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Functional and behavioral consequences of Parkinson's disease-associated *LRRK2-G2019S* mutation

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Abstract

LRRK2 mutation is the most common inherited, autosomal dominant cause of Parkinson's disease (PD) and has also been observed in sporadic cases. Most mutations result in increased *LRRK2* kinase activity. *LRRK2* is highly expressed in brain regions that receive dense, convergent innervation by dopaminergic and glutamatergic axons, and its levels rise developmentally coincident with glutamatergic synapse formation. The onset and timing of expression suggests strongly that *LRRK2* regulates the development, maturation and function of synapses. Several lines of data in mice show that *LRRK2-G2019S*, the most common *LRRK2* mutation, produces an abnormal gain of pathological function that affects synaptic activity, spine morphology, persistent forms of synapse plasticity and behavioral responses to social stress. Effects of the mutation can be detected as early as the second week of postnatal development and can last or have consequences that extend into adulthood and occur in the absence of dopamine loss. These data suggest that the generation of neural circuits that support complex behaviors is modified by *LRRK2-G2019S*. Whether such alterations impart vulnerability to neurons directly or indirectly, they bring to the forefront the idea that neural circuits within which dopamine neurons eventually degenerate are assembled and utilized in ways that are distinct from circuits that lack this mutation and may contribute to non-motor symptoms observed in humans with PD.

The relevance of brain development and critical periods to Parkinson's disease

Waves of protein-based signaling networks become activated and inactivated during development in a tightly orchestrated and genetically controlled process that produces appropriately wired neural circuits [1]. As young vertebrates interact with the world, a collaboration between behavioral experience and synaptic activity during an early, restricted postnatal period (a so-called critical period) shapes and refines nascent neural circuits in ways that become permanent and are essential for normal function. Disease-causing mutations in genes that are expressed during this period can have a strong impact on

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Author Contribution

D.L.B., G.W.H., B.A.M.-A. and A.H. generated the ideas presented and D.L.B. wrote the first draft. G.W.H., B.A. M.-A. and A.H. edited the manuscript.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

relationships between circuits, activity and environment, and it is well accepted that changes in these interactions contribute directly to the development and severity of nervous system disorders in which major disease-defining symptoms arise at early ages (e.g. autism) or by late adolescence (e.g. schizophrenia) [2–4]. A growing body of work studying patients and their relatives suggests that disease-causing gene mutations can significantly alter the structure and function of the brain at early stages in ways that may contribute to later-onset disorders, including Parkinson's disease (PD) [5]. While it is possible that such structural/functional changes and disease-defining symptoms sit in parallel, unrelated pathways, it is far more likely that early structural and functional changes are part of the disease progression. Thus, a complete understanding of the consequences of a gene mutation on neural circuits will provide key insights into disease onset and progression. Recent work supports the idea that the most common PD-related mutation, *LRRK2*-G2019S, increases neural activity at a time and in a manner that would be anticipated to alter striatal and other neural circuits [6,7]. Here, we review evidence from mouse models, showing that *LRRK2*-G2019S alters the development and function of striatal circuits in a manner that may contribute directly to the onset of the disease later in life or affect indirectly the nature and expression of both motor and non-motor symptoms.

LRRK2 hyperactivation and PD

LRRK2 encodes a multidomain 280 kDa protein with a catalytic core domain that supports both GTPase and kinase activities. Most pathogenic mutations are found within the catalytic core (Figure 1A). The G2019S mutation, which sits in the kinase domain, increases kinase activity by ~2-fold, whereas the next most common site of mutation R1141G/C/H lies in the GTPase domain and increases substrate phosphorylation several-fold [8]. Based on this and studies of additional mutations, the source of pathogenicity is thought to be due to hyperactivation of the kinase. Mechanisms driving LRRK2 pathogenicity may be central to other forms of PD. For example, a PD-causing mutation in VPS35 (D620N) increases LRRK2-mediated Rab phosphorylation substrates [9], and accumulation of extracellular α -synuclein aggregates is enhanced in the mouse brain and in neuron cultures expressing LRRK2-G2019S [10].

LRRK2 expression, localization and onset are concurrent with synapse development

LRRK2 is expressed at the right place and time to strongly influence the development of corticostriatal connections. *In situ* hybridization and immunohistochemical studies using verified antibodies show that within the brain, LRRK2 is most highly expressed in cerebral cortex and dorsal striatum [12–17]. Its expression levels rise postnatally over a time course that closely matches the generation of glutamatergic corticostriatal synapses [11,18] (Figure 1B). They show no such correlation with the development of thalamocortical or GABAergic synapses, suggesting that the influence of LRRK2 on synaptic circuits may be selective. Expression levels reach a maximum and level off [12,16] when experience is shaping connections [19–21]. This pattern of expression is typical for proteins that influence the development of neural circuits.

Cellular and functional data provide additional support that LRRK2 regulates synapses. Although immunocytochemical localization of endogenous LRRK2 has proved to be particularly difficult, biochemical data support that LRRK2 is present within synaptic fractions [15,22,23] and can be targeted to membranes in a regulated manner [24]. Neither the pace of synaptogenesis nor density of synapses appears to be affected by either *Lrrk2* deletion or mutation, but there is compelling evidence showing that LRRK2 regulates synapse function following synapse assembly [6,7,25].

LRRK2 and synaptic vesicle recycling

Functional studies in neurons lacking LRRK2 or having reduced LRRK2 expression generally agree that synaptic vesicle endocytosis is impaired [23,26–28]. The mechanisms driving impaired endocytosis are not yet clear, but Rab5b and potentially EndoA and Synaptojanin1 could be relevant LRRK2 kinase substrates [26,28,29] (Figure 2). LRRK2-G2019S and LRRK2-R1441C/G both appear to decrease synaptic vesicle endocytosis [28–30]. Since either loss or gain of kinase function reduces the efficiency or degree of endocytosis, the data suggest that LRRK2 kinase activity may be deployed in a cyclical fashion [26]. The mechanism, however, must also account for the fact that LRRK2 kinase inhibitors can rescue endocytic defects observed in neurons expressing LRRK2-G2019S with no detectable impact on endocytosis in wild-type (WT) control neurons [29]. Based on this, the endocytic defects seen with LRRK2-G2019S or R1441C/G may be regulating a pathway distinct from the decreased endocytosis observed following the loss of the entire, 280 kDa, multifunctional LRRK2 protein (and which can be rescued by expressing full-length WT LRRK2) [26].

Several studies support that cortical neurons expressing LRRK2-G2019S can also display increased exocytosis in neuron cultures or in preparations from young mice (Figure 2), an effect that is normalized by the addition of an LRRK2 kinase inhibitor [29,31]. This effect likely contributes to the increased spontaneous glutamatergic activity that is observed in postnatal day (P) 21–P28 striatal spiny projection neurons (SPNs) [6,7]. Additionally, P15 striatal SPNs lacking LRRK2 show increased EPSC amplitudes compared with WT neurons likely due to increased postsynaptic exocytosis and surface expression of AMPA receptors. In forebrain homogenates, the same study shows that LRRK2 binds to protein kinase A (PKA) regulatory subunit RII β and in cultured hippocampal neurons appears to negatively regulate PKA localization to synapses. In the absence of LRRK2, PKA activity was increased, and PKA-mediated phosphorylation and surface expression of GluA1 were enhanced. The R1441C mutation appears to reduce LRRK2/RII β binding, but it is not known whether or not LRRK2 kinase activity is involved in this mechanism [32]. PKA can also phosphorylate LRRK2, an action that may be important for LRRK2 localization and actions on particular substrates [33,34], but see also [35]. Both G2019S and R1441C/G mutations serve to increase the phosphorylation of LRRK2 substrate Rab proteins [8], making it also likely that decreased postsynaptic exocytosis also involves dysfunctional vesicle targeting and fusion (Figure 2).

Alpha-synuclein concentrates in presynaptic vesicles where it modulates endo- and exocytosis, suggesting a possible point of mechanistic convergence with LRRK2. However,

the effects of PD-associated mutation or overexpression of α -synuclein reduce synaptic vesicle release [36–38] or alter vesicle tethering [39] depending on the nerve terminal type, effects that are not at all similar to those observed with LRRK2 mutation. Perhaps more revealing is that in the presence of increased levels of α -synuclein, repetitive stimulation in a lamprey nerve preparation nearly eliminates endocytosis [40], suggesting that only certain kinds of activity may share convergent mechanisms.

LRRK2 and neural activity

Consistent with the effects of LRRK2-G2019S on presynaptic vesicle recycling and postsynaptic glutamate receptor expression, mutant LRRK2 alters synaptic activity recorded in cultured cortical neurons [41] and in acute striatal slices [6,7]. While there are some differences in approach or interpretation, the datasets from different laboratories are largely in agreement that SPNs expressing one or two copies of a knockin G2019S mutation show a developmentally transient increase in glutamatergic excitatory postsynaptic currents (EPSCs) that declines to WT levels by young adulthood [6,7]. The increase in activity is kinase-dependent, action potential driven, largely derived from the cerebral cortex, and observed in both dopamine receptor 1 (D₁R)- and dopamine receptor 2 (D₂R)-expressing SPNs [7]. Increased spontaneous EPSCs result from a gain in pathological function; activity in *Lrrk2*-D2017A (kinase-dead) mutants was no different from WT, and LRRK2 kinase inhibitors administered acutely reduced activity to WT levels (but not below WT levels) [7].

The G2019S mutation also affects dopaminergic actions in striatum. Striatal slices from 3-month-old mice expressing *Lrrk2*-G2019S showed increased peak dopamine levels detected in response to sustained stimulation compared with a decline in WT slices using fast scan voltammetry [6]. At 12 months, this effect was no longer evident in slices [6], and *in vivo*, microdialysis showed reduced extracellular dopamine in *Lrrk2*-G2019S striatum in the absence of stimulation [42]. Also at 12 months, mice overexpressing *Lrrk2*-G2019S showed reduced peak dopamine levels that were reduced further by repetitive stimulation [43]. It is not yet clear how *Lrrk2*-G2019S drives age-dependent changes in dopamine levels detected at baseline and in response to sustained stimulation, particularly since LRRK2 is expressed at very low levels in dopaminergic neurons. However, the low levels of LRRK2 do appear to confer a cell autonomous effect on DA release [29] and it seems likely that the striatum as a major postsynaptic target for dopamine fibers has a retrograde impact on DA release.

Increases in cortical glutamatergic activity onto SPNs might be predicted to drive changes in synapse formation. On a small scale, local stimulation of SPN dendrites in 2-week-old mice can prompt the genesis of new dendritic spines, and on a larger scale, silencing activity of either D₁R- or D₂R-expressing SPNs produces a circuit-wide imbalance that decreases or increases (respectively) the density of cortical synapses onto both D₁R- and D₂R SPNs [44]. However, measurements of pre- or postsynaptic protein levels, synapse or dendritic spine density have revealed no differences over the course of development that can be related to LRRK2 or mutant LRRK2 [6,7,32]. Adult *Lrrk2* knockout mice also show no differences in dendritic spine density in dorsal striatum [42]. Taken together, these data indicate that it is unlikely that normal LRRK2 is necessary and sufficient for proper synaptogenesis. However, at P15, *Lrrk2* knockout SPNs have thinner dendritic protrusions and decreased EPSC

amplitudes relative to WT SPNs, a developmentally transient phenotype that suggests LRRK2 expression may help to regulate glutamatergic synapse maturation [32]. Consistent with this idea, LRRK2-G2019S SPNs have larger dendritic spine heads and increased EPSC amplitudes at P21 [7]. Thus, it appears that the increased spontaneous EPSCs recorded in neurons expressing knockin *Lrrk2*-G2019S result from either enhanced presynaptic release, a decrease in the population of immature, postsynaptically silent (NMDAR-only) synapses, or both.

Synapse plasticity

The dramatic effects of the G2019S mutation on spontaneous glutamatergic activity in SPNs in mice are developmentally transient and not evident by adulthood. Nevertheless, the developmental timing suggests that this transient effect could mediate lasting changes in striatal circuit function. To test this, we asked whether a persistent form of synapse plasticity, long-term potentiation (LTP), would be altered in SPNs of adult *Lrrk2*-G2019S knockin mice. The data showed that while LTP can be readily elicited from SPNs in WT dorsal striatum, it was not only abolished in G2019S SPNs, but an LTP induction protocol produced long-term depression (LTD) instead [45]. When responses were compared between D₁R and D₂R-expressing SPNs, it was confirmed that LTP was absent in both SPN types, but that only D₂R SPNs showed a robust LTD, consistent with previous work indicating that LTD is more readily elicited in D₂R SPNs [46]. The impact of LRRK2-G2019S on LTP was evident at P21 and remained at P70 [45]. How LRRK2-G2019S interferes with LTP is not known, but likely involves its regulation of Rab proteins [8], since Rabs can regulate AMPA receptor insertion at synapses [47]. This idea gains strength from recent work, showing that PD-causing mutations in VPS35 enhance the kinase activity of LRRK2-G2019S [9; Figure 2] and also fail to sustain LTP in hippocampal neurons via a mechanism that appears to impair GluA1 insertion into the postsynaptic membrane [48,68]. It is also possible that LRRK2-G2019S impedes normal PKA function (see above [32]; Figure 2), since PKA-mediated phosphorylation of GluA1 is necessary for LTP [49]. No matter the underlying mechanism, the data suggest that rules for recruiting and modifying striatal circuits are different in neurons expressing LRRK2-G2019S.

In adult hippocampal slices from mice overexpressing (OE) either *Lrrk2*-WT or -G2019S, LTP is normal, but LTD is reduced selectively in G2019S OE neurons. While the mechanism is not fully understood, increased AMPA-to-NMDA ratios in G2019S OE neurons suggest that AMPA receptors fail to internalize in response to an LTD stimulus [50]. Although mechanisms underlying LTP and LTD in hippocampus are different from those in the dorsal striatum, the data from the two regions suggest that basic mechanisms regulating bidirectional changes in synapse strength are disrupted by LRRK2-G2019S in a cell-type specific manner.

Stress, experience and behavior

Bidirectional synapse plasticity is essential for acquiring and supporting the full range of learned behaviors. The absence of one arm of plasticity—LTP—in *Lrrk2*-G2019S knockin SPNs and LTD in *Lrrk2*-G2019S OE hippocampal neurons strongly suggests that learning,

memory and the performance of particular behaviors will be altered by G2019S. Since depression is a common early symptom in PD [51,52] and psychological stress can promote the onset of depression and exacerbate PD symptoms [53–55], we recently tested the effect of the *Lrrk2*-G2019S knockin mutation in chronic social defeat stress (CSDS), a validated depression model in male mice [56,57]. The data showed that following 10 days of CSDS in which 2- to 3-month-old mice were exposed for brief periods to physical subordination by a novel aggressor mouse, ~40% of WT mice were ‘resilient’ to the stress-experience and spent more time approaching and interacting with a novel social target mouse during a subsequent social interaction test. The remaining WT mice that had undergone CSDS were ‘susceptible’ to the social stress paradigm and spent more time avoiding the novel mouse [45]. This proportion of resilient and susceptible mice is consistent with previous work [57]. In stark contrast, all but one mouse in two separate cohorts of eight *Lrrk2*-G2019S mice each were resilient to CSDS (94% resilient), a result that strongly suggests that the mutation alters responsiveness to stress. Importantly, in the absence of CSDS, there were no differences between *Lrrk2*-G2019S and WT mice in exploratory behavior or social interaction.

Lack of self-care is associated with depression in humans, and self-care in mice can be measured using a sucrose splash test [58]. The data showed that *Lrrk2*-G2019S mice spent significantly more time grooming in the splash test than WT mice post-CSDS. In the absence of CSDS, there were no differences between WT and *Lrrk2*-G2019S mice in this or other anxiety-related tests, consistent with findings from other studies of *Lrrk2*-G2019S knockin and knockout mice [42,59], indicating that it was the response to CSDS that produces strikingly distinct behavioral outcomes [45].

While the mechanisms driving this different reaction to social stress are not fully understood, the data point towards an inability of LRRK2-G2019S synapses in the nucleus accumbens (NAc), a brain region involved in reward and depression [60–62], to undergo appropriate, behaviorally driven synaptic adaptations to CSDS that may involve AMPA receptor function and/or trafficking. Previous studies have established that a variety of synaptic and other cellular adaptations in the NAc are critical for the expression of both resilience and susceptibility to CSDS [56,63,64]. In WT mice, glutamatergic synapses in the NAc contain a mixture of both calcium-permeable (CP) and calcium-impermeable AMPAR subunits at baseline. WT mice that are susceptible to CSDS exhibit a significant incorporation of CP-AMPA receptors in comparison with those that are resilient [64,65]. In contrast, in *Lrrk2*-G2019S knockin mice, glutamatergic synapses in the NAc mostly lack CP-AMPA receptors at baseline and, following CSDS, fail to incorporate them [45]. This apparent inability to synaptically incorporate CP-AMPA receptors cannot be attributed to differences between genotypes in the expression levels of GluA1 or GluA2, as the major AMPAR subunit subtypes in striatum are detected at normal levels by immunoblot or immunostaining [45]. As discussed above, cellular data suggest that these differences in AMPA receptor trafficking could be driven by altered Rab activity [8,47] or by changes in the regulation of PKA [32] or a combination of both.

These data indicate a strong effect of the G2019S mutation on a complex behavior in adult mice. This stands in contrast with several studies showing at most mild hyperkinesia in young adult mice (10–12 months) expressing endogenous or transgenic LRRK2-G2019S

[6,42,59,66,67]. It may be the case that robust behavioral phenotypes may emerge only following a significant or stressful challenge, such as that imposed by CSDS. This may speak to the two-hit theory of PD, wherein genetic susceptibility is one component that, together with adverse environmental factors, predisposes the brain to PD. It will also be interesting to test these behaviors in R1441C/G/H mice where based on the strong effect of these mutations on LRRK2 kinase activity one would predict an even more robust response.

Findings showing *Lrrk2*-G2019S knockin mice to be highly resilient to CSDS are unexpected because depression and anxiety are commonly seen in patients with PD [51,52]. It is possible that the mice are showing an adaptive behavior in response to the mutation that could become maladaptive in older animals. In this context, it is interesting that a recent paper suggests that mice older than 10 months of age and overexpressing human *LRRK2*-G2019S can display anxiety and depression-like behaviors compared with non-transgenic mice expressing normal levels of endogenous LRRK2 [67]. A possible (and logical) mechanism for an age-related transition sits with dopamine, serotonin, or other neurochemical systems: mice expressing LRRK2-G2019S display an age-related decline in dopamine release and SPNs appear to respond to dopamine differently in *Lrrk2*-G2019S knockin compared with WT neurons [6,42,43].

Conclusion

Despite evidence that PD-linked mutant LRRK2 alters early postnatal synaptic structure and function in developing striatal circuits, and that aberrant behavioral phenotypes occur in *Lrrk2* knockin mutants and overexpressors at early and late adult ages, respectively, a causal link between early increases in corticostriatal activity and later behavioral abnormalities remains elusive. One possible link may lie in lasting changes in synaptic plasticity; perhaps, the disrupted synaptic potentiation and depression associated with G2019S expression has lasting consequences rendering insufficient synaptic changes in response to environmental cues. Regardless, continued investigation into the precise mechanisms by which LRRK2-G2019S affects synaptic form and function at all ages will likely shed light on the etiology of PD.

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Abbreviations

AMPAR	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptor
CP	calcium permeable
CSDS	chronic social defeat stress

D₁R	dopamine receptor 1
D₂R	dopamine receptor 2
EPSCs	excitatory postsynaptic currents
LTD	long-term depression
LTP	long-term potentiation
NAc	nucleus accumbens
PD	Parkinson's disease
PKA	protein kinase A
SPNs	spiny projection neurons
WT	wild type

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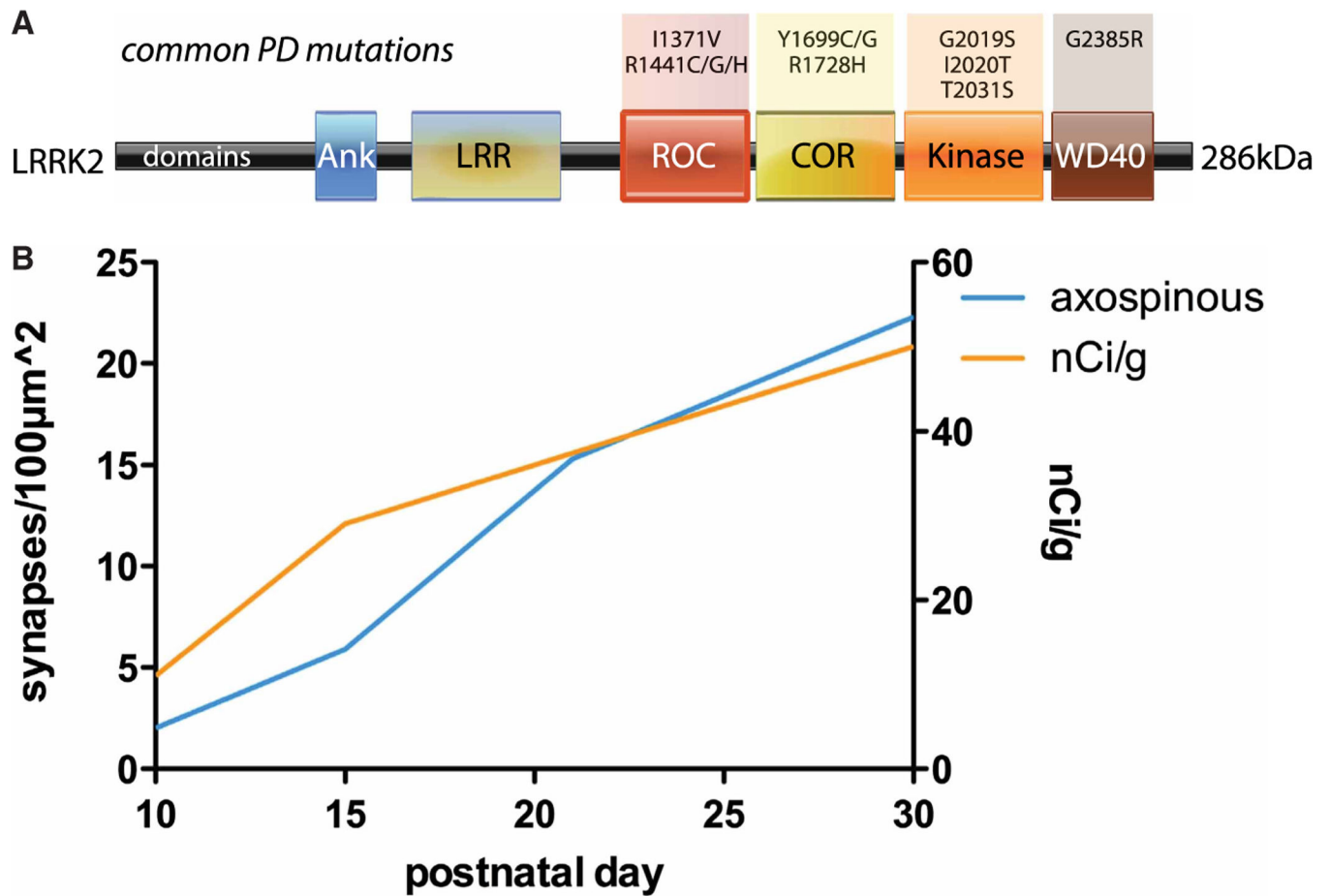


Figure 1. LRRK2 structure and its expression during synaptogenesis.

(A) Schematic showing principal protein domains in LRRK2 and sites of common PD-causing mutations. (B) LRRK2 levels rise over a time course that is shared by the generation of corticostriatal synapses. Estimates of synapse density over time (blue) are plotted on the left-hand Y-axis from data published by Sharpe and Tepper [11]; development of asymmetric synapses on dendritic spines serves as a proxy for the generation of corticospinal synapses. Estimates of LRRK2 mRNA levels over time (orange) are plotted on the right-hand Y-axis and are based on *in situ* hybridization data published in Westerlund et al. [12].

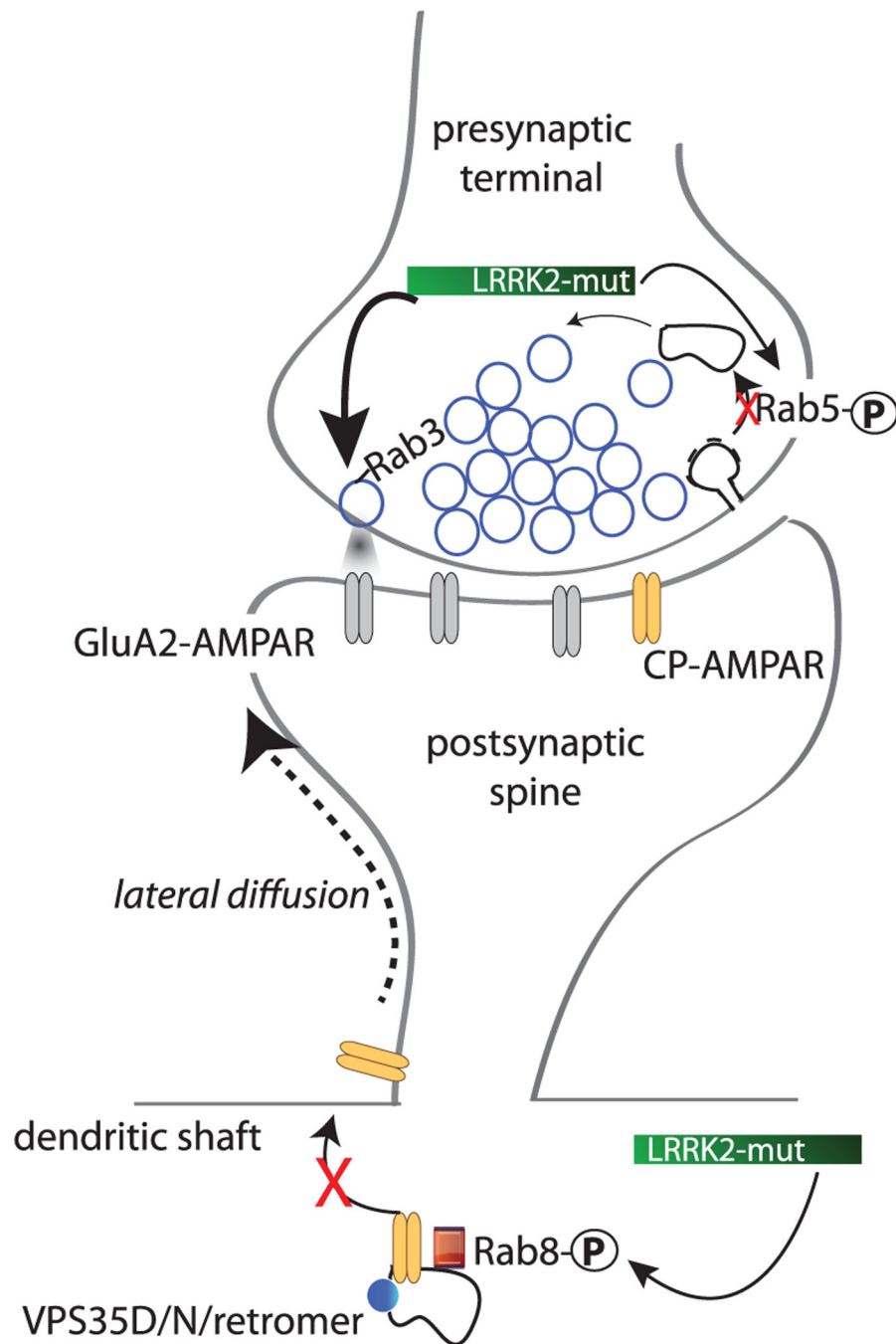


Figure 2. Potential pre- and postsynaptic mechanisms by which mutant LRRK2 can influence synaptic function.

Cartoon depicts a glutamatergic synapse and highlights possible sites of LRRK2-G2019S interaction/interference with the normal processes of presynaptic vesicle recycling and insertion of new CP-AMPA receptor subunits into the plasma membrane. CP: calcium permeable; LRRK2-mut: PD-causing LRRK2 mutations, particularly G2019S.