*, 14622–14627.

Tozzi, A., Durante, V., Bastioli, G., Mazzocchetti, P., Novello, S., Mechelli, A., Morari, M., Costa, C., Mancini, A., & Di Filippo, M. (2018). Dopamine D2 receptor activation potently inhibits striatal

glutamatergic transmission in a G2019S LRRK2 genetic model of Parkinson's disease. *Neurobiology of Diseases*, *118*, 1–8.

Vilas, D., Ispierto, L., Álvarez, R., Pont-Sunyer, C., Martí, M. J., Valldeoriola, F., Compta, Y., de Fabregues, O., Hernández-Vara, J., Puente, V., & Calopa, M. (2015). Clinical and imaging markers in premotor LRRK2 G2019S mutation carriers. *Parkinsonism & Related Disorders*, *21*, 1170–1176.

Vilas, D., Segura, B., Baggio, H. C., Pont-Sunyer, C., Compta, Y., Valldeoriola, F., José, M. M., Quintana, M., Bayés, A., Hernández-Vara, J., & Calopa, M. (2016). Nigral and striatal connectivity alterations in asymptomatic LRRK2 mutation carriers: A magnetic resonance imaging study. *Movement Disorders*, *31*, 1820–1828.

Volta, M., Beccano-Kelly, D. A., Paschall, S. A., Cataldi, S., MacIsaac, S. E., Kuhlmann, N., Kadgien, C. A., Tatarnikov, I., Fox, J., Khinda, J., & Mitchell, E. (2017). Initial elevations in glutamate and dopamine neurotransmission decline with age, as does exploratory behavior, in LRRK2 G2019S knock-in mice. *Elife*, *6*, e28377.

Volta M., Cataldi S., Beccano-Kelly D., Munsie L., Tatarnikov I., Chou P., Bergeron S., Mitchell E., Lim R., Khinda J., Lloret A., Bennett C. F., Paradiso C., Morari C., Farrer M. J. & Milnerwood A. J. (2015) Chronic and acute LRRK2 silencing has no long-term behavioral effects, whereas wild-type and mutant LRRK2 overexpression induce motor and cognitive deficits and altered regulation of dopamine release. *Parkinsonism Relat Disord 21*, 1156–1163. https://doi.org/10.1016/j.parkreldis.2015.07.025

Volta, M., & Melrose, H. (2017). LRRK2 mouse models: Dissecting the behavior, striatal neurochemistry and neurophysiology of PD pathogenesis. *Biochemical Society Transactions*, *45*, 113–122.

Wallings, R. L., Herrick, M. K., & Tansey, M. G. (2020). LRRK2 at the interface between peripheral and central immune function in Parkinson's. *Front Neurosci*, *14*, 443.

Wandinger-Ness, A., & Zerial, M. (2014). Rab proteins and the compartmentalization of the endosomal system. *Cold Spring Harbor Perspectives in Biology*, *6*, a022616.

Wang, H., & Pickel, V. M. (2002). Dopamine D2 receptors are present in prefrontal cortical afferents and their targets in patches of the rat caudate-putamen nucleus. *The Journal of Comparative Neurology*, *442*, 392–404.

Watanabe, S., & Boucrot, E. (2017). Fast and ultrafast endocytosis. *Current Opinion in Cell Biology*, *47*, 64–71.

West, A. B., Cowell, R. M., Daher, J. P. L., Moehle, M. S., Hinkle, K. M., Melrose, H. L., Standaert, D. G., & Volpicelli-Daley, L. A. (2014). Differential LRRK2 expression in the cortex, striatum, and substantia nigra in transgenic and nontransgenic rodents. *The Journal of Comparative Neurology*, *522*, 2465–2480.

Whiffin, N., Armean, I. M., Kleinman, A., Marshall, J. L., Minikel, E. V., Goodrich, J. K., Quaife, N. M. et al (2020). The effect of LRRK2 loss-of-function variants in humans. *Nature Medicine*, *26*, 869–877.

Wienisch, M., & Klingauf, J. (2006). Vesicular proteins exocytosed and subsequently retrieved by compensatory endocytosis are nonidentical. *Nature Neuroscience*, *9*, 1019–1027.

Wucherpfennig, T., Wilsch-Bräuninger, M., & González-Gaitán, M. (2003). Role of Drosophila Rab5 during endosomal trafficking at the synapse and evoked neurotransmitter release. *Journal of Cell Biology*, *161*, 609–624.

Xiong, Y., Dawson, T. M., & Dawson, V. L. (2017). Models of LRRK2-associated Parkinson's disease. *Advances in Neurobiology*, *14*, 163–191.

Xiong, Y., Neifert, S., Karuppagounder, S. S., Liu, Q., Stankowski, J. N., Lee, B. D., Ko, H. S., Grima, J. C., Mao, X., & Jiang, H. (2018). Robust kinase- and age-dependent dopaminergic and norepinephrine neurodegeneration in LRRK2 G2019S transgenic mice. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 1635–1640.

Yue, M., Hinkle, K. M., Davies, P., Trushina, E., Fiesel, F. C., Christenson, T. A., Schroeder, A. S., Zhang, L., Bowles, E., Behrouz, B., & Lincoln, S. J. (2015). Progressive dopaminergic alterations and mitochondrial abnormalities in LRRK2 G2019S knock-in mice. *Neurobiology of Diseases*, *78*, 172–195.

Yun, H. J., Park, J., Ho, D. H., Kim, H., Kim, C.-H., Oh, H., Ga, I., Seo, H., Chang, S., Son, I., & Seol, W. (2013). LRRK2 phosphorylates Snapin and inhibits interaction of Snapin with SNAP-25. *Experimental & Molecular Medicine*, *45*, e36. https://doi.org/10.1038/emm.2013.68

Zeisel, A., Munoz-Manchado, A. B., Codeluppi, S., Lonnerberg, P., La Manno, G., Jureus, A., Marques, S., Munguba, H., He, L., Betsholtz, C., Rolny, C., Castelo-Branco, G., Hjerling-Leffler, J., & Linnarsson, S. (2015). Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science*, *347*, 1138–1142. https://doi.org/10.1126/science.aaa1934

Zhang, H., & Sulzer, D. (2012). Regulation of striatal dopamine release by presynaptic auto- and heteroreceptors. *Basal Ganglia*, *2*, 5–13. https://doi.org/10.1016/j.baga.2011.11.004

Zhang, P., Fan, Y., Ru, H., Wang, L., Magupalli, V. G., Taylor, S. S., Alessi, D. R., & Wu, H. (2019). Crystal structure of the WD40 domain dimer of LRRK2. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 1579–1584.

Zhao, Y., Keshiya, S., Atashrazm, F., Gao, J., Ittner, L. M., Alessi, D. R., Halliday, G. M., Fu, Y., & Dzamko, N. (2018). Nigrostriatal pathology with reduced astrocytes in LRRK2 S910/S935 phosphorylation deficient knockin mice. *Neurobiology of Diseases*, *120*, 76–87.

Zimprich, A., Biskup, S., Leitner, P., Lichtner, P., Farrer, M., Lincoln, S., Kachergus, J., Hulihan, M., Uitti, R. J., Calne, D. B., Stoessl, A. J., Pfeiffer, R. F., Patenge, N., Carbajal, I. C., Vieregge, P., Asmus, F., Müller-Myhsok, B., Dickson, D. W., Meitinger, T., … Gasser, T. (2004). Mutations in LRRK2 cause autosomal-dominant Parkinsonism with pleomorphic pathology. *Neuron*, *44*, 601–607. https://doi.org/10.1016/j.neuron.2004.11.005

How to cite this article: Pischedda F, Piccoli G. LRRK2 at the pre-synaptic site: A 16-years perspective. *J Neurochem*. 2021;157:297–311. https://doi.org/10.1111/jnc.15240