Glutamate-induced excitotoxicity in Parkinson's disease: The role of glial cells

L. Iovino, M. E. Tremblay, & L. Civiero

November 2020

Journal of Pharmacological Sciences 144 (2020) 151–164

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Critical Review

Glutamate-induced excitotoxicity in Parkinson's disease: The role of glial cells

L. Iovino [a], M.E. Tremblay [b], L. Civiero [a, c, *]

[a] Department of Biology, University of Padova, Italy
[b] Division of Medical Sciences, University of Victoria, Victoria, Canada
[c] IRCCS San Camillo Hospital, Venice, Italy

ARTICLE INFO

Article history:
Received 28 April 2020
Received in revised form
30 June 2020
Accepted 27 July 2020
Available online 1 August 2020

Keywords:
Parkinson's disease
Glutamate-induced excitotoxicity
Inflammation
Astrocytes
Microglia

ABSTRACT

Glutamate is the major excitatory neurotransmitter in the central nervous system. Glutamate transmission efficiency depends on the correct functionality and expression of a plethora of receptors and transporters, located both on neurons and glial cells. Of note, glutamate reuptake by dedicated transporters prevents its accumulation at the synapse as well as non-physiological spillover. Indeed, extracellular glutamate increase causes aberrant synaptic signaling leading to neuronal excitotoxicity and death. Moreover, extrasynaptic glutamate diffusion is strongly associated with glia reaction and neuroinflammation. Glutamate-induced excitotoxicity is mainly linked to an impaired ability of glial cells to reuptake and respond to glutamate, then this is considered a common hallmark in many neurodegenerative diseases, including Parkinson's disease (PD). In this review, we discuss the function of astrocytes and microglia in glutamate homeostasis, focusing on how glial dysfunction causes glutamate-induced excitotoxicity leading to neurodegeneration in PD.

Introduction

In the central nervous system (CNS), the balance between excitatory and inhibitory neuronal connections is crucial for maintaining proper function. The majority of excitatory signals are mediated by glutamate, which is the predominant neurotransmitter in the mammalian CNS.[1] Glutamatergic neurotransmission is responsible for many cognitive, motor, sensory and autonomic activities.[2–4] As a consequence, keeping extracellular glutamate levels in a physiological range is crucial to ensure proper neuronal transmission and viability. Indeed, impaired glutamate homeostasis has important neuropathological consequences and has been associated to several neurological or neurodegenerative disorders (NDs) such as epilepsy, multiple sclerosis, Alzheimer's disease (AD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD).[5]

At the excitatory synapse, glutamate is continuously released and recaptured through the well described 'glutamate–glutamine cycle'.[6,7] Glutamate is released by neurons into the synaptic cleft where it binds to both ionotropic and metabotropic receptors to transmit an excitatory message. It has been estimated that, following action potential, glutamate concentration in the synaptic cleft exceeds 1 mM for <10 ms.[8,9] Afterwards, glutamate is rapidly removed from the cleft and its concentration returns to nanomolar levels, thanks to high-affinity transporters expressed both on neurons and glial cells (Fig. 1).[6,10] In astrocytes, the enzyme glutamine synthetase (GS) converts glutamate into inert glutamine which, in turn, can be promptly released and taken up by neurons as a precursor for glutamate synthesis.[6,7] Neurons are able to uptake glutamate[11,12] and this strategy has been described as a way to replenish the presynaptic glutamate stores, bypassing the glutamate–glutamine cycle.[11] Glutamate uptake occurs via five specialized glutamate transporters, defined as excitatory amino acid transporters (EAATs) 1 to 5 [6,10]. Two of these, the EAAT1 and EAAT2 (respectively named GLAST and GLT-1 in rodent brain) are considered, in terms of function and distribution, to be the most represented glutamate transporters in the CNS.[13,14] In more detail, EAAT1 is mainly expressed on astrocytes, while EAAT2, that is responsible for 90% of the brain glutamate reuptake, is highly expressed on astrocytes, but also on neurons[11,15] (Fig. 1). Moreover, EAAT1 and EAAT2 are present in oligodendrocytes,[16,17] while

* Corresponding author. Department of Biology, University of Padova, Italy.
E-mail address: laura.civiero@unipd.it (L. Civiero).
Peer review under responsibility of Japanese Pharmacological Society.

https://doi.org/10.1016/j.jphs.2020.07.011

Abbreviations

6-OHDA	6-hydroxydopamine; α-syn: α-synuclein
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	brain-derived neurotrophic factor
C1q	complement protein 1q
C3	complement 3 protein
CCL	chemokine ligands
CNS	central nervous system
Cx32	connexin 32
D1/D2 Rs	dopaminergic receptors
DA	dopaminergic
EAATs	excitatory aminoacid transporters
eCB	endocannabinoids
FGF	fibroblast growth factor
GFAP	glial fibrillary acidic protein
GP	globus pallidus
GS	glutamine synthetase
GSH	glutathione
HD	Hungtington's disease
IL	interleukine
iNOS	inducible nitric oxide synthase
JNK	c-Jun N-terminal kinase
KI	knock-in
KO	knock-out
LRRK2	Leucine-rich repeat kinase 2
L-Dopa	levodopa
LTP	long term potentiation
mGluR	metabotropic glutamate receptors
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	magnetic resonance imaging
NADPH	nicotinamide adenine dinucleotide phosphate hydrogen
NDs	neurodegenerative diseases
Nedd4-2	neuronal precursor cell expressed developmentally down-regulated 4-2
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	nerve growth factor
NMDA	N-methyl-D-aspartate
NO	nitric oxide
PD	Parkinson's disease
PET	positron emission tomography
PFF	preformed fibril
PI3K	phosphoinositide 3-kinase
PK2	prokineticin-2
PKA	protein kinase A
ROS	reactive oxygen species
sEPSC	spontaneous excitatory postsynaptic currents
SNpc	substantia nigra pars compacta
SNr	substantia nigra pars reticulate
SPET	single photon emission computed tomography
SPNs	spiny projection neurons
STN	subthalamic nucleus
TH	tyrosine hydroxylase
TNF-α	tumor necrosis factor-α
TrKB	tyrosine receptor kinase B
VgluT2	vesicular glutamate transporter type 2
xCs	cysteine–glutamate exchange system

microglia, which do not express glutamate transporters at steady-state, start to synthetize EAATs when they become reactive[18–20] (Fig. 1). Overall, the expression pattern of glutamate transporters on glial cells implies a crucial role in the maintenance of glutamate homeostasis both in health and disease.

Glutamate accumulation at the synapse over physiological range is toxic and triggers apoptotic cell death due to Ca^{2+} overload upon overstimulation of glutamate receptors.[5] Glutamate-induced excitotoxicity is considered a common hallmark of many NDs, including PD, and has been linked to alterations in the expression of glutamate transporters and receptors possibly in relation to inflammatory processes. Indeed, neuronal death but also glutamate spillover from the synaptic area can contribute to neuroinflammation.[21] Neuroinflammation is a complex phenomenon during which microglia and astrocytes become reactive and start to secrete excessive amounts of proinflammatory cytokines as well as chemokines.[22] Moreover, inflammation in the periphery is transmitted to the brain through changes in the integrity of the blood brain barrier.[23] Both peripheral and central inflammation are observed in NDs.[24,25] Of note, chemokines impact on glutamate homeostasis both downregulating EAATs expression and upregulating glutamate receptors, thus potentiating glutamatergic neurotransmission and excitotoxicity.[21]

PD is the second most common neurodegenerative disorder that affects movements as well as cognition. It is characterized by i) the loss of dopaminergic (DA) neurons in the *substantia nigra pars compacta* (SNpc) that project to the dorsal striatum, ii) a massive accumulation of aggregated α-synuclein (α-syn) as the main component of Lewy bodies and Lewy neurites and iii) extended neuroinflammation.[26] PD mainly occurs as sporadic condition, but it is now confirmed that a substantial proportion of risk to develop the pathology is driven by genetic variants.[27] Moreover, highly-penetrant rare mutations in *SNCA, LRRK2, VPS35, PRKN, PINK1* and *DJ-1* have been linked to familiar forms of PD.[28] Despite PD being generally described as a DA disease, it is now accepted that alterations of glutamatergic neurotransmission play a major role in PD progression.[29] Indeed, glutamate intervenes at crucial points of fronto-basal circuits involved in the modulation of voluntary movements (Fig. 2A). The striatum circuitry is mainly composed of inhibitory spiny projection neurons (SPNs) that receive DA innervation from SNpc and glutamatergic inputs from the cerebral cortex (CTX) and thalamus.[29,30] SPNs are divided into two subpopulations: the striatonigral neurons are connected to the *substantia nigra pars reticulata* (SNr), while the striatopallidal neurons project to neurons of the internal segment of the *globus pallidus* (GPi) directly (direct pathway) or communicate with the external segment of the *globus pallidus* (GPe) and the subthalamic nucleus (STN) through glutamate release (indirect pathway).[29,30] In addition, a direct glutamatergic connection exists between the STN and the SNpc.[29,30] The coordinated activity of the direct and indirect pathways becomes impaired in PD due to the progressive loss of nigral DA neurons.[29] A decreased stimulation of the DA receptors (D1R) affects the direct pathway leading to a reduced inhibition of the output signals while the lack of DA receptor (D2R)-mediated stimulation of the indirect pathway results in a disinhibition of the STN causing a glutamatergic overstimulation of the output signals[29] (Fig. 2B). Overall, unbalance of the direct–indirect pathways prevents the activation of motor thalamic nuclei, which results in a reduced stimulation of motor areas in the frontal cortex and hence in a decreased capacity to perform voluntary movements.[29] Of note, high-frequency

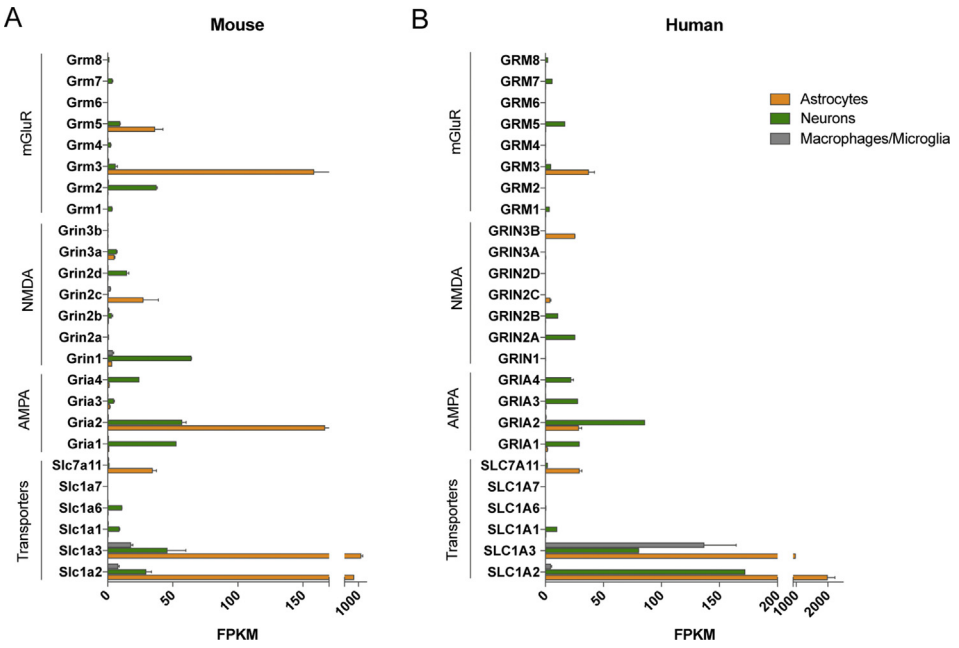

Fig. 1. Expression pattern of glutamate receptors and transporters in brain cells. Transcriptomic data showing the expression of mouse (A) and human (B) genes in astrocytes, neurons, as well as microglia/macrophages.[176,177] Mouse and human astrocytes n = 12 and n = 6 cells, human and mouse neurons n = 1 and n = 2 cells, human and mouse microglia n = 4 and n = 2 cells. The majority of genes involved in glutamate handling are considerably expressed in astrocytes and neurons but not in microglia. Abbreviation: RPKM, reads per kilobase of transcript per million mapped reads.

stimulation of cortico-striatal connectivity or inhibition of glutamate transporters can increase glutamate striatal levels in mice. This promotes glutamate spillover that can modulate/suppress DA evoked release by activating the group I metabotropic glutamate receptors (mGluR) 1 in SNpc DA terminals.[31]

The loss of SNpc DA terminals in the striatum was proposed to be the first trigger for neurodegeneration, occurring as a 'dying-back' process that affects the neuronal cell body at later stages.[32] But, what is the contribution of altered glutamate homeostasis to the DA neurodegeneration in the striatum? Are astrocytes and microglia involved by a loss or gain-of-function in glutamate-induced excitotoxicity and how do their activities coordinate together? In this review, we extensively discuss the evidence of impaired glutamatergic neurotransmission and glutamate clearance in genetic and idiopathic forms of PD, as well as the state of art concerning the role of glial cells in glutamate-mediated neuroinflammation, analyzing also these cells as a potential and attractive target for PD cure.

Evidence of impaired glutamate homeostasis in PD

Several clinical results obtained using magnetic resonance imaging (MRI), positron emission tomography (PET) and single photon emission computed tomography (SPET) have revealed slight alterations in glutamate content in the brain of PD patients that indicate increased glutamate neurotransmission.[33–35] Of note, a two-fold increase in glutamate level (~70 μmol/L) was also measured in the plasma from PD patients compared to controls (~30 μmol/L), reflecting increases in cerebral glutamatergic activity.[36,37] Whether glutamate alteration causes or exacerbates neurodegeneration in PD patients is still unclear. One possibility is that, with the progression of the disease, the sustained glutamatergic excitation of the SNpc neurons caused by STN disinhibition can further promote DA neuronal loss. However, several studies conducted in both acute and genetic *in vitro* and *in vivo* models of PD suggest that i) glutamate dyshomeostasis might induce DA neuronal death and ii) both nigral and striatal glutamatergic imbalance are involved in PD. The marked alterations of glutamate homeostasis and glutamatergic neurotransmission is detailed below and summarized in Table 1.

Acute exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) neurotoxins is largely used to investigate biochemical and cellular dysfunctions in PD cellular and animal models.[38] Of note, *in vivo* microdialysis experiments demonstrated an increase of basal extracellular glutamate levels by two-fold in the striatum of 1 month 6-OHDA-injected rats and MPTP-injected mice.[39,40] Also, a three-fold increase in glutamate extracellular concentration has been reported in the SNpc of MPTP-treated mice.[41] In the same study, the authors demonstrated that the affinity of glutamate transporters for their substrate was increased by MPTP treatment, without changing the rate of transport.[41] In primary astrocytes, Zhang et al. reported that treatment with MPTP reduces cellular viability, glutamate uptake and membrane expression of GLT-1 protein.[42] Specifically, glial cells biotransform MPTP into MPP^+ which activates the release of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB).[42] Consequently, the activation of NF-κB stimulates c-Jun N-terminal kinase (JNK)/c-Jun signaling and negatively modulates GLT-1 expression thus promoting cell death.[42] In the same report, they have shown that ceftriaxone, a β-lactame antibiotic, restores astrocytic viability, GLT-1 protein and mRNA expression, and promotes glutamate reuptake by suppressing the NF-κB/JNK/c-Jun signaling pathway in primary astrocytes.[42] More recently, the same group obtained similar data *in vivo* in mice intraperitoneally-injected with MPTP.[43] The animal model displays typical behavioral and histopathological deficits of PD coupled with downregulation of GLT-1 protein and mRNA levels, extracellular glutamate accumulation, excitotoxicity, as well as astrocytic and microglial reactivity.[43] The decrease in GLT-1 levels has been attributed to the increased ubiquitination of the transporter by the

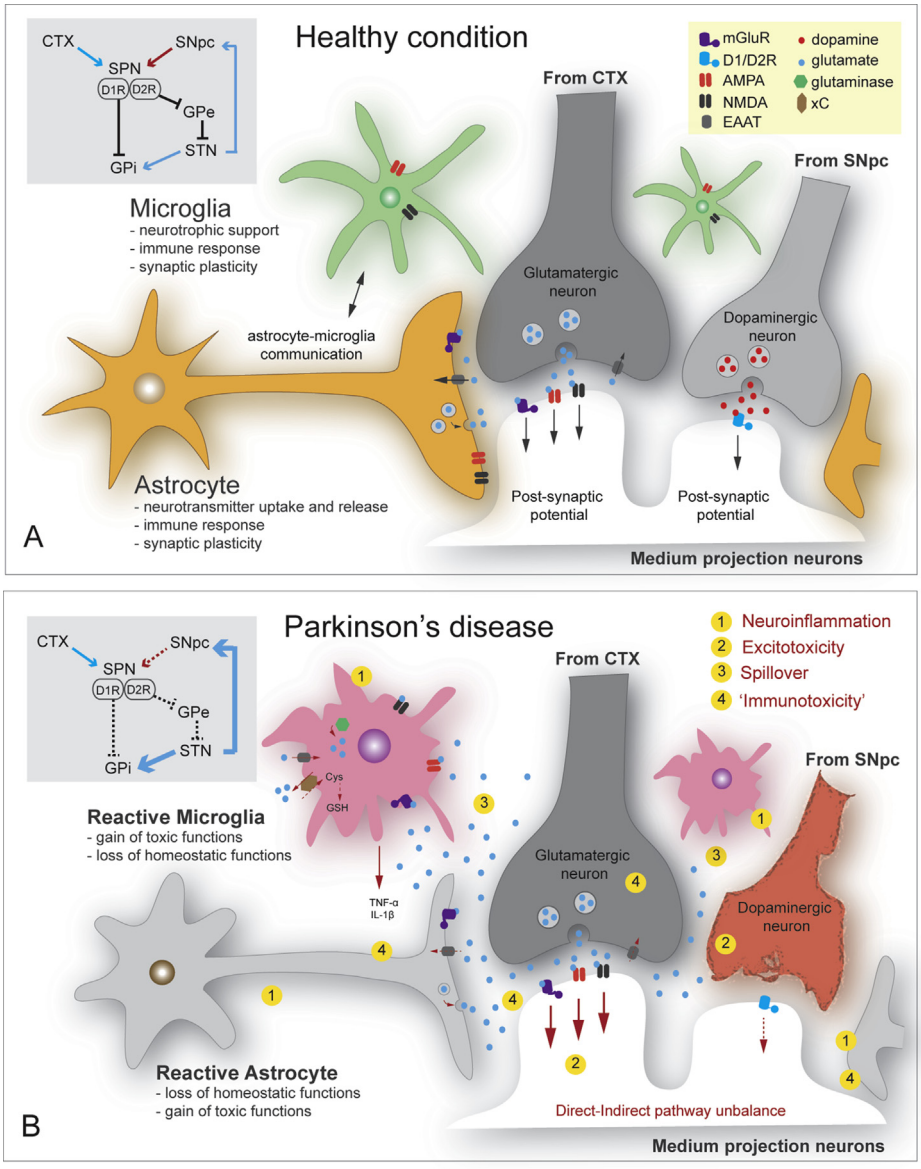

Fig. 2. Glutamate handling at the striatum and the role of glial cells. Striatal events are detailed in healthy condition with a focus on astrocytes and microglia functions (A). Impact of astrocytes and microglia loss or gain-of-functions in glutamate uptake, spillover and inflammation resulting in exacerbated neurodegeneration (B). Fronto-basal circuits involved in the modulation of voluntary movements and impaired connectivity caused by DA degeneration in PD (grey box).

neuronal precursor cell expressed developmentally down-regulated 4–2 (Nedd4-2), a member of the ubiquitin ligase enzymes E3 [43]. The increased Nedd4-2-mediated GLT-1 ubiquitination leads to GLT-1 internalization from the plasma membrane and lysosomal degradation.[43] Indeed, *in vivo* knockdown of Nedd4-2 ameliorates locomotor phenotypes, reverts DA neuron degeneration and attenuates the inflammatory activation of astrocytes and microglia suggesting that glutamate dyshomeostasis might be upstream of DA neurodegeneration.[43] Similar to MPTP, the injection of 6-OHDA in rat striatum causes a downregulation of GLT-1 and GLAST expression in the striatum, but not in the SNpc of 6-OHDA animals.[44] Again, treatment with ceftriaxone reduces striatal DA degeneration and the locomotor defects of 6-OHDA-injected rats.[45] The authors suggested that the antibiotic stimulates astrocytic GLT-1 expression, thus preventing overactivation of glutamatergic ionotropic receptors, i.e. N-methyl-D-aspartate (NMDA) receptors, and the subsequent Ca^{2+} overload in DA neurons. Recently, a novel molecular mechanism underlying GLT-1 downregulation in PD has been proposed by Wu et al. In MPP^{+}-treated astrocytes and MPTP-injected mice, the authors have identified miR-543–3p as a suppressor of GLT-1 protein and mRNA expression, and hence of its transport activity.[46] Furthermore, miR-543–3p inhibition was shown to increase GLT-1 expression and function thus relieving dyskinesia in the PD model.[46] A similar regulatory mechanism but mediated by miR-342–3p has been proposed by the same group.[47] Furthermore, intranigral application of glutamate receptor antagonists, like antagonists of group I metabotropic receptors, that comprise mGluR1 and mGluR5, appears to be efficient in preventing DA neurodegeneration in a 6-OHDA rat model.[48] Also, intrastriatal injection on MK-801, an antagonist of NMDA ionotropic receptors, prevented the Levodopa (L-Dopa)-induced increase in glutamate in 6-OHDA-lesioned rats.[49] Taking together these findings indicate that intervening with both nigral and striatal glutamate handling could block or slow DA neurodegeneration in PD.

Compelling evidence demonstrate an aberrant glutamate reuptake in several genetic animal models of PD. Mutations in and

Table 1
Reported glutamatergic defects in PD models.

PD models		Glutamatergic phenotype	References
Acute models	MPTP-lesioned mice	<< GLT-1 protein and mRNA expression in primary astrocytes and in the striatum	37,38
	6-0HDA-lesioned rats	<< GLT-1 and GLAST protein in the striatum but not in the substantia nigra	39
Genetic models	*SNCA mice*	<< GLT -1 and GLAST protein in A53T astrocytes from brainstem	48
		>> Release of glutamate in forebrain synaptoneurosomes of ASOTg mice	50
	PARK7 KO mice	<< GLT-1 but not of GLAST in primary cortical neurons and in whole brain	52
	PINK1 KO rats	<< Basal striatal glutamate neurotransmission	58
		>> glutamate striatal levels upon potassium stimulation	58
	PRNK rat neurons	<< Cell surface distribution of AMPA and NMDA receptors	59
	LRRK2 mice	>> EPSCs of SNPs in acute slices from Lrrk2 KO pups	65,68
		>> EPSCs of cortical neurons from Lrrk2 G2019S mice	69,70
		>> EPSCs of SNPs in acute slices from Lrrk2 G2019S adult mice	
		= EPSCs of SNPs in ≥6 months KI mice	72
Genetic manipulation of EAAT	Astrocytic downregulation of GLT-1 in the SNpc induces PD phenotypes in terms of progressive motor deficits and nigral DA neuronal death in mice		74

duplication or triplication of the *SNCA* gene, that encodes for the protein α-syn, were found to be associated with PD onset in human.[50–52] In a mouse model overexpressing the human PD-related A53T α-syn mutation selectively in astrocytes, it was described that A53T α-syn mutation results in a decreased expression level of functional GLT-1 and GLAST in the brainstem.[53] The authors hypothesized (but not demonstrated) that reduced expression of the two glutamate transporters could induce glutamate-induced excitotoxicity.[53] Furthermore, extended astrogliosis has been reported in the cerebral cortex, striatum, brainstem and spinal cord; microgliosis was observed in the brainstem, cerebellum, SNpc, but not in the cortex of this animal model.[53] Also, DA neuron degeneration has been found in the SNpc and in the ventral tegmental area of the same model.[53] A clear indication that aggregated α-syn (both fibrillar and oligomeric) might directly influence glutamatergic transmission comes from electrophysiology measurements.[54] In a study carried out on SH-SY5Y neuroblastoma cells and hippocampal mouse slices, Diógenes et al. have shown that exposure to α-syn oligomers, but not monomers or fibrils, increases basal synaptic transmission through NMDA receptor activation, triggering an enhanced contribution of glutamatergic Ca^{2+}-permeable (α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) AMPA ionotropic receptors.[54] They demonstrated that hippocampal brain slices treated with α-syn oligomers do not respond with further potentiation to a theta-burst stimulation, leading to impaired long-term potentiation (LTP).[54] This study provided new insights into a possible mechanism trough which α-syn could exert its toxicity in PD. Finally, a direct stimulation of glutamate release by both monomeric and fibrillar α-syn has been reported using synaptoneurosomes, an *in vitro* model that mimics functional synapses.[55] Moreover, α-syn increases Ca^{2+} depolarization-dependent presynaptic glutamate release as demonstrated using forebrain synaptoneurosomes from transgenic mice overexpressing the human α-syn protein throughout the brain (ASOTg).[55] Overall, these data suggest that α-syn can exert its toxicity by impacting glutamate clearance and transmission both in neurons and astrocytes.

Mutations in *PARK7* gene, that encodes for Protein/Nucleic Acid Deglycase (DJ-1) protein, are linked to early onset, familial forms of PD.[56] Downregulated levels of the glutamate transporter GLT-1 have been recognized in *Park7* knockout (KO) and mutant (M26I, E64D, and L166P) astrocytes.[57] DJ-1 regulates the assembly of lipid rafts, highly organized membrane microdomains that are involved in membrane receptor trafficking, endocytosis, and signal transduction.[58] At the membrane, GLT-1 assembles in the lipid rafts where it undergoes recycling.[59] In DJ-1 KO models, downregulation of the functional GLT-1, but not GLAST, correlates with a decreased level of two proteins crucially involved in lipid rafts assembly, flotillin-1 and caveolin-1, pointing to DJ-1 as a regulator of GLT-1 cellular localization.[57] Interestingly, the overexpression of wild-type but not DJ-1 familial mutants (M26I and L166P) in a DJ-1-null background reverts the decrease in glutamate uptake and GLT-1 downregulation.[57] DJ-1 protein is also involved in cellular protection against oxidative stress, especially reactive oxygen species (ROS) damage.[56] In addition to its association with neuroinflammation, cellular aging and degeneration, ROS production can reduce the uptake of glutamate.[60,61] Therefore, DJ-1 loss-of-function might intervene in PD pathology by inducing ROS-derived glutamate-induced excitoxicity. As a vicious circle, dysfunctional glutamate homeostasis could increase glutamate extracellular content which in turn would stimulate increases in Ca^{2+} levels at the mitochondria, eliciting overproduction of ROS, causing oxidative stress and its deleterious outcomes.

Mutations in *PINK1* (encoding for PINK1, PTEN-induced kinase 1) and *PRKN* (encoding for Parkin, E3-ubiquitin ligase) have been identified in familial recessive forms of PD.[62] Both PINK1 and Parkin are mitochondrial proteins involved in mitophagy, a cellular process that mediates mitochondrial quality control.[62] Age-dependent abnormalities in striatal glutamatergic transmission have been found in one study carried out in Pink1 KO rats.[63] Creed et al. applied *in vivo* microdialysis to measure both basal and potassium-induced release of different neurotransmitters and their metabolites in the striatum of awake *versus* freely moving rats.[63] Pink1 KO rats displayed a significant decrease of basal glutamate release between 4 and 12 months of age.[63] However, upon potassium stimulation, Pink1 KO rats showed an age-dependent increase in striatal glutamate levels.[63] Instead, no significant changes in both basal and evoked potassium-induced release of glutamate were reported for Parkin KO rats compared to controls and over time with age by the authors.[63] However, Zhu et al. revealed that overexpressing four Parkin forms commonly associated with PD (T240M, R275W, R334C, G430D) in hippocampal neurons from Parkin-null background rats impairs glutamatergic synaptic function.[64] Specifically, Parkin mutations disrupted glutamatergic synaptic transmission and plasticity by altering NMDA and AMPA cell-surface levels and, consequently, the receptor-mediated currents thus impairing LTP.[64] Moreover, they showed that Parkin regulates NMDA receptor trafficking through its direct ubiquitination while all Parkin mutants display a non-efficient receptor ubiquitination.[64] In the same study, PD pathological mutations in Parkin impacted the ability to regulate AMPA receptor trafficking by preventing the binding and retention of the postsynaptic scaffold Homer1.[64] Overall, this paper points to a major impact of Parkin pathological mutations in the biology of glutamate receptors. While neurons only were studied

so far, the pathological implication of mutated Parkin in glial cells, especially astrocytes and microglia, remains to be investigated. In addition, the impaired glutamate homeostasis observed in the whole brain of Pink1 KO mice raises the intriguing possibility that glial cells, possibly acting together, are responsible for the phenotype in whole brain and neurons.

The majority of inherited forms of PD are caused by mutations in the *PARK8* gene encoding for Leucine-rich repeat kinase 2 (LRRK2).[65] Mutations reside in the GTPase or kinase domains of LRRK2.[65] Among them, the pathogenic G2019S mutation is located in the kinase domain of the protein and is the most frequent variant identified in familial forms of PD.[66,67] The G2019S mutation induces a significant increase in LRRK2 kinase activity both *in vitro* and *in vivo* [68,69]. In the brain, LRRK2 is most highly expressed in striatal SPNs where it plays an important role in establishing and shaping the activity of corticostriatal circuits.[70] Indications of a physiological role of LRRK2 in modulating synaptic activity come from many studies carried out silencing Lrrk2 expression in cortical neurons or in SPNs of Lrrk2 KO mice. In 2011, Piccoli et al. demonstrated that Lrrk2 silencing in primary cortical neurons affects evoked postsynaptic currents and induces a redistribution of presynaptic vesicles, alteration of recycling dynamics, and increase of vesicle kinetics.[71] Furthermore, they found that LRRK2 protein interacts with several proteins involved in vesicular recycling, such as adaptor proteins 1 and 2, α-actinin 2, the clathrin coat assembly protein AP180, synapsin 1, VAMP2, SNAP25, dynamin 1 and synaptophysin.[71,72] Moreover, Parisiadou et al. revealed that Lrrk2 KO in mice have altered dendritic spine number and morphology on the SPNs, contributing to an abnormal synaptogenesis during development.[73] To determine if these alterations correlate with changes in SPNs synaptic transmission, they measured glutamatergic spontaneous excitatory postsynaptic currents (sEPSC) in striatal slices from Lrrk2 KO pups, revealing that EPSCs frequency is significantly reduced.[73] They finally demonstrated that Lrrk2 negatively modulates protein kinase A (PKA) activity during synaptogenesis and in response to D1 activation.[73] The absence of Lrrk2 promoted the synaptic translocation of PKA and increased PKA-mediated phosphorylation of AMPA glutamate receptor GluR1, resulting in abnormal synaptogenesis and transmission in the developing SPNs.[73] Furthermore, PKA-dependent phosphorylation of GluR1 was aberrantly enhanced in the striatum of P21 and 18-month-old Lrrk2 KO mice after treatment with a D1 agonist.[73]

Compelling evidence of an association between LRRK2 and glutamate in PD pathogenesis derives from studies carried out on mice harboring the common G2019S mutation. The majority of LRRK2 animal models display nearly absent or subtle DA neurodegeneration suggesting that glutamate dyshomeostasis is an early event in LRRK2-PD. An *in vitro* study by Beccano-Kelly et al. comparing primary cortical neuronal cultures derived from Lrrk2 KO and G2019S knock-in (KI) mice revealed that glutamate release is augmented in KI *versus* KO mice, as demonstrated by a >35% increase in sEPSC.[74] An independent study also revealed a four-fold increase in SPN sEPSC frequency at postnatal day 21 in KI mice and demonstrated that the application of LRRK2 kinase inhibitors normalized the electrical activity, suggesting that excessive neural activity in KI SPNs is LRRK2 kinase dependent.[70] Accordingly, Volta et al. demonstrated that sEPSC frequency, but not amplitude, is significantly changed in SNP acute striatal slices of aged 1–3 months KI mice compared to control.[75] These specific increases in glutamate release frequency were not maintained in older animals (>12 months).[75] From a behavioral point of view, young mice also manifested exploratory hyperactivity in cylinder exploration test.[75] In addition to early glutamatergic changes, also DA neurotransmission increases in SPNs from young (3 months) KI mice and decreases with aging (>12 months).[75] Of note, Lrrk2 G2019S-linked alterations in young adult mice are followed by hypodopaminergia, as well as mitochondrial and tau pathology.[76] A similar description of hyperactive phenotype in KI mice, starting at 3 months and continuing during aging, has been reported by Tozzi et al.[77] In agreement with Volta's results, no change in sEPSC was observed in SPNs of acute slices from 6 months old KI mice.[77] Moreover, the authors focused on DA receptors since they are primarily involved in the modulation of striatal synaptic transmission.[77] In order to analyze the effect of D2 receptor stimulation on striatal SPNs, they recorded sEPSC of KI SPNs in the presence of quinpirole, a D2 receptor agonist. Interestingly, they found that quinpirole does not modify sEPSC amplitude or frequency in wild-type animals.[77] By contrast, quinpirole significantly reduced sEPSC frequency without affecting amplitude in KI mice, suggesting a presynaptic modulation of this drug on striatal neurotransmission.[77] The authors evaluated the role of endocannabinoids (eCB) to explain the inhibitory presynaptic effects of quinpirole.[77] eCB are released into the extracellular space upon intracellular Ca^{2+} changes in SPNs and can bind CB1 inhibitory receptors on presynaptic glutamatergic neurons, thus promoting glutamate release inhibition.[77] In this line, they found that application of CB1 inhibitors prevented quinpirole-induce inhibitory responses.[77] At a first glance, these findings suggest that LRRK2 G2019S mutation profoundly impact glutamatergic neurotransmission by acting on neurons. However, LRRK2 is expressed at comparable levels also in astrocytes where it is emerging to play a functional role. Of note, Xiong et al. developed human TH promoter-controlled tetracycline-sensitive LRRK2 G2019S transgenic mice and showed using these mice that the mutation leads to an age- and kinase-dependent cell-autonomous neurodegeneration of DA neurons with a concomitant increase of reactive, glial fibrillary acidic protein (GFAP) positive astrocytes in the striatum.[78] Additional studies are required to shed light on possible implication of mutated LRRK2 in astrocytes and glia-mediated glutamate clearance.

Finally, recent observations clearly associate aberrant glutamate homeostasis with PD pathological manifestations. Indeed, exclusive downregulation of astrocytic GLT-1 in the SNpc, using adeno-associated viral vectors, induced PD phenotypes in terms of progressive motor deficits and nigral DA neuronal death in mice.[79] As reported for the acute and genetic PD models described above, the authors detected reactive astrocytes and microglia in the SNpc upon GLT-1 knockdown.[79] Furthermore, RNA sequencing analysis revealed altered gene expression patterns following GLT-1 knockdown in the SNpc.[79] Specifically, the results revealed disrupted Ca^{2+} signaling pathways that might be associated with apoptotic DA neuronal death in the SNpc.[79]

Concluding, increasing evidence highlight defects in glutamate handling in PD and robust data from genetics and pharmacology suggest that recovering aberrant glutamate homeostasis might be key to stop and revert neurodegenerative phenotypes.

The role of glial cells in PD pathology

Astrocytes and microglia are highly specialized glial cells of the CNS[6,21] (Fig. 2A). Astrocytes maintain neuronal vitality, supporting neurons in their metabolic and synaptic functions.[6] They possess a multitude of receptors to respond to neurotransmitters, including glutamate.[6] As a consequence, astrocytes promptly release “gliotransmitters” such as ATP, D-serine, GABA and glutamate that can modulate neuronal activity.[6,80] On one hand, as mentioned, they clear up the extrasynaptic space via the reuptake of glutamate as well as other neurotransmitters.[6] On the other hand, astrocytes control glutamate extra-synaptic diffusion thus protecting from glutamate spillover.[9,21] Finally, they cooperate with microglia in

maintaining the innate immune response of the CNS to trauma, injury or disease.[81] Microglia, the well-recognized resident immune cells, are specialized in coordinating immune response and protecting the CNS parenchyma from disease.[82,83] In normal physiological conditions, microglia are present in a "surveillance" phenotype, in which they appear highly ramified, with their dynamic processes continuously monitoring and maintaining the CNS homeostatic status.[21,82,83] By secreting neurotrophic factors such as nerve growth factor (NGF) and fibroblast growth factor (FGF), microglia help to maintain neuronal cell survival as well as circuit formation, integrity and plasticity.[84] Also, ramified microglia extend processes, contact synapses and play a functional dynamic role in synaptic plasticity using different mechanisms such as phagocytosis of synaptic elements.[84] Additionally, when an immune response recruits microglia, astrocytes surround the area and create a barrier to prevent the spread of toxic signals into the surrounding healthy CNS tissue.[85]

Accumulating evidence suggest that the "activation" or reactivity of astrocytes and microglia to challenges, referred as astrogliosis and microgliosis, play a pivotal role in both PD initiation and progression[86] (Fig. 2B). Immunohistochemical studies, labeling GFAP, a marker of reactive astrocytes, revealed the presence of astrogliosis in α-synucleinopathies[87] and α-syn-positive inclusions in astrocytes of idiopathic PD patients in different brain regions, including the striatum and cerebral cortex.[87–89] Furthermore, *in vivo* PET screening studies using [^{11}C](R)-PK11195, a radiotracer for pro-inflammatory glial cell activity (mainly used to label microglia, but also associated with astrocytic reactivity), revealed increased binding in the pons, basal ganglia and frontal and temporal cortical regions of PD patients.[90,91] The presence of reactive microglia, positively stained for the major histocompatibility complex class II antigen, tumor necrosis factor- α (TNF-α) and interleukin (IL)-6 has also been found in the SN and putamen of idiopathic PD patients.[92]

Once reactive, astrocytes and microglia change their morphology and acquire novel functions, producing and responding to anti-/pro-inflammatory cytokines and chemokines, which control, maintain and enhance the inflammatory condition.[93–95] Cytokines such as TNF-α and the chemokine ligands (CCL) 2 and 3 were shown to mediate microglial communication with astrocytes in mouse primary cultures, leading to astrocytic reactivity, for instance after exposure to the neurotoxin manganese.[96] In addition, microglia interact with astrocytes in rat primary cultures via ATP signaling onto astrocytic P2Y1, leading to neuroprotection after exposure to the neurotoxin methylmercury.[97] In acute mouse hippocampal slices, microglia-derived ATP was previously shown to stimulate astrocytic P2Y1 at steady-state, which resulted in an increased frequency of EPSCs via a mGluR5-dependent mechanism.[98] Depending on the context, reactive microglia can become deramified and ameboid, exacerbate their phagocytosis, exert anti-inflammatory and neuroprotective actions, or secrete pro-inflammatory cytokines and chemokines, such as inducible nitric oxide synthase (iNOS), ROS, reactive nitrogen species and CCL2 that attract peripheral monocytes into the brain and propagate the inflammatory state.[21,99,100] Furthermore, cytokines released by reactive microglia can activate receptor-mediated proapoptotic pathways on DA neurons, induce nitric oxide (NO) generation and increase the production of ROS, which lead to DNA damage, protein disruption and lipid peroxidation.[100] The latter causes increased prostaglandin E2 production by microglia resulting in direct toxicity to the DA neurons.[100] Increased levels of IL-6, IL-1β, TNF- α, and their receptors have been observed in both SN and striatum in a mouse model of acute PD.[101–103] In fact, application of the antibiotic minocycline, which blocks the production of IL-1 β, iNOS and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH)-oxidase, was shown to be neuroprotective in acute MPTP- and 6-OHDA PD models.[101,104–106] It has also been reported that pro-inflammatory microglia gradually outnumber the anti-inflammatory microglia in an MPTP-induced PD model.[100,107,108] Indeed, intraperitoneal injection of MPTP in mice increases the expression of IL-1 β, TNF-α and other inflammatory cytokines *in vitro* within nigral microglial cells and this phenotype is associated with progressive DA degeneration.[100,107,108]

Astrocytes can acquire different reactive phenotypes. In analogy with microglia, reactive astrocytes can be considered harmful to the CNS, since they produce proinflammatory factors, such as TNF-α, IL-1β, and ROS.[109] On the other side, in their reactive state astrocytes can be trophic and neuroprotective, by secreting neurotrophic factors like NGF, brain-derived neurotrophic factor (BDNF) and increasing the expression of glutamate reuptake systems.[109] Recently, Liddelow et al. reported the robust expression of neurotoxic reactive astrocytes in PD human brain.[94] Using *in situ* hybridization and immunohistochemical experiments on human brain slices from idiopathic PD patients, they found in the SN of PD patients a strong colocalization between GFAP and component complement 3 protein (C3), two markers of astrocytes with neurotoxic behaviors.[94] In the same study, they described that proinflammatory reactive microglia, secreting IL-1α, TNFα, and complement protein 1q (C1q), promote the astrocytic shift from neuroprotective to neurotoxic.[94] A recent study, carried out on the α-syn preformed fibril (α-syn PFF) model of sporadic PD indicates that α-syn fibrils induce microglial proinflammatory phenotype that, in turn, stimulates an astrocyte neurotoxic phenotype.[110] Similarly, astrocytes exposure to a α-syn PFF microglial conditioned medium results in a shift toward a toxic phenotype as suggested by the robust expression of GFAP and C3.[106]

Finally, a clear indication that astrocytes and microglia contribute to PD pathology comes from genetics. As we have already discussed, PD genes are expressed in astrocytes and microglia, and compelling evidence indicate that astrocytes and microglia harboring PD gene mutations display loss-of neuroprotective as well as gain of novel toxic functions possibly interfering with glutamate homeostasis or signaling.[86,111,112]

Glia-mediated handling of glutamate

Both astrocytes and microglia contribute to mediating glutamatergic transmission (Fig. 2A). As mentioned, astrocytes are responsible for most of the glutamate reuptake via their expression of EAAT1 and EAAT2 and they can sense glutamate extracellular content since they express AMPA and NMDA as well as group II mGluR3 and group I mGluR5.[6] The transformation into GFAP-positive, neurotoxic astrocytes can negatively impact on their ability to reuptake glutamate as identified in HD and ALS mouse models.[113–115] Vacuolization and astrogliosis are associated with a focal loss of the GLT-1 glutamate transporter. On this ground, as discussed above, astrogliosis and toxic reactive astrocytes have been observed also in PD brain, suggesting a similar reduction in glutamate buffering activity (Fig. 2B). Furthermore, it is worth noting that other mechanisms could be brought into action in non-physiological glutamate release. Na^+ gradient is indispensable for the inward direction of glutamate uptake and perturbation of Na^+ dynamics has been associated to many neurological disorders, including PD.[116,117] As a consequence, a reverse outward transport of glutamate could cause extracellular glutamate accumulation.

On the other hand, microglia in their surveillance state do not intervene in glutamate reuptake since they do not express glutamate transporters at rest (Fig. 2A).[21] However, microglia can start to synthetize *de novo* glutamate transporters when they become reactive, probably in response to the reduced ability of astrocytes to

reuptake glutamate[18,20,118] (Fig. 2B). Indeed, an increased microglial expression of EAAT1 has been found in cerebral cortex and striatum of human Creutzfeldt-Jakob disease as well as fatal familial insomnia.[118] Also, EAAT1 has been found to be upregulated in the brain of human immunodeficiency virus (HIV)-Infected patients.[20] Moreover, microglial GLAST and GLT-1 expression was found to increase in a rat model of cortical impact injury, while increases of EAAT2 and GS, which converts glutamate into glutamine, were observed in macaques infected with the simian immunodeficiency virus (SIV), an established animal model of HIV infection.[19]

Microglia also express glutamate receptors, both ionotropic and metabotropic, and can release glutamate.[21,119,120] Microglia express AMPA-type GluR1−GluR4 and kainate receptors, and were shown to respond to an NMDA agonist in the cortex of neonatal rats, indicating their display of functional NMDA receptors.[121,122] Also, microglia express members of all three groups of metabotropic glutamate receptors. Cultured rat microglia express group II mGluR2 and mGluR3 and group III mGluR4, mGluR6 and mGluR8, but not mGluR7.[119,120,123,124] In addition, microglia produce glutaminase, which generates glutamate from glutamine, and release glutamate through connexin 32 (Cx32) hemichannels. This mechanism was shown to cause microglial release of glutamate, leading to excitotoxic damage to neurons and dendrites in hippocampal culture from a mouse model of Rett syndrome.[125] In pathological contexts, other important non-vesicular mechanisms are involved in glutamate release by microglia[21] (Fig. 2B). The cysteine-glutamate transporters (also named cysteine−glutamate exchange system or xCs) is a Na^+-independent amino acid transporter that intakes one extracellular cystine extruding one glutamate.[1,5,21,126,127] Of note, it has been shown that xCs are activated during inflammation (see next paragraph for details).

Concluding, both astrocytes and microglia are supplied by a plethora of glutamate transporters and receptors, as well as enzymes, confirming that they are key players in glutamate signaling, clearance and release (Fig. 1). Therefore, any loss-of neuroprotective or gain of neurotoxic phenotypes by astrocytes and microglia involving dysregulation of their glutamate handling may contribute to glutamate-mediated toxicity in PD.

Inflammation: cause or consequence of glutamate alteration in PD?

As discussed inflammation and glutamate-induced excitotoxicity are two crucial processes involved in the onset and progression of PD, with a major contribution of glial cells.[25,60] However, it is still unclear whether inflammation is a cause or a consequence of glutamate dyshomeostasis. In PD, pathological mutations interfere *per se* with the biology of glutamate transporters and receptors supporting the idea that glutamate dyshomeostatsis may be the first or at least an early event. On this line, acute genetic manipulation of GLT-1 in SNpc astrocytes resulted in PD pathological and motor phenotypes in mice that were associated with progressing microgliosis and astrogliosis.[79] Indeed, glutamate accumulation and spillover overstimulate both neuronal and glial receptors. Coherently, transcriptomic data reveal that astrocytic GLT-1 ablation in mouse brain results in dysfunction of innate and adaptive immune pathways.[128] However, compelling evidence suggest that microglial reactivity contributes to non-physiologic glutamate release and promotes aberrant extrasynaptic signaling through ionotropic and metabotropic glutamate receptors.[21]

It is worth noting that some pathological PD-mutations are directly implicated in brain inflammation processes. For instance, LRRK2 that is highly expressed in astrocytes and microglia has been functionally linked to pathways pertinent to immune cell function, such as cytokine release, autophagy and phagocytosis.[129] Abolishing LRRK2 expression in BV-2 microglial-like cells[130] or mouse primary microglia[131] results in an higher migration capacity, while mouse primary microglia from KI mice have diminished ADP-induced migration.[132] In rats carrying the G2019S LRRK2 mutation, elevated neuroinflammation has been reported using PET imaging with the [^{11}C]PBR28 radioligand and immunohistochemistry.[133] Increased levels of reactive GFAP positive astrocytes were also found in a rat model carrying the G2019S mutation.[78]

As discussed above, microglia can increase on one hand glutamate release during inflammation by expressing the glutaminase enzyme, which further potentiates glutamatergic signaling.[21,134] On the other hand, reactive microglia can contribute to promoting glutamate-induced excitotoxicity in PD via the release of immune molecules[21,134] (Fig. 2B). This concept has been recently reviewed by Blaylock and defined as “Immunoexcitotoxicity”, to indicate the mechanism by which exacerbated levels of pro-inflammatory cytokines produced by microglia and astrocytes can contribute to neurodegeneration, with a particular focus on PD pathology.[134] It has been shown that TNF-α released by reactive microglia, binding to its receptors TNFR1, enhances the trafficking of Ca^{2+} permeable AMPA receptors at synapses.[134–136] Similarly, IL-1β enhances synaptic expression of NMDA receptors in the hippocampus of rats.[134,137] Furthermore, TNF-α can further contribute to glutamate toxicity inhibiting glutamate reuptake via NF-kB activation in rat organotypic hippocampal slices.[138] Moreover, high levels of TNF-α were measured in the striatum and cerebrospinal fluid of PD patients,[139] while TNF-immunoreactive microglial cells were found in the SNpc of PD versus control subjects.[140]

Cysteine intake allows for the synthesis of glutathione (GSH), the most abundant anti-oxidant molecule in the brain.[126] In PD, it has been shown that xCs release excessive extracellular glutamate.[126,141] Glutamate can bind the xCs leading to their toxic paralysis that results in GSH depletion and cell death.[21] This process has been referred as ‘oxidative glutamate toxicity’, in contrast to the excitotoxic glutamate toxicity resulting from overstimulation of ionotropic receptors.[5,21,126] Of note, inflammatory molecules such as TNF and lipopolysaccharide induce microglial expression of xCs.[21,142–144] As a result, microglia become capable of releasing glutamate, thus propagating excitotoxicity.[21,142–144] Moreover, IL1-β induces the expression of xCs transport on astrocytes via NF-kB signaling.[145] Increases in xCs expression have been observed by Western blot analysis in the striatum of acute-PD rat and mouse models[146,147] as well as in genetic mouse models of AD.[5,126] Intriguingly, a similar mechanism of action has been recently described in primary cultured microglia stimulated with α-syn aggregates.[148] In this study, the authors found that α-syn aggregates stimulate microglial reactivity via binding to both Toll-like receptor 2 and purinergic P2X7 receptors.[148] Then, the activation of an intracellular signaling cascade that involves phosphoinositide 3-kinase (PI3K) and NADPH oxidase promotes microglial glutamate release.[148] The inhibition of the xCs prevented the release of glutamate induced by aggregated α-syn, clearly indicating that xCs is a final effector of this process.[148] Furthermore, DA, via the activation of D1 dopamine receptors and the inhibition PI3K, induces a complete suppression of glutamate release by primary cultured microglia.[148] As a consequence, the authors concluded that the deficit in DA that characterizes PD may further aggravate this process in a vicious circle mechanism.[148]

Glutamate that accumulates at the synapse binds glutamatergic receptors located on microglia and can stimulate or inhibit their production and release of cytokines such as TNF-α[21](Fig. 2B). In fact, activation of kainate and NMDA receptors enhances microglial release of TNF-α, IL-1 and NO, while AMPA receptor activation inhibits TNF-α release.[119] Moreover, group I mGluR5 activation reduces TNF-α -production[149] while, activation of group II mGlu2 and

mGlu3 receptors potentiates microglial neurotoxicity[123] and increased TNF-α levels.[123,150] Conversely, the activation of group III metabotropic receptors has a neuroprotective role.[124] Antagonists of metabotropic receptors were revealed to attenuate neurodegeneration in MPTP rat and monkey models.[151,152]

As previously mentioned, astrocytes acquire in PD a neurotoxic phenotype, which has been proposed to contribute to neurodegeneration. Neurotoxic astrocytes release, as the pro-inflammatory activated microglia, pro-inflammatory cytokines.[94] The assumption of this proinflammatory phenotype also reduces the physiological ability of astrocytes to preserve brain integrity. As already discussed, neurotoxic reactive astrocytes in HD and ALS models displayed a reduced expression level of glutamate transporters.[113,114] This lead us to speculate that the reduction in glutamate transporter levels observed in both genetic and acute PD models could result from the presence of toxic astrocytes instead of neuroprotective ones, and that astrocytes in PD are mainly implicated with the course of pathology through the loss of their important physiological function. The exacerbation of microglial glutamate reuptake in this context would be insufficient to slow down or halt the disease progression and in turn, could further compromise the also important physiological functions of these resident immune cells.

Concluding, it is clear that reactive glial cells promotes an irreversible cascade of events that involve inflammation, glutamate-induced excitotoxicity and that, only as a final step, culminate with neurodegeneration. In this context, new therapeutic strategies aimed to control gliosis or to restore physiological glial cell functions could allow to treat, stop and/or prevent PD.

Targeting glial cells to moderate glutamate-induced toxicity

PD is the second most common ND affecting more than 6 million people worldwide and is currently without a cure.[153] Most of the current treatments are only able to manage few symptoms, such as motor ones, but do not prevent neurodegeneration and cognitive decline. Many of the therapeutic strategies aim to restore the function of DA neurons and are effective only within a narrow temporal window.[154] Prolonged treatment with L-Dopa is often accompanied by several side effects such as dyskinesia that limit its use.[155] There is therefore an urgent need to find innovative and more efficient treatments. Given the crucial role of glutamate-induced excitotoxicity in PD due to neuronal apoptosis induced via Ca^{2+} overload in cells, therapeutic strategies aiming to improve of all those mechanisms that promote glutamate homeostasis (i.e. potentiation of the expression of EAATs or inhibition of glutamate receptors) could be promising for the development of a PD cure.[15,156]

Glutamate transporters are also emerging as promising therapeutic targets.[15] As mentioned above, the downregulation of glutamate transporters is a common characteristic of NDs associated with cognitive deficits, and has been commonly found in PD. As already discussed, ceftriaxone treatment reverts the PD phenotype in MPTP and 6-OHDA models, by inducing GLT-1 expression.[42,45] Also, the finding that MPTP injection in the striatum decreased GLT-1 levels via Nedd4-2-mediated aberrant ubiquitination of the transporter, while promoting its lysosomal degradation, revealed that targeting Nedd4-2 could be beneficial to restore glutamate physiological levels.[43] More recently, it has been shown that treatment with ginsenoside Rb1, the main active ingredient of *Panax ginseng*, by promoting the expression of GLT-1, ameliorates motor deficits, prevents DA neurodegeneration and suppresses α-syn expression and astrogliosis in a MPTP mouse model.[157] Recent findings also point to vesicular glutamate transporter type 2 (VgluT2) as neuroprotective.[158] A novel report indicates that selective deletion of VgluT2 in DA neurons of conditional VgluT2-KO mice abolishes glutamate release from DA neurons and reduced their expression of BDNF and tyrosine receptor kinase B (TrkB), exacerbating the pathological effects of exposure to the MPTP.[158] Viral rescue of VgluT2 expression in DA neurons restored BDNF/TrkB expression as well as attenuated MPTP-induced DA neurodegeneration and locomotor impairment.[158] On this line, therapeutic strategies aimed to restore the physiological content of glutamate transporters could provide a great opportunity to modulate glutamate-induced excitotoxicity and to prevent excitotoxicity upstream. An extensive review on the potential therapeutic role of glutamate transporters in NDs has been recently published.[15]

Glutamate spillover induces the hyperactivation of glutamate receptors. Several studies propose to inhibit ionotropic glutamate receptors, in particular AMPA and NMDA, considering their major role in inducing excitotoxicity.[159] Antagonists of NMDA ionotropic glutamatergic receptors have been shown to prevent the locomotor behavioral phenotypes in many NDs, using for instance 6-OHDA rats[160] and MPTP-lesioned monkeys,[161,162] indicating that NMDA receptor blockade is efficacious in chronic models of PD. Also, combined with L-Dopa, AMPA receptor antagonists were able to improve the ability of L-Dopa to reverse motor deficits in SNc-lesioned rats and primates.[163–165] Nevertheless, the clinical application of glutamate ionotropic receptor antagonist drugs reveals no successful therapeutic outcomes because of their adverse side effects like psychosis, hallucinations, and impaired learning, likely due to the effects of altering glutamatergic signaling in healthy areas of the brain.[166] A recent research points to kainate glutamate receptors as a target to prevent DA neuron degeneration in a MPTP mouse model of PD.[167] On the other hand, metabotropic receptors seem to be more attractive therapeutic targets since they are not directly involved in excitotoxicity, but intervene in modulating glutamate activity.[168] Antagonists of these receptors are emerging as promising, without important side effects.[168] The activation of mGluR4 has been shown to prevent neuronal loss and inflammation resulting from microglial reactivity.[169] Furthermore, since mGluR4 are expressed on the terminals of GABAergic striatopallidal axons, their activation or potentiation has been shown to reduce excessive GABA release from these terminals and induces anti-PD effects.[170] On the other side, activation of mGluR2 has been recently found to prevent dyskinesia and behavioral alteration in a marmoset MPTP model of PD.[171]

Accumulated evidence suggest that anti-inflammatory drugs could have potential protective effects on DA neurons loss in PD animal models.[172] Other studies revealed a protective effect of non-aspirin non-steroidal anti-inflammatory drug use in PD patients, coherently with the presence of an extensive neuroinflammatory state in PD pathogenesis.[173] More recently, a novel attractive mechanism to inhibit neuroinflammation through regulating microglial reactivity was proposed using a MPTP mouse model of PD.[174] In this study, the authors identified Src, a non-receptor tyrosine kinase, and its pathway signaling as an attractive target to modulate neuroinflammation by demonstrating that Src regulates microgliosis *in vitro* and *in vivo*.[174]

The link between glutamate-induced excitotoxicity, inflammation and gliosis has received more attention. On the basis of the novel discoveries concerning the role of astrocytes and microglia in PD onset and progression, novel strategies have been proposed to promote glial beneficial phenotypes. Glial cell reactivity often results in “loss-of-function” changes and promotes pathogenesis *in vivo*. Recent findings in a MPTP mouse model of PD demonstrated that treatment with the secreted protein prokineticin-2 (PK2) promotes neuroprotective astrocytic phenotypes and increases expression of glutamate transporters (i.e. GLAST), thus promoting

glutamate reuptake and homeostasis.[175] Also, PK2-induced astrocytic reactivity leads to an increase in antioxidant and anti-inflammatory proteins, along with decreased inflammatory factors in both primary astrocytes and in the striatum.[175] On the basis of these results, the authors proposed that PK2 treatment could be a potential novel therapeutic strategy to prevent glutamate-induced excitotoxicity and inflammation not only in PD, but also in other related chronic NDs.

Despite all the novel discoveries suggesting that the modulation of astrogliosis and microgliosis could be a promising therapeutic target for PD, to date no therapies are in use to modulate glial activity in PD.

Conclusions

Impaired glutamate homeostasis in the striatum is emerging as a key feature of PD pathology. In the last decades, many studies have increased the current knowledge on the biology of glial cells and growing evidence indicates that astrocytes and microglia are active and crucial players in brain glutamate handling. On the other hand, increasing data strongly reveal that gain- or loss-of-function of these glial cells lead to important neuropathological consequences that markedly impact on brain functionality and behavior. On this line, recent findings point to the involvement of reactive astrocytes and microglia in several events that promote the onset and progression of PD, such as glutamate-induced excitotoxicity and neuroinflammation, opening a novel and thrilling field of investigation that needs to be pursued. Considering the absence of efficient therapeutic strategies for PD cure, and the increasing need to find treatments that are not only symptomatic, astrocytes and microglia are emerging as valuable candidates in this scenario. As discussed in the review, some appealing pharmacological targets might be identified by studying in depth the role of glial cells in glutamate homeostasis at the synapse. Future studies aimed to identify the basic molecular mechanisms that link the glutamate-induced excitotoxicity and inflammation processes to PD onset and progression, need to receive attention, since they could help to bridge the gap between research discoveries and clinical applications.

Contributions

LC and LI conceived and wrote the manuscript. MET contributed to writing and revising the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by UniPD (STARs 2019: Suppoting TAlents in ReSearch) and the Italian Ministry of Health (GR-2016-02363461) to LC. LI is a post-doctoral fellow supported by the Department of Biology, UniPD.

Declaration of competing interest

The Authors have no conflicts of interest to declare.

Acknowledgements

University of Padova to support LC as assistant professor and Canada Research Chair (Tier II) in Neurobiology of Aging and Cognition to MET.

References

1. Zhou Y, Danbolt NC. Glutamate as a neurotransmitter in the healthy brain. *J Neural Transm (Vienna)*. 2014;121(8):799–817. https://doi.org/10.1007/s00702-014-1180-8.
2. McDonald JW, Johnston MV. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Res Brain Res Rev*. 1990;15(1):41–70. https://doi.org/10.1016/0165-0173(90)90011-c.
3. Baj A, Moro E, Bistoletti M, Orlandi V, Crema F, Giaroni C. Glutamatergic signaling along the microbiota-gut-brain Axis. *Int J Mol Sci*. 2019;20(6). https://doi.org/10.3390/ijms20061482.
4. Robinson MB, Coyle JT. Glutamate and related acidic excitatory neurotransmitters: from basic science to clinical application. *Faseb J*. 1987;1(6):446–455. https://doi.org/10.1096/fasebj.1.6.2890549.
5. Lewerenz J, Maher P. Chronic glutamate toxicity in neurodegenerative diseases-what is the evidence? *Front Neurosci*. 2015;9:469. https://doi.org/10.3389/fnins.2015.00469.
6. Verkhratsky A, Nedergaard M. Physiology of astroglia. *Physiol Rev*. 2018;98(1):239–389. https://doi.org/10.1152/physrev.00042.2016.
7. Sonnewald U, Schousboe A. Introduction to the glutamate-glutamine cycle. *Adv Neurobiol*. 2016;13:1–7. https://doi.org/10.1007/978-3-319-45096-4_1.
8. Moussawi K, Riegel A, Nair S, Kalivas PW. Extracellular glutamate: functional compartments operate in different concentration ranges. *Front Syst Neurosci*. 2011;5:94. https://doi.org/10.3389/fnsys.2011.00094.
9. Rose CR, Felix L, Zeug A, Dietrich D, Reiner A, Henneberger C. Astroglial glutamate signaling and uptake in the Hippocampus. *Front Mol Neurosci*. 2017;10:451. https://doi.org/10.3389/fnmol.2017.00451.
10. Malik AR, Willnow TE. Excitatory amino acid transporters in physiology and disorders of the central nervous system. *Int J Mol Sci*. 2019;20(22). https://doi.org/10.3390/ijms20225671.
11. O'Donovan SM, Sullivan CR, McCullumsmith RE. The role of glutamate transporters in the pathophysiology of neuropsychiatric disorders. *NPJ Schizophr*. 2017;3(1):32. https://doi.org/10.1038/s41537-017-0037-1.
12. Furness DN, Dehnes Y, Akhtar AQ, et al. A quantitative assessment of glutamate uptake into hippocampal synaptic terminals and astrocytes: new insights into a neuronal role for excitatory amino acid transporter 2 (EAAT2). *Neuroscience*. 2008;157(1):80–94. https://doi.org/10.1016/j.neuroscience.2008.08.043.
13. Kim K, Lee S-G, Kegelman TP, et al. Role of excitatory amino acid transporter-2 (EAAT2) and glutamate in neurodegeneration: opportunities for developing novel therapeutics. *J Cell Physiol*. 2011;226(10):2484–2493. https://doi.org/10.1002/jcp.22609.
14. Hayashi MK. Structure-function relationship of transporters in the glutamate-glutamine cycle of the central nervous system. *Int J Mol Sci*. 2018;19(4). https://doi.org/10.3390/ijms19041177.
15. Pajarillo E, Rizor A, Lee J, Aschner M, Lee E. The role of astrocytic glutamate transporters GLT-1 and GLAST in neurological disorders: potential targets for neurotherapeutics. *Neuropharmacology*. 2019;161:107559. https://doi.org/10.1016/j.neuropharm.2019.03.002.
16. Pitt D, Nagelmeier IE, Wilson HC, Raine CS. Glutamate uptake by oligodendrocytes: implications for excitotoxicity in multiple sclerosis. *Neurology*. 2003;61(8):1113–1120. https://doi.org/10.1212/01.wnl.0000090564.88719.37.
17. DeSilva TM, Kabakov AY, Goldhoff PE, Volpe JJ, Rosenberg PA. Regulation of glutamate transport in developing rat oligodendrocytes. *J Neurosci*. 2009;29(24):7898–7908. https://doi.org/10.1523/JNEUROSCI.6129-08.2009.
18. van Landeghem FK, Stover JF, Bechmann I, et al. Early expression of glutamate transporter proteins in ramified microglia after controlled cortical impact injury in the rat. *Glia*. 2001;35(3):167–179. https://doi.org/10.1002/glia.1082.
19. Chretien F, Vallat-Decouvelaere A-V, Bossuet C, et al. Expression of excitatory amino acid transporter-2 (EAAT-2) and glutamine synthetase (GS) in brain macrophages and microglia of SIVmac251-infected macaques. *Neuropathol Appl Neurobiol*. 2002;28(5):410–417. https://doi.org/10.1046/j.1365-2990.2002.00426.x.
20. Vallat-Decouvelaere AV, Chrétien F, Gras G, Le Pavec G, Dormont D, Gray F. Expression of excitatory amino acid transporter-1 in brain macrophages and microglia of HIV-infected patients. A neuroprotective role for activated microglia? *J Neuropathol Exp Neurol*. 2003;62(5):475–485. https://doi.org/10.1093/jnen/62.5.475.
21. Haroon E, Miller AH, Sanacora G. Inflammation, glutamate, and glia: a trio of trouble in mood disorders. *Neuropsychopharmacology*. 2017;42(1):193–215. https://doi.org/10.1038/npp.2016.199.
22. Skaper SD, Facci L, Zusso M, Giusti P. An inflammation-centric view of neurological disease: beyond the neuron. *Front Cell Neurosci*. 2018;12:72. https://doi.org/10.3389/fncel.2018.00072.
23. Patel JP, Frey BN. Disruption in the blood-brain barrier: the missing link between brain and body inflammation in bipolar disorder? *Neural Plast*. 2015;2015:708306. https://doi.org/10.1155/2015/708306.
24. de Vries HE, Kooij G, Frenkel D, Georgopoulos S, Monsonego A, Janigro D. Inflammatory events at blood-brain barrier in neuroinflammatory and neurodegenerative disorders: implications for clinical disease. *Epilepsia*. 2012;53(Suppl 6):45–52. https://doi.org/10.1111/j.1528-1167.2012.03702.x.

25. Troncoso-Escudero P, Parra A, Nassif M, Vidal RL. Outside in: unraveling the role of neuroinflammation in the progression of Parkinson's disease. *Front Neurol*. 2018;9:860. https://doi.org/10.3389/fneur.2018.00860.
26. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature*. 1997;388(6645):839–840. https://doi.org/10.1038/42166.
27. Billingsley KJ, Bandres-Ciga S, Saez-Atienzar S, Singleton AB. Genetic risk factors in Parkinson's disease. *Cell Tissue Res*. 2018;373(1):9–20. https://doi.org/10.1007/s00441-018-2817-y.
28. Hernandez DG, Reed X, Singleton AB. Genetics in Parkinson disease: mendelian versus non-Mendelian inheritance. *J Neurochem*. 2016;139(Suppl 1):59–74. https://doi.org/10.1111/jnc.13593.
29. Blandini F, Porter RH, Greenamyre JT. Glutamate and Parkinson's disease. *Mol Neurobiol*. 1996;12(1):73–94. https://doi.org/10.1007/BF02740748.
30. Kreitzer AC. Physiology and pharmacology of striatal neurons. *Annu Rev Neurosci*. 2009;32:127–147. https://doi.org/10.1146/annurev.neuro.051508.135422.
31. Zhang H, Sulzer D. Glutamate spillover in the striatum depresses dopaminergic transmission by activating group I metabotropic glutamate receptors. *J Neurosci*. 2003;23(33):10585–10592.
32. Burke RE, O'Malley K. Axon degeneration in Parkinson's disease. *Exp Neurol*. 2013;246:72–83. https://doi.org/10.1016/j.expneurol.2012.01.011.
33. O'Gorman Tuura RL, Baumann CR, Baumann-Vogel H. Beyond dopamine: GABA, glutamate, and the axial symptoms of Parkinson disease. *Front Neurol*. 2018;9:806. https://doi.org/10.3389/fneur.2018.00806.
34. Weingarten CP, Sundman MH, Hickey P, Chen N. Neuroimaging of Parkinson's disease: expanding views. *Neurosci Biobehav Rev*. 2015;59:16–52. https://doi.org/10.1016/j.neubiorev.2015.09.007.
35. Gröger A, Kolb R, Schäfer R, Klose U. Dopamine reduction in the substantia nigra of Parkinson's disease patients confirmed by in vivo magnetic resonance spectroscopic imaging. *PloS One*. 2014;9(1). https://doi.org/10.1371/journal.pone.0084081. e84081.
36. Iwasaki Y, Ikeda K, Shiojima T, Kinoshita M. Increased plasma concentrations of aspartate, glutamate and glycine in Parkinson's disease. *Neurosci Lett*. 1992;145(2):175–177. https://doi.org/10.1016/0304-3940(92)90015-y.
37. Figura M, Kuśmierska K, Bucior E, et al. Serum amino acid profile in patients with Parkinson's disease. *PloS One*. 2018;13(1). https://doi.org/10.1371/journal.pone.0191670. e0191670.
38. Simola N, Morelli M, Carta AR. The 6-hydroxydopamine model of Parkinson's disease. *Neurotox Res*. 2007;11(3–4):151–167. https://doi.org/10.1007/bf03033565.
39. Meshul CK, Emre N, Nakamura CM, Allen C, Donohue MK, Buckman JF. Time-dependent changes in striatal glutamate synapses following a 6-hydroxydopamine lesion. *Neuroscience*. 1999;88(1):1–16. https://doi.org/10.1016/s0306-4522(98)00189-4.
40. Robinson S, Freeman P, Moore C, Touchon JC, Krentz L, Meshul CK. Acute and subchronic MPTP administration differentially affects striatal glutamate synaptic function. *Exp Neurol*. 2003;180(1):74–87. https://doi.org/10.1016/s0014-4886(02)00050-x.
41. Meredith GE, Totterdell S, Beales M, Meshul CK. Impaired glutamate homeostasis and programmed cell death in a chronic MPTP mouse model of Parkinson's disease. *Exp Neurol*. 2009;219(1):334–340. https://doi.org/10.1016/j.expneurol.2009.06.005.
42. Zhang Y, Zhang X, Qu S. Ceftriaxone protects astrocytes from MPP(+) via suppression of NF-κB/JNK/c-Jun signaling. *Mol Neurobiol*. 2015;52(1):78–92. https://doi.org/10.1007/s12035-014-8845-z.
43. Zhang Y, He X, Meng X, et al. Regulation of glutamate transporter trafficking by Nedd4-2 in a Parkinson's disease model. *Cell Death Dis*. 2017;8(2). https://doi.org/10.1038/cddis.2016.454. e2574.
44. Chung EKY, Chen LW, Chan YS, Yung KKL. Downregulation of glial glutamate transporters after dopamine denervation in the striatum of 6-hydroxydopamine-lesioned rats. *J Comp Neurol*. 2008;511(4):421–437. https://doi.org/10.1002/cne.21852.
45. Chotibut T, Davis RW, Arnold JC, et al. Ceftriaxone increases glutamate uptake and reduces striatal tyrosine hydroxylase loss in 6-OHDA Parkinson's model. *Mol Neurobiol*. 2014;49(3):1282–1292. https://doi.org/10.1007/s12035-013-8598-0.
46. Wu X, Meng X, Tan F, et al. Regulatory mechanism of miR-543-3p on GLT-1 in a mouse model of Parkinson's disease. *ACS Chem Neurosci*. 2019;10(3):1791–1800. https://doi.org/10.1021/acschemneuro.8b00683.
47. Wu D-M, Wang S, Wen X, et al. Suppression of microRNA-342-3p increases glutamate transporters and prevents dopaminergic neuron loss through activating the Wnt signaling pathway via p21-activated kinase 1 in mice with Parkinson's disease. *J Cell Physiol*. 2019;234(6):9033–9044. https://doi.org/10.1002/jcp.27577.
48. Vernon AC, Zbarsky V, Datla KP, Croucher MJ, Dexter DT. Subtype selective antagonism of substantia nigra pars compacta Group I metabotropic glutamate receptors protects the nigrostriatal system against 6-hydroxydopamine toxicity in vivo. *J Neurochem*. 2007;103(3):1075–1091. https://doi.org/10.1111/j.1471-4159.2007.04860.x.
49. Jonkers N, Sarre S, Ebinger G, Michotte Y. MK801 suppresses the L-DOPA-induced increase of glutamate in striatum of hemi-Parkinson rats. *Brain Res*. 2002;926(1–2):149–155. https://doi.org/10.1016/s0006-8993(01)03147-x.
50. Singleton AB, Farrer M, Johnson J, et al. alpha-Synuclein locus triplication causes Parkinson's disease. *Science*. 2003;302(5646):841. https://doi.org/10.1126/science.1090278.
51. Chartier-Harlin M-C, Kachergus J, Roumier C, et al. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet*. 2004;364(9440):1167–1169. https://doi.org/10.1016/S0140-6736(04)17103-1.
52. Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science*. 1997;276(5321):2045–2047. https://doi.org/10.1126/science.276.5321.2045.
53. Gu X-L, Long C-X, Sun L, Xie C, Lin X, Cai H. Astrocytic expression of Parkinson's disease-related A53T alpha-synuclein causes neurodegeneration in mice. *Mol Brain*. 2010;3:12. https://doi.org/10.1186/1756-6606-3-12.
54. Diógenes MJ, Dias RB, Rombo DM, et al. Extracellular alpha-synuclein oligomers modulate synaptic transmission and impair LTP via NMDA-receptor activation. *J Neurosci*. 2012;32(34):11750–11762. https://doi.org/10.1523/JNEUROSCI.0234-12.2012.
55. Sarafian TA, Littlejohn K, Yuan S, et al. Stimulation of synaptoneurosome glutamate release by monomeric and fibrillated α-synuclein. *J Neurosci Res*. 2017;95(9):1871–1887. https://doi.org/10.1002/jnr.24024.
56. Repici M, Giorgini F. DJ-1 in Parkinson's disease: clinical insights and therapeutic perspectives. *J Clin Med*. 2019;8(9). https://doi.org/10.3390/jcm8091377.
57. Kim J-M, Cha S-H, Choi YR, Jou I, Joe E-H, Park SM. DJ-1 deficiency impairs glutamate uptake into astrocytes via the regulation of flotillin-1 and caveolin-1 expression. *Sci Rep*. 2016;6:28823. https://doi.org/10.1038/srep28823.
58. Simons K, Ehehalt R. Cholesterol, lipid rafts, and disease. *J Clin Invest*. 2002;110(5):597–603. https://doi.org/10.1172/JCI16390.
59. Butchbach MER, Tian G, Guo H, Lin C-LG. Association of excitatory amino acid transporters, especially EAAT2, with cholesterol-rich lipid raft microdomains: importance for excitatory amino acid transporter localization and function. *J Biol Chem*. 2004;279(33):34388–34396. https://doi.org/10.1074/jbc.M403938200.
60. Tilleux S, Hermans E. Neuroinflammation and regulation of glial glutamate uptake in neurological disorders. *J Neurosci Res*. 2007;85(10):2059–2070. https://doi.org/10.1002/jnr.21325.
61. Meiser J, Vazquez A, Hiller K. DJ1 at the interface between neurodegeneration and cancer. *Oncotarget*. 2017;8(6):9015–9016. https://doi.org/10.18632/oncotarget.14889.
62. Truban D, Hou X, Caulfield TR, Fiesel FC, Springer W. PINK1, Parkin, and mitochondrial quality control: what can we learn about Parkinson's disease pathobiology? *J Parkinsons Dis*. 2017;7(1):13–29. https://doi.org/10.3233/JPD-160989.
63. Creed RB, Menalled L, Casey B, et al. Basal and evoked neurotransmitter levels in Parkin, DJ-1, PINK1 and LRRK2 knockout rat striatum. *Neuroscience*. 2019;409:169–179. https://doi.org/10.1016/j.neuroscience.2019.04.033.
64. Zhu M, Cortese GP, Waites CL. Parkinson's disease-linked Parkin mutations impair glutamatergic signaling in hippocampal neurons. *BMC Biol*. 2018;16(1):100. https://doi.org/10.1186/s12915-018-0567-7.
65. Cookson MR. The role of leucine-rich repeat kinase 2 (LRRK2) in Parkinson's disease. *Nat Rev Neurosci*. 2010;11(12):791–797. https://doi.org/10.1038/nrn2935.
66. Ozelius LJ, Senthil G, Saunders-Pullman R, et al. LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N Engl J Med*. 2006;354(4):424–425. https://doi.org/10.1056/NEJMc055509.
67. Goldwurm S, Di Fonzo A, Simons EJ, et al. The G6055A (G2019S) mutation in LRRK2 is frequent in both early and late onset Parkinson's disease and originates from a common ancestor. *J Med Genet*. 2005;42(11):e65. https://doi.org/10.1136/jmg.2005.035568.
68. Greggio E, Zambrano I, Kaganovich A, et al. The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation. *J Biol Chem*. 2008;283(24):16906–16914. https://doi.org/10.1074/jbc.M708718200.
69. Civiero L, Vancraenenbroeck R, Belluzzi E, et al. Biochemical characterization of highly purified leucine-rich repeat kinases 1 and 2 demonstrates formation of homodimers. *PloS One*. 2012;7(8). https://doi.org/10.1371/journal.pone.0043472. e43472.
70. Matikainen-Ankney BA, Kezunovic N, Mesias RE, et al. Altered development of synapse structure and function in striatum caused by Parkinson's disease-linked LRRK2-g2019S mutation. *J Neurosci*. 2016;36(27):7128–7141. https://doi.org/10.1523/JNEUROSCI.3314-15.2016.
71. Piccoli G, Condliffe SB, Bauer M, et al. LRRK2 controls synaptic vesicle storage and mobilization within the recycling pool. *J Neurosci*. 2011;31(6):2225–2237. https://doi.org/10.1523/JNEUROSCI.3730-10.2011.
72. Piccoli G, Onofri F, Cirnaru MD, et al. Leucine-rich repeat kinase 2 binds to neuronal vesicles through protein interactions mediated by its C-terminal WD40 domain. *Mol Cell Biol*. 2014;34(12):2147–2161. https://doi.org/10.1128/MCB.00914-13.
73. Parisiadou L, Yu J, Sgobio C, et al. LRRK2 regulates synaptogenesis and dopamine receptor activation through modulation of PKA activity. *Nat Neurosci*. 2014;17(3):367–376. https://doi.org/10.1038/nn.3636.
74. Beccano-Kelly DA, Kuhlmann N, Tatarnikov I, et al. Synaptic function is modulated by LRRK2 and glutamate release is increased in cortical neurons of G2019S LRRK2 knock-in mice. *Front Cell Neurosci*. 2014;8:301. https://doi.org/10.3389/fncel.2014.00301.

75. Volta M, Beccano-Kelly DA, Paschall SA, et al. Initial elevations in glutamate and dopamine neurotransmission decline with age, as does exploratory behavior, in LRRK2 G2019S knock-in mice. *Elife*. 2017;6. https://doi.org/10.7554/eLife.28377.
76. Yue M, Hinkle KM, Davies P, et al. Progressive dopaminergic alterations and mitochondrial abnormalities in LRRK2 G2019S knock-in mice. *Neurobiol Dis*. 2015;78:172–195. https://doi.org/10.1016/j.nbd.2015.02.031.
77. Tozzi A, Durante V, Bastioli G, et al. Dopamine D2 receptor activation potently inhibits striatal glutamatergic transmission in a G2019S LRRK2 genetic model of Parkinson's disease. *Neurobiol Dis*. 2018;118:1–8. https://doi.org/10.1016/j.nbd.2018.06.008.
78. Xiong Y, Neifert S, Karuppagounder SS, et al. Robust kinase- and age-dependent dopaminergic and norepinephrine neurodegeneration in LRRK2 G2019S transgenic mice. *Proc Natl Acad Sci USA*. 2018;115(7):1635–1640. https://doi.org/10.1073/pnas.1712648115.
79. Zhang Y, Meng X, Jiao Z, Liu Y, Zhang X, Qu S. Generation of a novel mouse model of Parkinson's disease via targeted knockdown of glutamate transporter GLT-1 in the substantia nigra. *ACS Chem Neurosci*. 2020;11(3):406–417. https://doi.org/10.1021/acschemneuro.9b00609.
80. Araque A, Carmignoto G, Haydon PG, Oliet SHR, Robitaille R, Volterra A. Gliotransmitters travel in time and space. *Neuron*. 2014;81(4):728–739. https://doi.org/10.1016/j.neuron.2014.02.007.
81. Ransohoff RM, Brown MA. Innate immunity in the central nervous system. *J Clin Invest*. 2012;122(4):1164–1171. https://doi.org/10.1172/JCI58644.
82. Norris GT, Kipnis J. Immune cells and CNS physiology: microglia and beyond. *J Exp Med*. 2019;216(1):60–70. https://doi.org/10.1084/jem.20180199.
83. Greenhalgh AD, David S, Bennett FC. Immune cell regulation of glia during CNS injury and disease. *Nat Rev Neurosci*. 2020;21(3):139–152. https://doi.org/10.1038/s41583-020-0263-9.
84. Reemst K, Noctor SC, Lucassen PJ, Hol EM. The indispensable roles of microglia and astrocytes during brain development. *Front Hum Neurosci*. 2016;10:566. https://doi.org/10.3389/fnhum.2016.00566.
85. Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol*. 2010;119(1):7–35. https://doi.org/10.1007/s00401-009-0619-8.
86. Joe E-H, Choi D-J, An J, Eun J-H, Jou I, Park S. Astrocytes, microglia, and Parkinson's disease. *Exp Neurobiol*. 2018;27(2):77–87. https://doi.org/10.5607/en.2018.27.2.77.
87. Forno LS, DeLanney LE, Irwin I, Di Monte D, Langston JW. Astrocytes and Parkinson's disease. *Prog Brain Res*. 1992;94:429–436. https://doi.org/10.1016/s0079-6123(08)61770-7.
88. Braak H, Sastre M, Del Tredici K. Development of alpha-synuclein immunoreactive astrocytes in the forebrain parallels stages of intraneuronal pathology in sporadic Parkinson's disease. *Acta Neuropathol*. 2007;114(3):231–241. https://doi.org/10.1007/s00401-007-0244-3.
89. Wakabayashi K, Hayashi S, Yoshimoto M, Kudo H, Takahashi H. NACP/alpha-synuclein-positive filamentous inclusions in astrocytes and oligodendrocytes of Parkinson's disease brains. *Acta Neuropathol*. 2000;99(1):14–20. https://doi.org/10.1007/pl00007400.
90. Gerhard A, Pavese N, Hotton G, et al. In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol Dis*. 2006;21(2):404–412. https://doi.org/10.1016/j.nbd.2005.08.002.
91. Edison P, Ahmed I, Fan Z, et al. Microglia, amyloid, and glucose metabolism in Parkinson's disease with and without dementia. *Neuropsychopharmacology*. 2013;38(6):938–949. https://doi.org/10.1038/npp.2012.255.
92. Ohashi T, Goitsuka R, Watari T, Tsujimoto H, Hasegawa A. Elevation of feline interleukin 6-like activity in feline immunodeficiency virus infection. *Clin Immunol Immunopathol*. 1992;65(3):207–211. https://doi.org/10.1016/0090-1229(92)90148-h.
93. Sochocka M, Diniz BS, Leszek J. Inflammatory response in the CNS: friend or foe? *Mol Neurobiol*. 2017;54(10):8071–8089. https://doi.org/10.1007/s12035-016-0297-1.
94. Liddelow SA, Guttenplan KA, Clarke LE, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. 2017;541(7638):481–487. https://doi.org/10.1038/nature21029.
95. Chhor V, Le Charpentier T, Lebon S, et al. Characterization of phenotype markers and neuronotoxic potential of polarised primary microglia in vitro. *Brain Behav Immun*. 2013;32:70–85. https://doi.org/10.1016/j.bbi.2013.02.005.
96. Kirkley KS, Popichak KA, Afzali MF, Legare ME, Tjalkens RB. Microglia amplify inflammatory activation of astrocytes in manganese neurotoxicity. *J Neuroinflammation*. 2017;14(1):99. https://doi.org/10.1186/s12974-017-0871-0.
97. Shinozaki Y, Nomura M, Iwatsuki K, Moriyama Y, Gachet C, Koizumi S. Microglia trigger astrocyte-mediated neuroprotection via purinergic gliotransmission. *Sci Rep*. 2014;4:4329. https://doi.org/10.1038/srep04329.
98. Pascual O, Ben Achour S, Rostaing P, Triller A, Bessis A. Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. *Proc Natl Acad Sci USA*. 2012;109(4):E197–E205. https://doi.org/10.1073/pnas.1111098109.
99. Cherry JD, Olschowka JA, O'Banion MK. Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. *J Neuroinflammation*. 2014;11:98. https://doi.org/10.1186/1742-2094-11-98.
100. Liu C-Y, Wang X, Liu C, Zhang H-L. Pharmacological targeting of microglial activation: new therapeutic approach. *Front Cell Neurosci*. 2019;13:514. https://doi.org/10.3389/fncel.2019.00514.
101. Wu DC, Jackson-Lewis V, Vila M, et al. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J Neurosci*. 2002;22(5):1763–1771.
102. Reynolds AD, Glanzer JG, Kadiu I, et al. Nitrated alpha-synuclein-activated microglial profiling for Parkinson's disease. *J Neurochem*. 2008;104(6):1504–1525. https://doi.org/10.1111/j.1471-4159.2007.05087.x.
103. Lofrumento DD, Saponaro C, Cianciulli A, et al. MPTP-induced neuroinflammation increases the expression of pro-inflammatory cytokines and their receptors in mouse brain. *Neuroimmunomodulation*. 2011;18(2):79–88. https://doi.org/10.1159/000320027.
104. Cankaya S, Cankaya B, Kilic U, Kilic E, Yulug B. The therapeutic role of minocycline in Parkinson's disease. *Drugs Context*. 2019;8. https://doi.org/10.7573/dic.212553, 212553.
105. He Y, Appel S, Le W. Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum. *Brain Res*. 2001;909(1–2):187–193. https://doi.org/10.1016/s0006-8993(01)02681-6.
106. Du Y, Ma Z, Lin S, et al. Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. *Proc Natl Acad Sci USA*. 2001;98(25):14669–14674. https://doi.org/10.1073/pnas.251341998.
107. Pisanu A, Lecca D, Mulas G, et al. Dynamic changes in pro- and anti-inflammatory cytokines in microglia after PPAR-γ agonist neuroprotective treatment in the MPTPp mouse model of progressive Parkinson's disease. *Neurobiol Dis*. 2014;71:280–291. https://doi.org/10.1016/j.nbd.2014.08.011.
108. Yao K, Zhao Y-F. Aging modulates microglia phenotypes in neuroinflammation of MPTP-PD mice. *Exp Gerontol*. 2018;111:86–93. https://doi.org/10.1016/j.exger.2018.07.010.
109. Liddelow SA, Barres BA. Reactive astrocytes: production, function, and therapeutic potential. *Immunity*. 2017;46(6):957–967. https://doi.org/10.1016/j.immuni.2017.06.006.
110. Yun SP, Kam T-I, Panicker N, et al. Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease. *Nat Med*. 2018;24(7):931–938. https://doi.org/10.1038/s41591-018-0051-5.
111. Booth HDE, Hirst WD, Wade-Martins R. The role of astrocyte dysfunction in Parkinson's disease pathogenesis. *Trends Neurosci*. 2017;40(6):358–370. https://doi.org/10.1016/j.tins.2017.04.001.
112. Lecours C, Bordeleau M, Cantin L, Parent M, Paolo TD, Tremblay M-È. Microglial implication in Parkinson's disease: loss of beneficial physiological roles or gain of inflammatory functions? *Front Cell Neurosci*. 2018;12:282. https://doi.org/10.3389/fncel.2018.00282.
113. Howland DS, Liu J, She Y, et al. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proc Natl Acad Sci USA*. 2002;99(3):1604–1609. https://doi.org/10.1073/pnas.032539299.
114. Behrens PF, Franz P, Woodman B, Lindenberg KS, Landwehrmeyer GB. Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. *Brain*. 2002;125(Pt 8):1908–1922. https://doi.org/10.1093/brain/awf180.
115. Li K, Li J, Zheng J, Qin S. Reactive astrocytes in neurodegenerative diseases. *Aging Dis*. 2019;10(3):664–675. https://doi.org/10.14336/AD.2018.0720.
116. Fontana ACK. Current approaches to enhance glutamate transporter function and expression. *J Neurochem*. 2015;134(6):982–1007. https://doi.org/10.1111/jnc.13200.
117. Mitani A, Tanaka K. Functional changes of glial glutamate transporter GLT-1 during ischemia: an in vivo study in the hippocampal CA1 of normal mice and mutant mice lacking GLT-1. *J Neurosci*. 2003;23(18):7176–7182.
118. Chrétien F, Le Pavec G, Vallat-Decouvelaere A-V, et al. Expression of excitatory amino acid transporter-1 (EAAT-1) in brain macrophages and microglia of patients with prion diseases. *J Neuropathol Exp Neurol*. 2004;63(10):1058–1071. https://doi.org/10.1093/jnen/63.10.1058.
119. Pocock JM, Kettenmann H. Neurotransmitter receptors on microglia. *Trends Neurosci*. 2007;30(10):527–535. https://doi.org/10.1016/j.tins.2007.07.007.
120. Murugan M, Ling E-A, Kaur C. Glutamate receptors in microglia. *CNS Neurol Disord - Drug Targets*. 2013;12(6):773–784. https://doi.org/10.2174/18715273113126660174.
121. Noda M, Nakanishi H, Nabekura J, Akaike N. AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia. *J Neurosci*. 2000;20(1):251–258.
122. Acarin L, González B, Castellano B, Castro AJ. Microglial response to N-methyl-D-aspartate-mediated excitotoxicity in the immature rat brain. *J Comp Neurol*. 1996;367(3):361–374. https://doi.org/10.1002/(SICI)1096-9861(19960408)367:3<361. AID-CNE4>3.0.CO;2–3.
123. Taylor DL, Diemel LT, Cuzner ML, Pocock JM. Activation of group II metabotropic glutamate receptors underlies microglial reactivity and neurotoxicity following stimulation with chromogranin A, a peptide up-regulated in Alzheimer's disease. *J Neurochem*. 2002;82(5):1179–1191. https://doi.org/10.1046/j.1471-4159.2002.01062.x.
124. Taylor DL, Diemel LT, Pocock JM. Activation of microglial group III metabotropic glutamate receptors protects neurons against microglial neurotoxicity. *J Neurosci*. 2003;23(6):2150–2160.
125. Maezawa I, Jin L-W. Rett syndrome microglia damage dendrites and synapses by the elevated release of glutamate. *J Neurosci*. 2010;30(15):5346–5356. https://doi.org/10.1523/JNEUROSCI.5966-09.2010.
126. Lewerenz J, Hewett SJ, Huang Y, et al. The cystine/glutamate antiporter system x(c)(-) in health and disease: from molecular mechanisms to novel

therapeutic opportunities. *Antioxidants Redox Signal*. 2013;18(5):522–555. https://doi.org/10.1089/ars.2011.4391.
127. Malarkey EB, Parpura V. Mechanisms of glutamate release from astrocytes. *Neurochem Int*. 2008;52(1–2):142–154. https://doi.org/10.1016/j.neuint.2007.06.005.
128. Sharma A, Kazim SF, Larson CS, et al. Divergent roles of astrocytic versus neuronal EAAT2 deficiency on cognition and overlap with aging and Alzheimer's molecular signatures. *Proc Natl Acad Sci USA*. 2019;116(43):21800–21811. https://doi.org/10.1073/pnas.1903566116.
129. Wallings RL, Tansey MG. LRRK2 regulation of immune-pathways and inflammatory disease. *Biochem Soc Trans*. 2019;47(6):1581–1595. https://doi.org/10.1042/BST20180463.
130. Kim B, Yang M-S, Choi D, et al. Impaired inflammatory responses in murine Lrrk2-knockdown brain microglia. *PloS One*. 2012;7(4). https://doi.org/10.1371/journal.pone.0034693. e34693.
131. Ma B, Xu L, Pan X, et al. LRRK2 modulates microglial activity through regulation of chemokine (C-X3-C) receptor 1 -mediated signalling pathways. *Hum Mol Genet*. 2016;25(16):3515–3523. https://doi.org/10.1093/hmg/ddw194.
132. Choi I, Kim B, Byun J-W, et al. LRRK2 G2019S mutation attenuates microglial motility by inhibiting focal adhesion kinase. *Nat Commun*. 2015;6:8255. https://doi.org/10.1038/ncomms9255.
133. Schildt A, Walker MD, Dinelle K, et al. Single inflammatory trigger leads to neuroinflammation in LRRK2 rodent model without degeneration of dopaminergic neurons. *J Parkinsons Dis*. 2019;9(1):121–139. https://doi.org/10.3233/JPD-181446.
134. Blaylock RL. Parkinson's disease: microglial/macrophage-induced immunoexcitotoxicity as a central mechanism of neurodegeneration. *Surg Neurol Int*. 2017;8:65. https://doi.org/10.4103/sni.sni_441_16.
135. Bernardino L, Xapelli S, Silva AP, et al. Modulator effects of interleukin-1beta and tumor necrosis factor-alpha on AMPA-induced excitotoxicity in mouse organotypic hippocampal slice cultures. *J Neurosci*. 2005;25(29):6734–6744. https://doi.org/10.1523/JNEUROSCI.1510-05.2005.
136. Wang D. Tumor necrosis factor-alpha alters electrophysiological properties of rabbit hippocampal neurons. *J Alzheimers Dis*. 2019;68(3):1257–1271. https://doi.org/10.3233/JAD-190043.
137. Gardoni F, Boraso M, Zianni E, et al. Distribution of interleukin-1 receptor complex at the synaptic membrane driven by interleukin-1β and NMDA stimulation. *J Neuroinflammation*. 2011;8(1):14. https://doi.org/10.1186/1742-2094-8-14.
138. Zou JY, Crews FT. TNF alpha potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: neuroprotection by NF kappa B inhibition. *Brain Res*. 2005;1034(1–2):11–24. https://doi.org/10.1016/j.brainres.2004.11.014.
139. Mogi M, Harada M, Riederer P, Narabayashi H, Fujita K, Nagatsu T. Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. *Neurosci Lett*. 1994;165(1–2):208–210. https://doi.org/10.1016/0304-3940(94)90746-3.
140. Boka G, Anglade P, Wallach D, Javoy-Agid F, Agid Y, Hirsch EC. Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. *Neurosci Lett*. 1994;172(1–2):151–154. https://doi.org/10.1016/0304-3940(94)90684-x.
141. Bridges R, Lutgen V, Lobner D, Baker DA. Thinking outside the cleft to understand synaptic activity: contribution of the cystine-glutamate antiporter (System xc-) to normal and pathological glutamatergic signaling. *Pharmacol Rev*. 2012;64(3):780–802. https://doi.org/10.1124/pr.110.003889.
142. Piani D, Fontana A. Involvement of the cystine transport system xc- in the macrophage-induced glutamate-dependent cytotoxicity to neurons. *J Immunol*. 1994;152(7):3578–3585.
143. Figuera-Losada M, Rojas C, Slusher BS. Inhibition of microglia activation as a phenotypic assay in early drug discovery. *J Biomol Screen*. 2014;19(1):17–31. https://doi.org/10.1177/1087057113499406.
144. Mesci P, Zaïdi S, Lobsiger CS, et al. System xC- is a mediator of microglial function and its deletion slows symptoms in amyotrophic lateral sclerosis mice. *Brain*. 2015;138(Pt 1):53–68. https://doi.org/10.1093/brain/awu312.
145. Jackman NA, Uliasz TF, Hewett JA, Hewett SJ. Regulation of system x(c)(-) activity and expression in astrocytes by interleukin-1β: implications for hypoxic neuronal injury. *Glia*. 2010;58(15):1806–1815. https://doi.org/10.1002/glia.21050.
146. Massie A, Schallier A, Mertens B, et al. Time-dependent changes in striatal xCT protein expression in hemi-Parkinson rats. *Neuroreport*. 2008;19(16):1589–1592. https://doi.org/10.1097/WNR.0b013e328312181c.
147. Massie A, Schallier A, Kim SW, et al. Dopaminergic neurons of system x(c)$^-$-deficient mice are highly protected against 6-hydroxydopamine-induced toxicity. *Faseb J*. 2011;25(4):1359–1369. https://doi.org/10.1096/fj.10-177212.
148. Dos-Santos-Pereira M, Acuña L, Hamadat S, et al. Microglial glutamate release evoked by α-synuclein aggregates is prevented by dopamine. *Glia*. 2018;66(11):2353–2365. https://doi.org/10.1002/glia.23472.
149. Byrnes KR, Stoica B, Loane DJ, Riccio A, Davis MI, Faden AI. Metabotropic glutamate receptor 5 activation inhibits microglial associated inflammation and neurotoxicity. *Glia*. 2009;57(5):550–560. https://doi.org/10.1002/glia.20783.
150. Taylor DL, Jones F, Kubota ESFCS, Pocock JM. Stimulation of microglial metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor alpha-induced neurotoxicity in concert with microglial-derived Fas ligand. *J Neurosci*. 2005;25(11):2952–2964. https://doi.org/10.1523/JNEUROSCI.4456-04.2005.
151. Masilamoni GJ, Bogenpohl JW, Alagille D, et al. Metabotropic glutamate receptor 5 antagonist protects dopaminergic and noradrenergic neurons from degeneration in MPTP-treated monkeys. *Brain*. 2011;134(Pt 7):2057–2073. https://doi.org/10.1093/brain/awr137.
152. Hsieh M-H, Ho S-C, Yeh K-Y, et al. Blockade of metabotropic glutamate receptors inhibits cognition and neurodegeneration in an MPTP-induced Parkinson's disease rat model. *Pharmacol Biochem Behav*. 2012;102(1):64–71. https://doi.org/10.1016/j.pbb.2012.03.022.
153. Finsterwald C, Magistretti PJ, Lengacher S. Astrocytes: new targets for the treatment of neurodegenerative diseases. *Curr Pharmaceut Des*. 2015;21(25):3570–3581. https://doi.org/10.2174/1381612821666150710144502.
154. Oertel W, Schulz JB. Current and experimental treatments of Parkinson disease: a guide for neuroscientists. *J Neurochem*. 2016;139(Suppl 1):325–337. https://doi.org/10.1111/jnc.13750.
155. Stoker TB, Torsney KM, Barker RA. Emerging treatment approaches for Parkinson's disease. *Front Neurosci*. 2018;12:693. https://doi.org/10.3389/fnins.2018.00693.
156. Calabresi P, Di Filippo M, Gallina A, et al. New synaptic and molecular targets for neuroprotection in Parkinson's disease. *Mov Disord*. 2013;28(1):51–60. https://doi.org/10.1002/mds.25096.
157. Zhang Y-L, Liu Y, Kang X-P, et al. Ginsenoside Rb1 confers neuroprotection via promotion of glutamate transporters in a mouse model of Parkinson's disease. *Neuropharmacology*. 2018;131:223–237. https://doi.org/10.1016/j.neuropharm.2017.12.012.
158. Shen H, Marino RAM, McDevitt RA, et al. Genetic deletion of vesicular glutamate transporter in dopamine neurons increases vulnerability to MPTP-induced neurotoxicity in mice. *Proc Natl Acad Sci USA*. 2018;115(49):E11532–E11541. https://doi.org/10.1073/pnas.1800886115.
159. Johnson KA, Conn PJ, Niswender CM. Glutamate receptors as therapeutic targets for Parkinson's disease. *CNS Neurol Disord - Drug Targets*. 2009;8(6):475–491. https://doi.org/10.2174/187152709789824606.
160. Kelsey JE, Mague SD, Pijanowski RS, Harris RC, Kleckner NW, Matthews RT. NMDA receptor antagonists ameliorate the stepping deficits produced by unilateral medial forebrain bundle injections of 6-OHDA in rats. *Psychopharmacology*. 2004;175(2):179–188. https://doi.org/10.1007/s00213-004-1799-5.
161. Steece-Collier K, Chambers LK, Jaw-Tsai SS, Menniti FS, Greenamyre JT. Antiparkinsonian actions of CP-101,606, an antagonist of NR2B subunit-containing N-methyl-d-aspartate receptors. *Exp Neurol*. 2000;163(1):239–243. https://doi.org/10.1006/exnr.2000.7374.
162. Graham WC, Robertson RG, Sambrook MA, Crossman AR. Injection of excitatory amino acid antagonists into the medial pallidal segment of a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated primate reverses motor symptoms of parkinsonism. *Life Sci*. 1990;47(18):PL91–PL97. https://doi.org/10.1016/0024-3205(90)90376-3.
163. Löschmann PA, Lange KW, Kunow M, et al. Synergism of the AMPA-antagonist NBQX and the NMDA-antagonist CPP with L-dopa in models of Parkinson's disease. *J Neural Transm Park Dis Dement Sect*. 1991;3(3):203–213. https://doi.org/10.1007/bf02259538.
164. Löschmann PA, Kunow M, Wachtel H. Synergism of NBQX with dopamine agonists in the 6-OHDA rat model of Parkinson's disease. *J Neural Transm Suppl*. 1992;38:55–64.
165. Klockgether T, Turski L, Honoré T, et al. The AMPA receptor antagonist NBQX has antiparkinsonian effects in monoamine-depleted rats and MPTP-treated monkeys. *Ann Neurol*. 1991;30(5):717–723. https://doi.org/10.1002/ana.410300513.
166. Paoletti P, Neyton J. NMDA receptor subunits: function and pharmacology. *Curr Opin Pharmacol*. 2007;7(1):39–47. https://doi.org/10.1016/j.coph.2006.08.011.
167. Stayte S, Laloli KJ, Rentsch P, et al. The kainate receptor antagonist UBP310 but not single deletion of GluK1, GluK2, or GluK3 subunits, inhibits MPTP-induced degeneration in the mouse midbrain. *Exp Neurol*. 2020;323:113062. https://doi.org/10.1016/j.expneurol.2019.113062.
168. Crupi R, Impellizzeri D, Cuzzocrea S. Role of metabotropic glutamate receptors in neurological disorders. *Front Mol Neurosci*. 2019;12:20. https://doi.org/10.3389/fnmol.2019.00020.
169. Ponnazhagan R, Harms AS, Thome AD, et al. The metabotropic glutamate receptor 4 positive allosteric modulator ADX88178 inhibits inflammatory responses in primary microglia. *J Neuroimmune Pharmacol*. 2016;11(2):231–237. https://doi.org/10.1007/s11481-016-9655-z.
170. Jones CK, Bubser M, Thompson AD, et al. The metabotropic glutamate receptor 4-positive allosteric modulator VU0364770 produces efficacy alone and in combination with L-DOPA or an adenosine 2A antagonist in preclinical rodent models of Parkinson's disease. *J Pharmacol Exp Therapeut*. 2012;340(2):404–421. https://doi.org/10.1124/jpet.111.187443.
171. Sid-Otmane L, Hamadjida A, Nuara SG, et al. Selective metabotropic glutamate receptor 2 positive allosteric modulation alleviates L-DOPA-induced psychosis-like behaviours and dyskinesia in the MPTP-lesioned marmoset. *Eur J Pharmacol*. 2020;873:172957. https://doi.org/10.1016/j.ejphar.2020.172957.
172. Martinez B, Peplow PV. Neuroprotection by immunomodulatory agents in animal models of Parkinson's disease. *Neural Regen Res*. 2018;13(9):1493–1506. https://doi.org/10.4103/1673-5374.237108.
173. Ren L, Yi J, Yang J, Li P, Cheng X, Mao P. Nonsteroidal anti-inflammatory drugs use and risk of Parkinson disease: a dose-response meta-analysis. *Medicine*

(Baltim). 2018;97(37). https://doi.org/10.1097/MD.0000000000012172. e12172.
174. Yang H, Wang L, Zang C, et al. Src inhibition attenuates neuroinflammation and protects dopaminergic neurons in Parkinson's disease models. *Front Neurosci*. 2020;14:45. https://doi.org/10.3389/fnins.2020.00045.
175. Neal M, Luo J, Harischandra DS, et al. Prokineticin-2 promotes chemotaxis and alternative A2 reactivity of astrocytes. *Glia*. 2018;66(10):2137–2157. https://doi.org/10.1002/glia.23467.
176. Zhang Y, Chen K, Sloan SA, et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci*. 2014;34(36):11929–11947. https://doi.org/10.1523/JNEUROSCI.1860-14.2014.
177. Zhang Y, Sloan SA, Clarke LE, et al. Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. *Neuron*. 2016;89(1):37–53. https://doi.org/10.1016/j.neuron.2015.11.013.