REVIEW

Converging roles of ion channels, calcium, metabolic stress, and activity pattern of *Substantia nigra* dopaminergic neurons in health and Parkinson's disease

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Abstract

Dopamine-releasing neurons within the *Substantia nigra* (SN DA) are particularly vulnerable to degeneration compared to other dopaminergic neurons. The age-dependent, progressive loss of these neurons is a pathological hallmark of Parkinson's disease (PD), as the resulting loss of striatal dopamine causes its major movement-related symptoms. SN DA neurons release dopamine from their axonal terminals within the dorsal striatum, and also from their cell bodies and dendrites within the midbrain in a calcium- and activity-dependent manner. Their intrinsically generated and metabolically challenging activity is created and modulated by the orchestrated function of different ion channels and dopamine D2-autoreceptors. Here, we review increasing evidence that the mechanisms that control activity patterns and calcium homeostasis of SN DA neurons are not only crucial for their dopamine release within a physiological range but also modulate their mitochondrial and lysosomal activity, their metabolic stress levels, and their vulnerability to degeneration in PD. Indeed, impaired calcium homeostasis, lysosomal and mitochondrial dysfunction, and metabolic stress in SN DA neurons represent central converging trigger factors for idiopathic and familial PD. We summarize double-edged roles of ion channels, activity patterns, calcium homeostasis, and related feedback/feed-forward signaling mechanisms in SN DA neurons for maintaining and modulating their physiological function, but also for contributing to their vulnerability in PD-paradigms. We focus on the emerging roles of maintained neuronal activity and calcium homeostasis within a physiological bandwidth, and its modulation by PD-triggers, as well as on bidirectional functions of voltage-gated L-type calcium channels and

SN DA neurons – specific functions and fate in Parkinson's disease

Functional anatomy of the dopaminergic midbrain system

Dopamine-releasing catecholaminergic neurons within the midbrain are part of the basal ganglia network, and are crucial for a variety of fundamental brain functions, such as voluntary movement, goal-directed behavior and habit formation, and also motivation, emotion, cognition, reward, memory, associative learning, and decision making (Bjorklund and Dunnett 2007; Gerfen and Surmeier 2011; D'Ardenne *et al.* 2012; Pignatelli and Bonci 2015). Consequently, their loss or their dysfunction is associated with respective pathophysiologies, like schizophrenia, substance abuse, or Parkinson's disease (PD) (Wise 2009; Brisch *et al.* 2014; Kalia and Lang 2015).

The cell bodies of tyrosine hydroxylase-positive dopaminergic (DA) neurons within the midbrain are mainly arranged within three nuclei in close proximity: the ventral tegmental area (VTA, or A10 group), the retrorubral field (RRF, or A8 group), and the *Substantia nigra* (SN, or A9 group) (Dahlstrom and Fuxe 1964; Bjorklund and Dunnett 2007). Simplified, according to their widespread axonal projections, DA midbrain neurons are subdivided into the mesocorticolimbic system (VTA and RRF DA neurons), and the mesostriatal system (SN DA neurons). However, in-depth cell-specific analyses of DA projections and inputs, biophysical properties, neuronal wiring, gene expression, and physiological functions have revealed a much more complex picture, defining a variety of different subpopulations of midbrain DA neurons (Bromberg-Martin *et al.* 2010; Liss and Roeper 2010; Bourdy and Barrot 2012; Lammel *et al.* 2012; Ungless and Grace 2012; Watabe-Uchida *et al.* 2012; Jhou *et al.* 2013; Roeper 2013; Volman *et al.* 2013; Poulin *et al.* 2014; Beier *et al.* 2015; Hoglinger *et al.* 2015; Lerner *et al.* 2015).

Electrical activity patterns of SN DA neurons *in vivo* and *in vitro*

The number of SN DA neurons ranges from about 10.000 (bilaterally) in adult mice to about 400.000 in adult humans (Damier *et al.* 1999a; Brichta and Greengard 2014), and they are predominantly found within the *SN pars compacta*. They receive direct excitatory inputs from the subthalamic nucleus, the pedunculopontine nucleus, as well as from somatosensory and motor cortices, and they receive inhibitory inputs particularly from the (dorsolateral) striatum (Grillner and Mercuri 2002; Henny *et al.* 2012; Watabe-Uchida *et al.* 2012; Lerner *et al.* 2015).

In vivo, within the intact basal ganglia network, SN DA neurons predominantly display two types of firing patterns: tonic irregular single-spike activity in the frequency range of 1–10 Hz (compare Fig. 2) (Hage and Khaliq 2015), or phasic so-called burst activity at higher frequencies (~ 13–20 Hz in anesthetized animals, or up to 80 Hz in awake animals or humans), as originally described by the pioneering work of Grace and Bunney (Grace and Bunney 1984a,b) and Johnson (Johnson *et al.* 1992), reviewed, e.g. in (Johnson and Wu 2004; Bean 2007a; Deister *et al.* 2009; Lee and Tepper 2009; Paladini and Roeper 2014; Dragicevic *et al.* 2015). Burst activity of SN DA neurons causes a phasic supralinear increase in released dopamine, predominantly in response to novel, unexpected or salient events (Schultz *et al.* 2015), and it encodes the so-called '*Go*' signals that facilitate movement initiation and motor learning (Jin and Costa 2010; Costa 2011; Sommer *et al.* 2014). As illustrated in Figs 1 upper and 2, *in vitro*, even in complete synaptic isolation, SN DA neurons still display an intrinsically generated, very regular activity with relatively low frequencies (~ 0.5–4 Hz), the so-called pacemaker activity (Grace and Onn 1989; Bean 2007a; Chan *et al.* 2007; Lammel *et al.* 2008; Margolis *et al.* 2010).

SN DA neurons are particularly vulnerable to degenerative triggers

Although seemingly more homogeneous than the VTA/RRF DA neurons, recent work identified distinct subpopulations of DA neurons also within the SN: the medial and lateral SN DA neurons, with axonal projections to the dorsomedial and dorsolateral striatum, respectively (Bjorklund and Dunnett 2007; Schiemann *et al.* 2012; Lerner *et al.* 2015), as well as the ventral tier and dorsal tier SN DA neurons (Damier *et al.* 1999a; Watabe-Uchida *et al.* 2012; Lehericy *et al.* 2014). However, dorsal tier SN DA neurons are more 'VTA/RRF DA-type' like, in view of their electrophysiological properties, their expression of the cytosolic calcium-binding protein calbindin-D28K, the absence of DCC (deleted in colorectal cancer, the receptor for the axon guidance molecule netrin), and their lower susceptibility to degenerative triggers and to cell death in PD (Yamada *et al.* 1990; Damier *et al.* 1999a; Neuhoff *et al.* 2002; Lammel *et al.* 2008; Margolis *et al.*
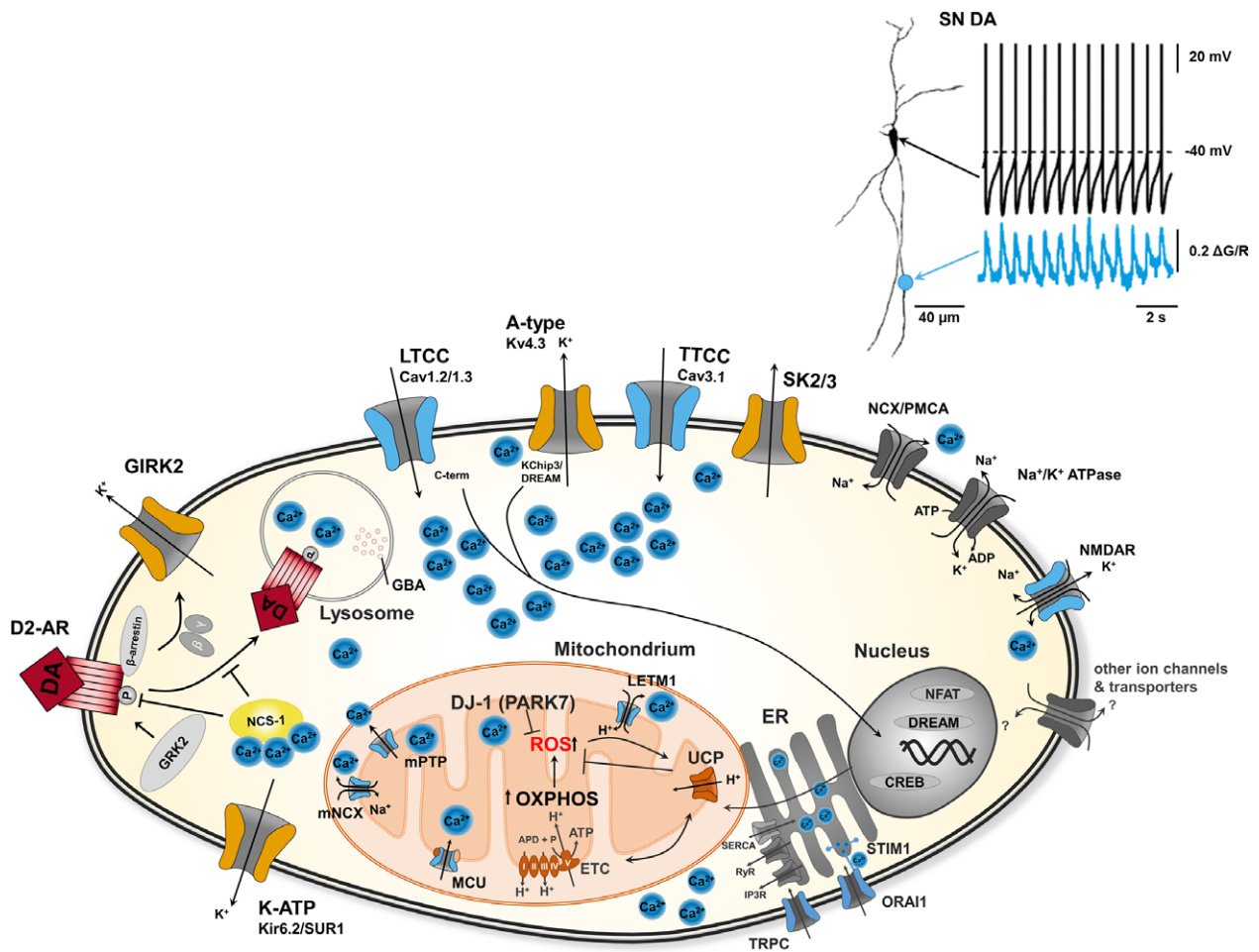
Fig. 1 Converging pathways of ion channel activities, Ca^{2+} homeostasis and metabolic stress in *Substantia nigra* dopaminergic neurons in health and Parkinson's disease. **Upper**: Whole-cell current clamp recording of a SN DA neuron (shown left as a projection image), illustrating the typical low-frequency pacemaker activity (black trace, mV). Lower blue trace depicts parallel 2-photon laser scanning fluo-4 Ca^{2+} imaging of the same neuron, illustrating the dendritic Ca^{2+} oscillations ($\Delta G/R$), that are fully blocked by the L-type Ca^{2+} channel blocker isradipine particularly in the proximal dendrites, while activity of SN DA neurons remains largely unaffected (figure adapted from and methods detailed in Guzman *et al.* 2010). **Lower**: Cartoon illustrating distinct ion channels, receptors and transporters that generate or modulate the activity patterns of SN DA neurons *in vivo* and *in vitro*, and that are associated with oscillating Ca^{2+} levels and signaling pathways, affecting mitochondrial and lysosomal function as well as gene expression in health and in Parkinson's disease, (see text for details). Note that only a selection of ion channels that are expressed in SN DA neurons is depicted. Voltage-gated LTCCs (particular of the Cav1.3 type) as well as metabolically gated K-ATP channels (of the Kir6.2/SUR1 type) seem to be crucial for physiological SN DA function, but have both also been linked to SN DA degeneration and PD. *Abbreviations:* A-type Kv/KChip: A-type voltage-gated K^+ channel; CREB: cAMP response element-binding protein; D2-AR: dopamine D2 autoreceptor; DJ-1: PARK7 gene product; DREAM: DRE antagonist modulator; ER: endoplasmatic reticulum; ETC: electron transport chain; GBA: glucocerebrosidase; GIRK: G-protein-coupled inwardly rectifying K^+ channel; GRK2: G-protein-dependent kinase 2; IP3R: inositol-3-phosphate receptor; K-ATP: ATP-sensitive K^+ channel; LETM1: high Ca^{2+} affine leucine zipper EF-hand containing transmembrane protein 1; LTCC (Cav1.3): Cav1.3 L-type voltage-gated Ca^{2+} channel; mCU: mitochondrial Ca^{2+} uniporter; MNCX: mitochondrial Na^+/Ca^{2+} exchanger; mPTP: mitochondrial permeability transition pore; NCS-1: neuronal Ca^{2+} sensor 1; NCX: Na^+/Ca^{2+} exchanger; NMDA-R: N-methyl-D-aspartate glutamate receptor; OXPHOS: oxidative phosphorylation; P: phosphate; PD: Parkinson's disease; PMCA: plasma membrane Ca^{2+} ATPase; ROS: reactive oxygen species; RyR: ryanodine receptor; SERCA: sarcoplasmic/endoplasmic reticulum (ER) Ca^{2+} ATPase; SK: small conductance Ca^{2+} sensitive K^+ channel; STIM: stromal interaction molecule; TCA: tricarboxylic acid cycle, TRP: transient receptor potential; TTCC: T-type voltage-gated Ca^{2+} channel; UCP: uncoupling protein.

2010; Brichta and Greengard 2014). In contrast, ventral tier SN DA neurons build the 'classical' calbindin-D28K-negative and DCC-positive mesostriatal SN DA population

(located particularly in the ventrolateral SN) that is crucial for control and modulation of voluntary movements and for habit learning/formation. These SN DA neurons are partic-

ularly vulnerable to degeneration in PD, and their loss causes the major motor symptoms of PD patients: bradykinesia, rigor, resting tremor, and postural instability (Fearnley and Lees 1991; Agid *et al.* 1993; Damier *et al.* 1999b; Braak *et al.* 2004; Osborne *et al.* 2005; Kordower *et al.* 2013; Brichta and Greengard 2014; Dopeso-Reyes *et al.* 2014; Kalia and Lang 2015). It is noteworthy that other, non-DA neurons are also progressively degenerating in PD, particularly noradrenergic neurons within the locus coeruleus (LC), but also neurons, e.g. within the pedunculopontine nucleus, or the dorsal motor nucleus (Jellinger 1991; Zarow *et al.* 2003; Del Tredici and Braak 2013; Goedert *et al.* 2013; Greene 2014; Sanchez-Padilla *et al.* 2014).

In this review, we focus on the 'classical' mesostriatal (or nigrostriatal) SN DA neurons, and on their particularly high vulnerability to degeneration in PD. The cause for the differential vulnerability of the different types of SN DA and VTA DA neurons to degenerative triggers is still not clear; however, it is present not only in PD and its animal models, but it also occurs in the non-PD brain during aging (Damier *et al.* 1999b; Hindle 2010; Collier *et al.* 2011; Reeve *et al.* 2014; Rodriguez *et al.* 2015). Indeed, aging is the most prominent risk factor for PD, and PD is discussed to reflect a form of premature or accelerated aging (Surmeier 2007; Hindle 2010; Collier *et al.* 2011).

Impaired calcium homeostasis, mitochondrial and lysosomal dysfunction, and metabolic stress are central trigger factors for PD

Although the cause of most PD cases is unclear (idiopathic), in addition to age, a variety of environmental and genetic (PARK-genes) PD trigger factors have been identified, as detailed in a range of excellent reviews (Hirsch and Hunot 2009; Hardy 2010; Winklhofer and Haass 2010; Gasser *et al.* 2011; Deleidi and Gasser 2013; Kieburtz and Wunderle 2013; Ramanan and Saykin 2013; Singleton *et al.* 2013; Sulzer and Surmeier 2013). Mutations in PARK-genes lead to familial Mendelian forms of PD. Particularly, ion channels and activity-related oscillatory intracellular Ca^{2+} load (compare Fig. 1, upper insert), mitochondrial and lysosomal dysfunction, oxidative and metabolic stress, as well as toxic protein propagation, protein mishandling, impairment of the ubiquitin–proteasome pathway and inflammation contribute to SN DA degeneration and sporadic as well as familial PD (Gonzalez-Hernandez *et al.* 2010; Guzman *et al.* 2010; Obeso and Lanciego 2011; Coskun *et al.* 2012; Sulzer and Surmeier 2013; Beilina and Cookson 2016; Gan-Or *et al.* 2015; Pacelli *et al.* 2015; Roselli and Caroni 2015). In view of PD as a multifactorial disease, there is increasing evidence that all these intrinsic as well as extrinsic factors seem to intermingle and aggravate each other, and affect particularly SN DA neurons and their vulnerability in PD, as discussed in the next paragraphs.

Mitochondrial (dys–)function and metabolic stress in SN DA neurons

SN DA neurons appear to be intrinsically particularly vulnerable to metabolic stress and mitochondrial dysfunction. Thus, it is not too surprising that inhibitors of the complex I of the mitochondrial electron transfer chain (ETC), like MPTP, induce selective SN DA neuron degeneration and parkinsonism in humans and animals, and are widely used to generate PD-animal models (Langston and Ballard 1983; Schapira 2010; Blesa and Przedborski 2014). The dopamine metabolism itself already seems to cause intrinsically high levels of metabolic stress and free reactive oxygen and nitrogen species (ROS/RNS) in SN DA neurons (Berman and Hastings 1999; Sulzer 2007; Surmeier *et al.* 2011). ROS/RNS can damage proteins, lipids, and DNA of mitochondria, leading to their dysfunction, while mitochondrial dysfunction in turn provides a source of metabolic stress and ROS/RNS – propagating a self-accelerating cycle of increasing metabolic stress in DA neurons (Henchcliffe and Beal 2008; Schapira 2008; Vila *et al.* 2008). Mitochondria build a dynamic network (Youle and van der Bliek 2012), where important metabolic pathways take place, like the tricarboxylic acid (TCA) cycle and the oxidative phosphorylation within the ETC, and thus ATP production, as well as control of metabolic stress and cell death, e.g. via apoptosis (Perier *et al.* 2012). Consequently, cells possess defense responses against mitochondrial dysfunction. This is particularly important for the highly vulnerable SN DA neurons, which are subjected to continued metabolic stress. In this context, a double-edged role of neuromelanin is discussed. It is build by catecholamine metabolites, and accumulates particularly in highly vulnerable SN DA (and LC) neurons with age. On one hand, neuromelanin seems to protect SN DA neurons from (iron-catalyzed) dopamine oxidation, ROS production, metabolic stress, and from degeneration, while its neuronal release seems to have opposite effects on SN DA neurons, as it can propagate microglia activation, neuroinflammation, and neurodegeneration (Hirsch *et al.* 1988; Zucca *et al.* 2015). Additional defense mechanisms in SN DA neurons against mitochondrial dysfunction and metabolic stress are mild mitochondrial uncoupling by uncoupling proteins (UCPs), and a variety of other antioxidative mechanisms, mediated e.g. by PARK-gene functions (Liss *et al.* 2005; Guzman *et al.* 2010; Sanchez-Padilla *et al.* 2014). The PARK7 gene product DJ-1 (Bonifati *et al.* 2003) for instance suppresses mitochondrial metabolic stress and ROS levels particularly in SN DA neurons (Guzman *et al.* 2010). However, despite their defense mechanisms and their DNA-repair machinery (Alexeyev *et al.* 2013), mitochondrial DNA damage accumulates in SN DA neurons with age, likely contributing to their already high metabolic stress levels, and to their progressive degeneration (Bender *et al.* 2006; Kraytsberg *et al.* 2006; Reeve *et al.* 2008).

Lysosomal (dys-)function and metabolic stress in SN DA neurons

As a further protection mechanism against mitochondrial dysfunction and metabolic stress, defective mitochondria are disposed of by mitophagy, an autophagic-lysosomal elimination (Pilsl and Winklhofer 2012; Ryan *et al.* 2015). This dynamic process seems to be particularly important for SN DA neurons, as a variety of PARK-gene products are involved in this pathway, like the mitochondrial kinase PINK1/PARK6 (Valente *et al.* 2004; Hao *et al.* 2010), the mitochondrial protease HtrA2/Omi/PARK13 (Plun-Favreau *et al.* 2007), the E3-ubiquitin ligase parkin/PARK2, (Kitada *et al.* 1998), and the F-box only protein 7/PARK15 (Di Fonzo *et al.* 2009). Loss of function mutations in these PARK-genes trigger SN DA degeneration and cause autosomal recessive familial forms of PD (Gasser *et al.* 2011; van Brug *et al.* 2015). A variety of further PARK-genes are associated with the autophagy–lysosome pathway (Bourdenx *et al.* 2014; Gan-Or *et al.* 2015). For instance, the lysosomal p-type ATPase13A2/PARK13 (Ramirez *et al.* 2006; Kruger *et al.* 2011), or alpha-synuclein (PARK1, PARK4), which is partly degraded by lysosomes (Dehay *et al.* 2013; Hunn *et al.* 2015), the leucine-rich repeat kinase LRRK2/PARK8 (Funayama *et al.* 2002; Khan *et al.* 2005), the LIM homeodomain transcription factor LMX1b, crucial not only for SN DA development and maintenance but also for autophagy–lysosome pathway function (Laguna *et al.* 2015), and the lysosomal glucocerebrosidase (GBA) (Siebert *et al.* 2014; Schapira 2015).

Heterozygous mutations in the GBA gene occur in about 10% of 'sporadic' PD cases and have just recently emerged as a major risk factor for PD (Mazzulli *et al.* 2011; Schapira and Gegg 2013). Reduced GBA function triggers accumulation of misfolded alpha-synuclein and PD (Asselta *et al.* 2014; Schapira 2015). Mutant GBA-protein is trapped in the endoplasmic reticulum (ER), causing not only elevated metabolic stress but also elevated Ca^{2+} release from the ER (Westbroek *et al.* 2011; Tan *et al.* 2014; Kilpatrick *et al.* 2015). Furthermore, dopaminergic neurons derived from pluripotent stem cells of GBA-associated PD patients not only display lysosomal and autophagic dysfunction but also elevated intracellular Ca^{2+} levels and impaired calcium homeostasis (Schondorf *et al.* 2014). These findings point to another factor that contributes to metabolic stress, as well as to mitochondrial and lysosomal (dys)function in SN DA neurons: their calcium homeostasis.

Calcium homeostasis and metabolic stress in SN DA neurons

Intracellular Ca^{2+} levels, Ca^{2+} microdomains, Ca^{2+} buffering, and the mode of Ca^{2+} entry are tightly controlled in SN DA neurons, as Ca^{2+} modulates a variety of cellular functions, like excitability, neurotransmitter release, ATP-production, apoptosis, and general regulation of enzymes and gene expression (Dolmetsch 2003; Brini *et al.* 2014; Burgoyne and Haynes 2014). In view of SN DA neuron vulnerability to

metabolic stress, Ca^{2+} regulates mitochondrial motility (Wang and Schwarz 2009), stimulates the mitochondrial nitric oxide synthase, leading to elevated levels of ROS/RNS (Traaseth *et al.* 2004; Murphy 2009; Sanchez-Padilla *et al.* 2014), and it stimulates enzymes of the TCA cycle and the ETC, and thus ATP production, thereby further boosting ROS generation (Gleichmann and Mattson 2011). Ca^{2+} also stimulates calpain, a protease that is involved in alpha-synuclein processing, SN DA degeneration, and PD (Crocker *et al.* 2003; Dufty *et al.* 2007; Diepenbroek *et al.* 2014; Games *et al.* 2014). Furthermore, when mitochondrial Ca^{2+} levels rise above a certain threshold, opening of the mitochondrial permeability transition pore, and thus neurodegeneration is triggered (Martin *et al.* 2014). In view of differential vulnerability of DA neurons to degenerative triggers, physiological mitochondrial matrix Ca^{2+} levels appear to be higher in highly vulnerable SN DA neurons compared to more resistant VTA DA neurons (J. Surmeier, personal communication), possibly contributing to their differential vulnerability to metabolic stress and PD-trigger factors.

As illustrated in Fig. 1, Ca^{2+} can enter SN DA neurons from the extracellular space via channels and transporters located in their plasma membrane. More precisely, via voltage-gated calcium channels (VGCCs) and/or glutamate receptors like NMDA-R or AMPA-R (Bezprozvanny and Mattson 2008), or by store operated calcium channels, like STIM/ORAI and transient receptor potential channel complexes (Dietrich *et al.* 2014; Majewski and Kuznicki 2015). Ca^{2+} is also released from mitochondria (e.g. via the mitochondrial sodium–calcium exchanger) and the ER (via RyR and IP3 receptors) (Brini 2003; Malli *et al.* 2003). Spatial and temporal dynamics of cytosolic Ca^{2+} levels in SN DA neurons are further controlled by its extrusion, e.g. via plasma membrane Ca^{2+} ATPases, or sodium–calcium exchangers (NCX), and by intracellular Ca^{2+}-buffering (Schwaller 2010; Hajnoczky *et al.* 2014). Because of low levels of Ca^{2+}-binding proteins in SN DA neurons, like calbindin, Ca^{2+} is mainly buffered by uptake into the ER, via the sarco-ER Ca^{2+} ATPases (SERCAs), and by mitochondria (Verkhratsky 2005; Cali *et al.* 2012; Hoppins and Nunnari 2012). Mitochondria take up Ca^{2+} directly via a mitochondrial Ca^{2+} uniporter mCU, or a mitochondrial Ca^{2+}/H^+ exchanger Letm1 (Baughman *et al.* 2011; De Stefani *et al.* 2011; Waldeck-Weiermair *et al.* 2011), or indirectly via the ER-mitochondria pathway (Rizzuto *et al.* 2009; Drago *et al.* 2011; Bakowski *et al.* 2012). This pathway is involved in defining SN DA vulnerability and in PD (Cali *et al.* 2014; Guardia-Laguarta *et al.* 2015), and survival of cultured SN DA neurons is stimulated by modulating Ca^{2+} release from the ER (Guerreiro *et al.* 2015), while MPTP can induce mitochondrial as well as ER stress in DA neurons (Selvaraj *et al.* 2012).

Besides the ER-mitochondria compartment, lysosomes also buffer cytosolic Ca^{2+} – via uptake by a yet unidentified

Ca^{2+}/H^+ exchanger (Gomez-Suaga *et al.* 2012; Osellame and Duchen 2014). The Ca^{2+} concentration is assumed to be about 5000-fold higher in lysosomes than in the cytoplasm (Christensen *et al.* 2002; Xu and Ren 2015). In view of novel neuroprotective PD-therapies, ambroxol, a well-established drug for treating conditions with productive cough or viscous mucus (Weiser 2008), has recently emerged as a promising new tool. Ambroxol, which can pass the blood–brain barrier, reduces neuronal metabolic stress and was shown to improve GBA-activity and lysosomal function in human fibroblasts derived from PD-patients (Bendikov-Bar *et al.* 2013; Albin and Dauer 2014; McNeill *et al.* 2014; Siebert *et al.* 2014). Ambroxol normalizes PD-associated GBA dysfunction (and associated calcium homeostasis) seemingly by increasing its activity and its expression (Maegawa *et al.* 2009; McNeill *et al.* 2014; Ambrosi *et al.* 2015; Schapira 2015). Furthermore, recent findings indicate that Ambroxol can accumulate in lysosomes, and directly affect their H^+ and Ca^{2+} homeostasis, and it stimulates exocytosis from secretory lysosomes via pH-dependent Ca^{2+} release (Fois *et al.* 2015).

In conclusion, all these findings illustrate a complex homeostatic interplay of calcium homeostasis, lysosomal and mitochondrial function and metabolic stress, as well as PARK-gene products in SN DA neurons, whose physiological action is already metabolically challenging (see below). Accordingly, dysfunction of this complex interplay triggers degeneration, particularly of metabolically compromised SN DA neurons, and it constitutes a converging pathological PD-pathway. Consequently, its pharmacological modulation offers novel strategies for neuroprotective PD therapies – as currently under investigation for Ambroxol.

The electrical activity of SN DA neurons causes particular metabolic stress and triggers dopamine release

There is one central factor that critically modulates the complex interplay of calcium, mitochondrial and lysosomal function particularly in SN DA neurons that we have not yet considered: their electrical activity. Neuronal activity *per se* implies high energy demand and metabolic stress, mainly because of stimulation of the Na^+/K^+ ATPase, that is necessary to maintain the asymmetric ion distribution after action potentials (intracellular high K^+, low Na^+) and that is consuming about 50% and more ATP in active neurons (Laughlin *et al.* 1998; Howarth *et al.* 2012; Johar *et al.* 2014). Again, SN DA neurons seem to be particularly dependent on proper Na^+/K^+ ATPase activity, as mutations in its ATP1A3 subunit causes a form of rapid-onset dystonia parkinsonism, RDP (Heinzen *et al.* 2014). This might be attributed to the fact that the metabolic cost of SN DA neuron activity is particularly high, as specific voltage-gated calcium channels (VGCC, see below) are active during their physiological action, causing an activity-related, oscillatory increase in intracellular Ca^{2+} levels (compare Fig. 1, upper insert). These oscillatory Ca^{2+} changes cause related oscil-latory changes of mitochondrial membrane potentials, ROS-levels, and of plasma membrane Ca^{2+} ATPase activity (Fig. 1) (Guzman *et al.* 2010; Surmeier *et al.* 2011; Pissadaki and Bolam 2013). It is important to emphasize that more resistant VTA DA neurons display an electrical activity that is similar to that of highly vulnerable SN DA neurons; however, it is not associated with oscillatory elevated Ca^{2+} and metabolic stress levels (Guzman *et al.* 2010; Khaliq and Bean 2010). Highly vulnerable locus coeruleus neurons however display similar activity-dependent Ca^{2+} oscillations and related metabolic stress levels – that are interestingly sensitive to orexinergic peptides (orexins) (Sanchez-Padilla *et al.* 2014).

Taken together, the specific mode of electrical activity of SN DA neurons generates periodically elevated Ca^{2+} and metabolic stress levels that likely render them particularly vulnerable to additional metabolic stressors and PD-triggers. Not only because of the general metabolic cost of neuronal activity, but predominantly as a result of their Ca^{2+} channel activity. In conclusion, the elevated activity-related Ca^{2+} and metabolic stress levels in SN DA neurons provide a molecular rationale for their particularly high vulnerability to mitochondrial, lysosomal, and PARK-gene dysfunction, and thus for the differential vulnerability of SN DA and VTA DA neurons to degeneration in PD. Moreover, given its high metabolic costs, the oscillatory Ca^{2+} signal in SN DA neurons must be crucial for their specific physiological functions. Indeed, as SN DA neurons release dopamine in a calcium- and activity-dependent manner (Rice *et al.* 2011; Brimblecombe *et al.* 2015) – from presynaptic axonal sites into the striatum (De-Miguel and Nicholls 2015), as well as from somatodendritic sites into the midbrain (Ford *et al.* 2010; Rice and Patel 2015) – action potential frequency and its pattern, as well as intracellular presynaptic and somato-dendritic Ca^{2+} levels in fact mediate eventually all physiological functions of SN DA neurons.

Ion channels define activity pattern and Ca^{2+} homeostasis of SN DA neurons

Given the central role of SN DA neurons for voluntary movement control, and thus, e.g. context-dependent *fight-or-flight responses*, it is not surprising that a variety of intrinsic and extrinsic factors and mechanisms are crucial for generating, maintaining, and modulating activity patterns, calcium homeostasis, and dopamine release of SN DA neurons within their physiological bandwidth (Guzman *et al.* 2009; Surmeier and Schumacker 2013; Dragicevic *et al.* 2015; Kimm *et al.* 2015). Particularly important in this context are ion channels and receptors, as they not only generate but also homeostatically shape SN DA activity patterns to physiological demands; because of their specific expression pattern, their regulations and their complex interplay, as illustrated in Fig. 1 and as reviewed, e.g. in (Bean 2007a; Michel *et al.*
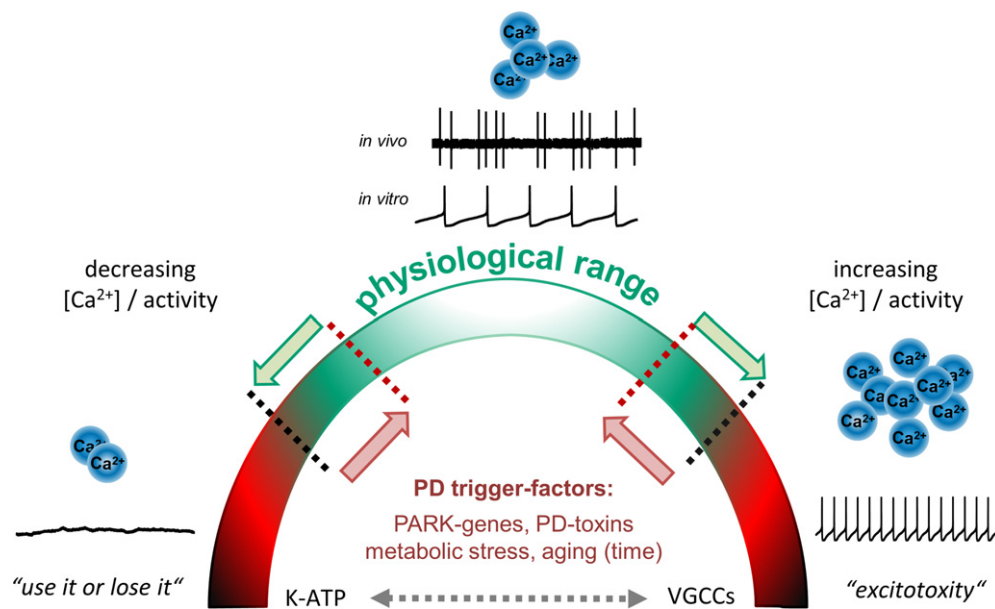
Fig. 2 Double-edged roles of activity and free intracellular Ca²⁺ levels for physiological functions of SN DA neurons and their vulnerability to PD-triggers. Shown are typical *in vivo* and *in vitro* single-spike activity patterns of SN DA neurons from adult mice (adapted from Dragicevic *et al.* 2015; methods detailed in Schiemann *et al.* 2012; Dragicevic *et al.* 2014). Note that the important – and energy demanding – burst activity mode of SN DA neurons is not shown. The cartoon summarizes that SN DA neurons possess several intrinsic feedback and feed-forward mechanisms to protect and to adapt their activity pattern as well as their oscillatory calcium homeostasis (crucial for dopamine release, and metabolic homeostasis) in both directions within a physiological bandwidth (indicated by green arrows/color and black dotted lines). Both, reduced as well as elevated activity and associated calcium homeostasis can trigger SN DA degeneration, as indicated by '*use it or lose it*' and '*excitotoxicity*'. PD-trigger factors could narrow the physiological bandwidth of these two parameters, and thus facilitate pathophysiological and degenerative pathways (indicated by red arrows/color and red dotted lines). VGCCs as well as K-ATP channels are from particular importance, as they have bidirectional physiological functions, can stimulate each other's activity (indicated by dotted gray double-arrow), and they both can trigger selective SN DA degeneration and PD (compare figure 1, for details see text). *Abbreviations:* K-ATP: ATP-sensitive potassium channel; PARK-gene: Parkinson's disease associated gene; PD: Parkinson's disease; VGCCs: voltage-gated calcium channels.

2007; Guzman *et al.* 2009; Liss and Roeper 2010; Paladini and Roeper 2014; Dragicevic *et al.* 2015). Here, we focus on how distinct ion channels (within a complex feedback and feed-forward signaling network) modulate activity patterns, intracellular calcium homeostasis, and metabolic sensitivity specifically of SN DA neurons and thus define not only their physiological functions, but also their vulnerability to degeneration in PD.

GIRK channel–coupled D2-autoreceptors mediate calcium- and dopamine–dependent activity control of SN DA neurons

The activity of SN DA neurons *in vitro* and *in vivo* is inhibited by dopamine itself in a negative feedback loop via dopamine autoreceptors of the D2-subtype (D2-AR) (De Mei *et al.* 2009; Beaulieu *et al.* 2015). D2-ARs belong to the seven-transmembrane G-protein-coupled receptor family, and are located at the presynapses as well as on the somatodendritic compartment of SN DA neurons. Upon dopamine binding, the G-protein beta-gamma subunit gets released from the D2 autoreceptor, and directly opens G-protein-coupled inwardly rectifying potassium channels

(GIRK2) (Luscher and Slesinger 2010). The GIRK2 activation in turn leads to hyperpolarization of the SN DA neuron membrane potential and thereby inhibits their electrical activity (Bozzi and Borrelli 2006; De Mei *et al.* 2009; Ford 2014; Dragicevic *et al.* 2015). In essence, in a negative feedback loop and via D2-ARs, dopamine itself modulates and adapts its own activity-dependent, presynaptic, and somatodendritic release from SN DA neurons, and consequently its physiological functions (De-Miguel and Nicholls 2015; Rice and Patel 2015). It is noteworthy, that this D2-AR/GIRK2 feedback regulation is particularly pronounced in highly vulnerable mature SN DA neurons, compared to more resistant VTA DA neurons (Lammel *et al.* 2008; Brichta and Greengard 2014; Dragicevic *et al.* 2015). Moreover, within the population of SN DA neurons, D2-AR/ GIRK2 activity control displays a homeostatic plasticity (Fig. 1). D2-AR responses of SN DA neurons are sensitized in a dopamine-, calcium-, and age-dependent manner: their dopamine autoinhibition – in response to phasically elevated *in vivo* dopamine levels (e.g. caused by cocaine or L-DOPA), during maturation, and/or because of elevated free intracel-

lular Ca^{2+} levels – is strengthened by functional coupling to the neuronal calcium sensor NCS-1 (Borgkvist *et al.* 2014; Dragicevic *et al.* 2014; Ng *et al.* 2016; Poetschke *et al.* 2015). As illustrated in Fig. 1, NCS-1 binds to D2-receptors at their third intracellular loop in a calcium-dependent fashion, and thereby prevents G-protein-coupled receptor kinase (GRK2) mediated phosphorylation, and thus beta-arrestin mediated internalization and D2-receptor desensitization (Kabbani *et al.* 2012; Beaulieu *et al.* 2015). We showed that this calcium-dependent D2-AR sensitization preferentially depends on VGCCs of the Cav1.3 channel subtype (that are discussed below) (Dragicevic *et al.* 2014).

In view of SN DA vulnerability in PD, sensitized D2-AR responses appear less pronounced in PARK7 KO (DJ-1) and in PARK8 transgenic (LRRK2-R1441C) mice, and their mature SN DA neurons display juvenile-like desensitizing D2-AR responses (Goldberg *et al.* 2005; Tong *et al.* 2009; Tong and Shen 2012). Moreover, remaining human SN DA neurons from PD patients display a transcriptional dysregulation of the NCS-1/D2-AR/GIRK2 signaling network (Dragicevic *et al.* 2014). These findings indicate that dopamine- and calcium-mediated sensitization of D2-AR responses also adapts SN DA neuron activity *in vivo* and in PD. In context of SN DA degeneration, sensitized D2-AR/GIRK2 responses will reduce elevated activity, and thus oscillatory calcium load, and related metabolic stress in SN DA neurons, and thus could protect them from overexcitability and excitotoxic cell death (compare Fig. 2). SN DA neurons are particularly prone to excitotoxicity, as it is triggered by elevated activity, perturbed calcium homeostasis, high levels of ROS/RNS, and metabolic stress (Prentice *et al.* 2015).

In conclusion, flexible dopamine-dependent D2-AR/GIRK2 responses in SN DA neurons allow modulation and homeostatic adaptation of their activity, their oscillatory calcium signaling and their dopamine release to physiological needs. Beyond that, D2-AR/GIRK2 sensitization, e.g. as a result of pathophysiologically elevated SN DA activity and/or calcium overload, will counteract excitotoxic cell death, and thus represents another defense mechanism against metabolic stress. As summarized next, in this view, two different classes of ion channels have emerged to be particularly relevant for SN DA neuron activity, related calcium and metabolic homeostasis, as well as for PD-pathophysiology: voltage-gated calcium channels (VGCCs), and metabolically gated ATP-sensitive potassium (K-ATP) channels.

Bidirectional roles of voltage–gated Ca^{2+} channels in SN DA neurons

A family of voltage-gated Ca^{2+} channels is expressed in SN DA neurons

Voltage-gated calcium channels (VGCCs) are transmembrane proteins that mediate membrane potential-dependent extracellular Ca^{2+} entry into cells, and their dysfunction is associated with a variety of diseases (Catterall *et al.* 2005; Simms and Zamponi 2014; Zamponi *et al.* 2015). VGCC subtypes exhibit distinct spatial, temporal, and disease-related expression patterns (Perez-Reyes 2003; Catterall *et al.* 2005; Adams and Snutch 2007). They are tightly regulated by Ca^{2+}, calmodulin, and related Ca^{2+} sensor proteins, which cause facilitation and inactivation of channel activity (Zuhlke *et al.* 1999; Catterall *et al.* 2013). In first instance, their open probability increases with depolarization of neuronal membrane potentials, and they undergo not only voltage-dependent but also Ca^{2+}-dependent channel inactivation. This provides an important autoregulatory feedback mechanism (Cens *et al.* 2006; Christel and Lee 2012; Catterall and Zheng 2015). Depending on their membrane potential activation thresholds, VGCCs are divided into high voltage-activated (HVA) and low voltage-activated (LVA) channels (Flockerzi *et al.* 1991; Snutch *et al.* 2001; Perez-Reyes 2003; Durante *et al.* 2004). Members of the HVA channel family require a stronger membrane depolarization for activation (mainly caused by action potentials), while LVA channels are already active at subthreshold membrane potentials, before the threshold for activation of voltage-gated sodium channels is reached and action potentials are generated (Armstrong and Matteson 1985; Simms and Zamponi 2014; Zamponi *et al.* 2015). The family of HVA channels consists of the so-called L-type calcium channels (LTCCs), with channel pores build by Cav1.1-1.4 alpha1-subunits, the P/Q-type (or Cav2.1), the N-type (Cav2.2), and the R-type channels (Cav2.3). LVA channels are constituted by T-type channels (TTCCs), build by Cav3.1–3.3 pore-forming alpha-subunits (Catterall *et al.* 2005; Simms and Zamponi 2014; Zamponi *et al.* 2015).

All these VGCC-classes are functionally expressed in SN DA neurons: Cav1.2 and Cav1.3 generate LTCC currents (both expressed in several splice-variants, and in complex with yet to be fully defined accessory β and α2-δ subunits), and Cav3.1 is the predominant form of T-type channels (Olson *et al.* 2005; Striessnig *et al.* 2006; Lipscombe *et al.* 2013; Dragicevic *et al.* 2014; Lieb *et al.* 2014; Poetschke *et al.* 2015; Scharinger *et al.* 2015). In view of their emerging role in SN DA neurons and in PD, it is important to note that Cav1.3 LTCCs activate at more negative (subthreshold) membrane potentials compared to the widely expressed Cav1.2 and other HVA channels (Koschak *et al.* 2001; Lipscombe *et al.* 2004; Lieb *et al.* 2014). The distinct types of VGCCs in SN DA neurons are crucial for their presynaptic as well as for their (mechanistically still not fully understood) somatodendritic dopamine release (Rice *et al.* 2011; Brimblecombe *et al.* 2015; Rice and Patel 2015). VGCC-activity causes Ca^{2+} mediated slow oscillatory membrane potentials in SN DA neurons (Chan *et al.* 2007; Puopolo *et al.* 2007; Guzman *et al.* 2009; Putzier *et al.* 2009), and an oscillatory, metabolically challenging Ca^{2+} influx, associated with their electrical activity, as described above (Bean 2007b).

PD-protective effects of voltage-gated L-type Ca²⁺ channel blockers

LTCCs in SN DA neurons, particularly those of the Cav1.3 subtype, have received much attention in recent years, since blood–brain barrier (BBB) permeable LTCC blockers of the dihydropyridine-type [DHPs, like isradipine (Striessnig *et al.* 1998)] apparently reduce the risk for developing PD by about 30% in humans, as epidemiologic data of retrospective studies indicate (Becker *et al.* 2008; Ritz *et al.* 2010; Marras *et al.* 2012; Pasternak *et al.* 2012; Gudala *et al.* 2015; Lang *et al* 2015). This PD-protective effect is assumed (but not yet clearly demonstrated) to be caused by inhibition of LTCCs in SN DA neurons, most likely of Cav1.3 channels, which in turn abolishes dendritic Ca²⁺ oscillations and associated oscillatory metabolic stress in mice (Guzman *et al.* 2010; Kang *et al.* 2013; Surmeier and Schumacker 2013; Huang *et al.* 2014; Ortner *et al.* 2014). These Ca²⁺ oscillations get stronger and more sensitive to DHPs with increasing distance from the soma (Wilson and Callaway 2000; Guzman *et al.* 2010; Jang *et al.* 2011; Dryanovski *et al.* 2013), and in distal dendrites, they are completely suppressed by low-doses of DHPs (Guzman *et al.* 2010; Dryanovski *et al.* 2013; Surmeier and Schumacker 2013). BBB permeable DHP-based LTCC blockers are already well-established drugs, commonly prescribed to treat hypertension (Epstein *et al.* 2007; Coca *et al.* 2013). Consequently, after a phase II safety study, they are already in an ongoing clinical phase III study (2013–2018; ClinicalTrials.gov Identifier: NCT00909545) to test their efficacy as neuroprotective PD therapy (Simuni *et al.* 2010, Parkinson Study 2013). Furthermore, given the widespread expression of Cav1.2 LTCCs but not of Cav1.3 throughout the body, in particular in the cardiovascular system (Sinnegger-Brauns *et al.* 2004; Hofmann *et al.* 2014), novel Cav1.3 selective drugs are under development (Kang *et al.* 2013; Huang *et al.* 2014; Ortner *et al.* 2014). However, the specific physiological functions of Cav1.2 and Cav1.3 and of associated Ca²⁺ oscillations in SN DA neurons are still not clear. This is an important issue in view of the aimed chronic pharmacological Cav1.3 block as novel therapeutic avenue for PD, as LTCCs seem to have bidirectional and context-dependent functions in SN DA neurons (Chan *et al.* 2007; Guzman *et al.* 2009; Dragicevic *et al.* 2014; Poetschke *et al.* 2015).

Cav1.3 L-type Ca²⁺ channels stabilize and stimulate activity of SN DA neurons

While originally thought to be crucial for the generation of intrinsic pacemaker activity in SN DA neurons (Nedergaard *et al.* 1993; Mercuri *et al.* 1994; Chan *et al.* 2007; Puopolo *et al.* 2007), it is now widely accepted that LTCCs are not essential for SN DA pacemaker activity generation, but are stabilizing pacemaker activity and its precision (Guzman *et al.* 2009; Drion *et al.* 2011; Branch *et al.* 2014; Dragicevic *et al.* 2014; Poetschke *et al.* 2015). SN DA neurons are able to compensate pharmacological LTCC block as well as germline loss of Cav1.3 LTCCs [Cav1.3 KO mice (Platzer *et al.* 2000; Striessnig and Koschak 2008)] by other ion channels, e.g. by functional down-regulation of inhibitory Kv1 potassium channels within minutes after DHP-mediated distortion of SN DA activity, or functional up-regulation of either hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels, or of TTCCs (Cav3.1) on longer time scales (Chan *et al.* 2007; Guzman *et al.* 2009; Poetschke *et al.* 2015). HCN channels (built by HCN2-4) facilitate SN DA pacemaker generation and its frequency (Franz *et al.* 2000; Neuhoff *et al.* 2002) in interplay with voltage-gated sodium channels (Bean 2007a). Cav3.1 TTCCs stabilize SN DA pacemaker frequency and its precision in an age-dependent manner (Poetschke *et al.* 2015), most likely by functional coupling to Ca²⁺ sensitive small conductance potassium channels (SK3), and thereby shaping SN DA action potential after-hyperpolarization (Wolfart *et al.* 2001; Wolfart and Roeper 2002; Stocker 2004; Bond *et al.* 2005; Gueguinou *et al.* 2014; Poetschke *et al.* 2015).

In addition to stabilizing SN DA activity, LTCCs allow maintenance of neuronal electrical activity, particularly in metabolic demand situations, because of their Ca²⁺-mediated stimulation of the TCA cycle and the ETC, and thus ATP production that is crucial for Na⁺/K⁺ ATPase activity and neuronal function. On the other hand, LTCC-mediated dendritic Ca²⁺ oscillations also stimulate Ca²⁺-related detrimental pathways, as discussed above. Thus, to avoid a vicious LTCC activity-dependent feed-forward spiral toward cell death, SN DA neurons are in need of respective protective feedback control mechanisms.

VGCCs can also inhibit SN DA activity, in interplay with K⁺ channels

Such a protection mechanism is provided by the Ca²⁺-dependent sensitization of inhibitory D2-AR/GIRK2 responses via NCS-1, that are sensitive to isradipine and depend on Cav1.3 function, as described above (Dragicevic *et al.* 2014). Hence, Cav1.3 channels have bidirectional functions in SN DA neurons: on one hand, they stimulate their electrical activity, their ATP production, and also their metabolic stress levels, while on the other hand, they can inhibit SN DA activity via stimulation of Ca²⁺- and NCS-1-dependent D2/GIRK2 activity (Borgkvist *et al.* 2014; Dragicevic *et al.* 2015).

TTCCs seem to have similar bidirectional functions in SN DA neurons as they not only stimulate but also inhibit pacemaker activity via stimulation of potassium channels: Cav3.1 channels can also sensitize Ca²⁺- and NCS-1 dependent D2-AR/GIRK2 signaling (Poetschke *et al.* 2015). Additionally, TTCCs can functionally couple and activate Ca²⁺-stimulated A-type K⁺ channels (An *et al.* 2000; Anderson *et al.* 2010; Jerng and Pfaffinger 2014; Turner and Zamponi

2014; Zamponi *et al.* 2015) – likely (but not yet demonstrated) also in SN DA neurons. These voltage-gated A-type channels are formed by Kv4.3 pore-forming α- and Ca^{2+}-sensitive KChip3 β-subunits, and they dynamically inhibit the electrical activity of SN DA neurons, similar as GIRK2 channels (Kimm and Bean 2014; Liss *et al.* 2001, 2002).

Beyond that, VGCCs can also alter ion channel function and neuronal activity at the level of gene expression, as they are particularly effective in activating Ca^{2+}-dependent transcription factors, like cAMP response element-binding protein (CREB), myocyte enhancer factor-2 (MEF-2), nuclear factor of activated T cells (NFAT), and downstream regulatory element antagonist modulator (DREAM) (Graef *et al.* 1999; Mandel and Goodman 1999; Mao *et al.* 1999; Dolmetsch *et al.* 2001; Osawa *et al.* 2001; Dolmetsch 2003; Screaton *et al.* 2004; Rivas *et al.* 2011; Putney 2012; Selvakumar *et al.* 2014). Moreover, the C-terminus of Cav1.3 and Cav1.2 itself can be cleaved, and translocate to the nucleus in a Ca^{2+}-dependent fashion, where the LTCC C-termini act as transcription factors (Dolmetsch 2003; Gomez-Ospina *et al.* 2006; Lu *et al.* 2015). Likewise, the A-type K^+ channel subunit KChip3 (a member of the NCS-1 like Ca^{2+} binding protein family) is known as the transcriptional repressor DREAM (Jo *et al.* 2001; Buxbaum 2004), and can shuttle from the membrane to the nucleus in inverse correlation with cellular Ca^{2+} levels (Fig. 1) (Li and Adelman 2000; Sours-Brothers *et al.* 2009; Mellstrom *et al.* 2014).

In conclusion, VGCCs modulate the electrical activity of SN DA neurons in *both* directions: in a positive feed-forward mechanism by stimulating action potential generation and ATP synthesis, and in a negative feedback mechanism by stimulation of inhibitory K^+ channels like GIRK2 or Kv4.3, via calcium-sensing proteins (e.g. NCS-1 and KChip3). Moreover, by regulating gene expression, they can modulate SN DA neuron function in opposite manners on different timescales (compare Figs 1 and 2).

Bidirectional roles of metabolically gated K–ATP channels in SN DA neurons

K-ATP channels act as metabolic sensors in SN DA neurons
ATP-sensitive potassium (K-ATP) channels are heteromultimeric transmembrane proteins, consisting of pore-forming α-subunits of the Kir6 type (Kir6.1 or Ki6.2) and regulatory β-subunits, the sulfonylurea receptors (SUR1 or SUR2A/B) members of the ATP-binding cassette transporter superfamily (Ashcroft and Gribble 1998; Proks and Ashcroft 2009). Similar as in pancreatic beta-cells, in mature SN DA neurons K-ATP channels are formed by Kir6.2 and SUR1 (Liss *et al.* 2005; Schiemann *et al.* 2012). However, K-ATP channels are expressed in a variety of additional neuronal and non-neuronal cells, and their dysfunction is associated with a variety of diseases (Seino and Miki 2003; McTaggart *et al.*

2010). Given the crucial role of K-ATP channel closure for triggering pancreatic insulin secretion in interplay with LTCCs (Ashcroft *et al.* 1984; Nichols 2006; Clark and Proks 2010; Seino 2012), K-ATP blockers (sulfonylureas like glibenclamide or tolbutamide) are well-established drugs for treatment of diabetes type II (T2D), while K-ATP activators like diazoxide are applied to treat hypertension (Miura and Miki 2003; Ashcroft 2007; Ashcroft and Rorsman 2013; de Wet and Proks 2015).

In view of PD and high vulnerability of SN DA neurons, K-ATP channels act as so-called metabolic sensors (Nichols 2006; Liss and Roeper 2010), as their open probability is regulated by ATP- and ADP levels, as well as by a variety of other metabolic signals, such as insulin, leptin, ghrelin, long-chain fatty acids (acyl-CoA), PIP_2, pH, hydrogen peroxide, ROS/RNS, and nitric oxide (as reviewed in (Ashcroft 2007; Dragicevic *et al.* 2015)). In essence, the open probability of K-ATP channels is higher in metabolic demand situations, and their activity hyperpolarizes excitable cells, and thus inhibits e.g. energy-demanding electrical activity of SN DA neurons (Liss and Roeper 2001; Miki and Seino 2005; Nichols 2006; Ashcroft and Rorsman 2013). Thereby, K-ATP channels protect cells from overexcitability and excitotoxicity – a well-described mechanism e.g. for ischemic preconditioning and myoprotection (Liss and Roeper 2001; Miki *et al.* 2001; Yamada and Inagaki 2005; Soundarapandian *et al.* 2007; Lefer *et al.* 2009; Flagg *et al.* 2010).

Considering the differential vulnerability of DA neurons to degeneration, it is important to note that although more resistant VTA DA neurons also express functional K-ATP channels of the same molecular make-up, they are not activated in response to metabolic stress that activates K-ATP channels in SN DA neurons (Liss *et al.* 1999a, 2005; Schiemann *et al.* 2012). This differential metabolic sensitivity of SN DA and VTA DA K-ATP channels is likely caused by differential mitochondrial uncoupling via UCPs that does not compromise ATP production, but lowers ROS and metabolic stress levels (Liss *et al.* 2005; Guzman *et al.* 2010).

Concisely, in a feedback mechanism, selective K-ATP channel activity in SN DA neurons reduces their electrical activity and thus need of energy, particularly in metabolically demanding situations. To avoid, however, a chronic loss of electrical activity and related dopamine release of metabolically challenged SN DA neurons, they are in need of respective feed-forward control mechanisms.

K-ATP channels can inhibit but also stimulate activity of SN DA neurons
Indeed, similar as VGCCs, K-ATP channels have also bidirectional effects in SN DA neurons: in first instance, their activation in response to metabolic stress (e.g. PD-triggers) reduces SN DA neuron activity as expected (Liss *et al.* 2005). However, we identified that *in vivo,* under physiological conditions, within the intact basal ganglia network,

K-ATP channel activity does not inhibit but in contrast stimulate the activity of SN DA neurons by facilitating their switch to NMDA glutamate receptor-mediated burst activity (Schiemann *et al.* 2012; da Silva and Costa 2012; Dragicevic *et al.* 2015). This K-ATP triggered burst activity of SN DA neurons, associated with a supralinear increase in dopamine release, stimulates novelty-induced exploration *in vivo* in mice (Deacon *et al.* 2006; Schiemann *et al.* 2012). Burst activity is particularly energy-demanding, and therefore contributes to metabolic stress; however, its K-ATP channel-mediated facilitation in SN DA neurons would allow and maintain goal-directed behavior in novel situations, particularly under metabolic demand, like food deprivation or *fight-or-flight* situations (Schiemann *et al.* 2012; Paladini and Roeper 2014; Dragicevic *et al.* 2015). It is noteworthy, that in subthalamic nucleus neurons, in contrast to SN DA neurons, NMDA-R activity stimulates K-ATP channels (*in vitro*), thereby reducing neuronal burst activity that is pathologically increased in PD (Shen and Johnson 2010).

In conclusion, K-ATP channels (Kir6.2/SUR1) can modulate the electrical activity of SN DA neurons in *both* directions: in a negative feedback mechanism by membrane hyperpolarization, and in a positive feed-forward mechanism, in functional interplay with NMDA glutamate receptors, by stimulation of burst activity (compare Fig. 1 and 2). Yet, a strong K-ATP activation, for instance in response to PD-triggers, will at a certain point no longer trigger burst activity of SN DA neurons, but in contrast reduce their activity, or even silence them, as illustrated in Fig. 2.

Bidirectional effects of acute and chronic K-ATP channel activity for SN DA neuron survival

Considering the preferential degeneration of SN DA neurons in PD, acute and chronic K-ATP channel activity has also bidirectional – protective but also detrimental – effects on SN DA survival. In line with a well-described general neuroprotective effect of K-ATP channel activity in metabolic stress situations, SN DA neurons of K-ATP channel-deficient mice [Kir6.2 KO (Minami *et al.* 2004)] display a significantly higher vulnerability *in vivo* in acute response to the PD-toxin MPTP (Liss *et al.* 2005). However, chronic K-ATP channel activity in SN DA neurons *in vivo* in response to sustained metabolic stress is not beneficial anymore, but in contrast seems to trigger degeneration as evident from K-ATP KO: germline loss of Kir6.2 K-ATP channels rescued SN DA neurons from selective degeneration in two different PD mouse models (Liss *et al.* 1999b, 2005), the *chronic* neurotoxic MPTP/probenecid PD-model (Jackson-Lewis and Przedborski 2007; Meredith and Rademacher 2011), as well as the homozygous *weaver GIRK2* mutant mouse, a *chronic* genetic model of PD (Navarro *et al.* 1996; Oo *et al.* 1996; Slesinger *et al.* 1996). In agreement with a neurodegenerative effect of chronic K-ATP channel activity, pharmacological K-ATP channel block protects cultured rat SN DA neurons

(Toulorge *et al.* 2010), and PC12 DA cells from degeneration (Nam *et al.* 2015).

Beyond that, a pathophysiological role of chronic K-ATP channel activity in human PD is indicated, as remaining SN DA neurons from PD patients express about 2-fold higher levels of SUR1, the subunit that is responsible for K-ATP channel trafficking to the plasma membrane (Sharma *et al.* 1999; Tucker *et al.* 1997), and about 10-fold higher levels of the NMDA-R pore forming subunit NR1 (Schiemann *et al.* 2012; Dragicevic *et al.* 2015). Consistent with these human expression data and our functional *in vivo* mouse data, SN DA neurons of awake PD patients display high levels of burst activity (Zaghloul *et al.* 2009; Schiemann *et al.* 2012). Furthermore, there is retrospective epidemiological evidence of a reduced risk for PD in T2D patients that were treated with K-ATP blockers (Powers *et al.* 2006; Scigliano *et al.* 2006; Schernhammer *et al.* 2011; Wahlqvist *et al.* 2012; Cereda *et al.* 2013; Lu *et al.* 2014; Brauer *et al.* 2015). Likewise, there is evidence for BBB penetrance of these K-ATP channel blockers (Ashcroft 2010; Lahmann *et al.* 2015). This evidence, however, is ambiguous and must be interpreted with caution, as T2D *per se* seems to affect the risk for PD (Hu *et al.* 2007; Cereda *et al.* 2012; Lima *et al.* 2014; Lu *et al.* 2014; Zhang and Tian 2014), and both diseases seem to share common pathophysiological pathways (Santiago and Potashkin 2013, 2014), which could skew protective effects of K-ATP blockers. Nevertheless, pharmacological K-ATP blockade, selectively in SN DA neurons, could provide another ion channel targeting neuroprotective strategy as a novel PD therapy. However, to the best of our knowledge, currently no clinical studies are further evaluating this avenue.

In summary, mouse model and human data identified that K-ATP channels in SN DA neurons act as bidirectional metabolic gatekeepers, adapting their activity and thus dopamine release to their metabolic state, based on feed-forward as well as feedback mechanisms: K-ATP channel-mediated feed-forward burst facilitation, e.g. in metabolic demand situations would enhance dopamine release and exploratory movement. However, it would also boost SN DA energy demand and metabolic stress, and consequently activate further K-ATP channels. This will, particularly in the presence of additional PD-stressors, in a feedback mechanism *reduce* activity and energy consumption of SN DA neurons. *In vivo*, K-ATP channel activity in acute response to pathophysiological metabolic stressors is beneficial, but their chronic pathophysiological activity in response to PD-triggers has opposite effects and triggers SN DA degeneration.

A flexible range of activity pattern and Ca²⁺ levels is vital for SN DA function and survival

Given the bidirectional functions of Cav1.3 LTCCs as well as of K-ATP channels for SN DA neuron activity, it is not

clear whether the supposed PD protective effect of pharmacological channel inhibition might be caused by a reduction or a stimulation of SN DA activity. Indeed, the relationship between ion channel activity, activity pattern, calcium homeostasis, physiological functions, and vulnerability of SN DA neurons in PD is not a simple one, but is rather complex and context dependent (Fig. 2).

A number of studies indicate that the *maintained activity* of SN DA neurons is not only crucial for dopamine release and fulfillment of their physiological functions but also for their maintenance and survival during development and adulthood (Michel *et al.* 2007, 2013; Toulorge *et al.* 2010, 2011; Dragicevic *et al.* 2015; Guerreiro *et al.* 2015; Wang *et al.* 2015). In accordance with the classical 'use it or lose it' principle of neuronal plasticity and neuronal loss (Swaab *et al.* 2002; Coyle 2003; Coulson *et al.* 2008; Valenzuela *et al.* 2012), reduced activity of SN DA neurons seems to facilitate their degeneration *in vivo* and *in vitro* (Salthun-Lassalle *et al.* 2004; Liss *et al.* 2005; Janezic *et al.* 2013). On the other hand, *reduced activity* has also been shown to be beneficial for SN DA survival, particularly in acute pathophysiological demand situations (Liss *et al.* 2005; Yamada and Inagaki 2005; Aumann *et al.* 2008; Virgili *et al.* 2013). In accordance with classic excitotoxic cell death pathways, elevated activity clearly is detrimental for SN DA survival, as it triggers energy demand, metabolic stress, and pathophysiological Ca^{2+} overload, and its detrimental consequences (Blandini 2010; Surmeier *et al.* 2011; Ambrosi *et al.* 2014; Prentice *et al.* 2015; Van Laar *et al.* 2015).

However, Ca^{2+} apparently also has context-dependent bidirectional effects on SN DA neuron physiology, viability, and vulnerability. As detailed above, Ca^{2+} can trigger excitotoxicity and other detrimental pathways, however, it is necessary not only for physiological SN DA functions but also for their survival (Michel *et al.* 2013). Findings from PINK1 (PARK6) and HtrA2/Omi (PARK13) PD-gene mice support this view, as their SN DA pacemaker activity is less precise, and burst firing seems to be facilitated in consequence of *reduced* Ca^{2+} release from ER and mitochondria, and thus by *reduced* cytosolic Ca^{2+} (Bishop *et al.* 2010).

Given these bidirectional roles of SN DA neuron activity and related Ca^{2+} signaling, intrinsic or synaptic mechanisms that modulate their activity should have variable, context-dependent effects on their viability and their vulnerability in PD (Fig. 2). On one hand, mechanisms that *maintain* or *stimulate* electrical activity, and thus dopamine release and the ability for voluntary movement – even or particularly under metabolic demand situations (e.g. food deprivation, or *fight-or-flight* situations) – could be beneficial or even life-saving for the organism. However, as a drawback, ongoing stimulated activity of SN DA neurons and associated metabolic stress might render SN DA neurons more vulnerable to excitotoxicity and PD-triggers. On the other hand,

mechanisms that *reduce* SN DA activity and calcium-mediated dopamine release, and thus *impair* voluntary movement, could be detrimental for the organism, particularly under situations were immediate and/or ongoing motion is required for survival. This would, however, protect SN DA neurons from excitotoxic events.

In conclusion, a context-dependent, flexible bandwidth of activity patterns and associated Ca^{2+} levels is necessary to enable respective physiological functions of SN DA neurons. Consequently, as illustrated in Figs 1 and 2, SN DA neurons possess several intrinsic mechanisms to protect and to adapt their activity pattern as well as their calcium homeostasis in both directions within a physiological range.

The physiological bandwidth of activity patterns and Ca^{2+} levels in SN DA neurons is defined by ion channels, and gets narrowed by PD–triggers (?)

An intricate network of ion channels that can mediate bidirectional functions on different timelines, and that complement and compensate each other as detailed above, is crucial to enable this physiological flexibility of SN DA neurons. In this sense, in an immediate scenario, LTCC activity and intracellular Ca^{2+} stabilize and stimulate SN DA activity and their ATP production in a feed-forward mechanism: the more active the neuron is, the more dopamine gets released, the more ATP is needed and would be produced because of activity-related VGCC activity and Ca^{2+} stimulation of TCA, ETC and further enzymes. Furthermore, LTCC activity can homeostatically adapt SN DA function to physiological needs via alterations of Ca^{2+}-dependent gene expression as already discussed above (Pathak *et al.* 2015). These short- and long-term physiological functions of VGCCs and Ca^{2+} in SN DA neurons would ensure their adaptive electrical activity, dopamine release, and context-specific movement control. The burst-triggering function of K-ATP channel activation in SN DA neurons further contributes to this positive safety net of reinforcement mechanisms (Schiemann *et al.* 2012). However, as detailed above, VGCCs as well as K-ATP channels not only facilitate and stabilize pacemaker activity, but they can also stimulate inhibitory responses that reduce SN DA activity and related Ca^{2+} levels in negative feedback loops, e.g. via NCS-1/D2/GIRK2 or A-type Kv4.3/KChip3 channel sensitization. Moreover, A-type channel function in SN DA neurons is also complex and context dependent (Hahn *et al.* 2003). On one hand, the glia cell line-derived neurotrophic factor GDNF that promotes maintenance and survival of SN DA neurons (de d'Anglemont Tassigny *et al.* 2015; Kramer and Liss 2015), can stimulate pacemaker frequency of SN DA neurons by A-type current inhibition (Yang *et al.* 2001), and Kv4-blockers infused *in vivo* into the striatum reduce PD-symptoms in mice (Aidi-Knani *et al.* 2015). However, on the other hand, over-expression of the mutated PARK1-gene

product A53T α-synuclein, which triggers SN DA degeneration, has GDNF-like effects on A-type currents *in vivo* in mouse SN DA neurons (Subramaniam *et al.* 2014). Moreover, remaining human SN DA neurons from PD patients display not only elevated burst activity, but also massively elevated levels of Kv4.3 mRNA – possibly as a compensatory protective feedback mechanism (Fig. 2) (Schiemann *et al.* 2012; Schlaudraff *et al.* 2014; Dragicevic *et al.* 2015). Beyond this already complex scenario, VGCCs, A-type Kv4.3 channels, GIRK2, and K-ATP channels modulate each other's activity: elevated Ca^{2+} levels and associated metabolic stress because of VGCC activation (in particular Cav1.3) can sensitize D2/GIRK2 responses, and increase the open probability of Ca^{2+}-sensitive A-type Kv4.3 channels as well as of metabolically sensitive K-ATP channels. Membrane hyperpolarization and reduced SN DA activity resulting from these K^+ channel activities in turn could increase VGCC currents, in particular of LVA Cav1.3 LTCCs and Cav3.1 TTCCs.

It should be emphasized again that less vulnerable VTA DA neurons, which are not essential for voluntary movement, do neither display VGCC-mediated intracellular Ca^{2+} oscillations during pacemaking, nor A-type K^+ channel-mediated frequency control, or K-ATP channel-mediated high metabolic sensitivity, as detailed above. However, other neurons that show high vulnerability to PD-triggers, and that are important for *fight-or-flight* responses, like the noradrenergic LC neurons, possess ion channel activities similar to those of SN DA neurons (Koyama *et al.* 1999; Samuels and Szabadi 2008; de Oliveira *et al.* 2010; Tovar *et al.* 2013; Sanchez-Padilla *et al.* 2014; Hopp *et al.* 2015; Matschke *et al.* 2015).

In conclusion, despite their complex homeostatic bidirectional regulatory mechanisms, the 'high calcium, high activity, high metabolism' phenotype of SN DA neurons means that they are energetically 'living on the edge'. Their massive axonal fields and lower number of mitochondria might further contribute to their fragile homeostasis (Liang *et al.* 2007; Surmeier *et al.* 2010; Bolam and Pissadaki 2012; Ciron *et al.* 2015; Hunn *et al.* 2015). Hence, any factor that perturbs their delicate metabolic balance (e.g. PD-triggers) might 'tip them over the edge'. Meaning that their feedback and feed-forward control mechanisms are no longer sufficient to keep SN DA activity and calcium homeostasis within a desired physiological range, and consequently detrimental pathways can trigger degeneration (Pissadaki and Bolam 2013; Surmeier and Schumacker 2013; Dragicevic *et al.* 2015). In other words, as illustrated in figure 2, PD-trigger factors (e.g. environmental factors or PARK-genes, impairing mitochondrial and/or lysosomal function) would narrow the physiological bandwidth of flexible SN DA activity and calcium signaling in both directions. Consequently, reduced as well as elevated activity- and calcium-levels could tip SN DA neurons more easily 'over their physiological edge' (Pissadaki and Bolam 2013). In this scenario, the same SN DA activity or oscillatory calcium signal that enables their physiological function, could – in the presence of PD-triggers – stimulate their degeneration, by e.g. inducing excitotoxicitiy or apoptosis as described above.

Summary

Taken together, the bidirectional effects of SN DA activity, calcium homeostasis and related ion channel activity on their viability and vulnerability illustrate the general dilemma of these neurons that seem to be committed to maintain their flexible physiological activity. On one hand, they need stimulatory intrinsic (and extrinsic) feed-forward mechanisms to ensure their homeostatic activity, calcium signaling, and dopamine release within the basal ganglia network, even under metabolically demanding situations, as well as to prevent cell death in a 'use it or lose it' context. On the other hand, SN DA neurons need respective inhibitory feedback mechanisms to prevent overexcitability, Ca^{2+} overload, excitotoxicity, and its related cell death mechanisms (compare Fig. 1 and 2).

These context-dependent stimulatory and inhibitory mechanisms are enabled by a network of ion channels (Fig. 1) that can mediate bidirectional functions, and enable a flexible bandwidth of SN DA activity patterns. PD-trigger factors could narrow this physiological bandwidth at both ends, and thus facilitate pathophysiological and degenerative pathways (Fig. 2). To make things worse, once the intricate steady-state of SN DA neurons gets out of balance, the different players that enable and maintain their physiological flexibility, could now – not least because of their complex interactions – augment detrimental pathophysiological changes of SN DA activity pattern and/or calcium load, leading to a vicious self-energizing spiral that becomes independent from its initial source (e.g. PD-triggers), and progressively fortify SN DA degeneration.

Voltage-gated calcium channels (particularly the Cav1.3 L-type and the Cav3.1 T-type) as well as metabolically gated K-ATP channels (particularly the Kir6.2/SUR1 type) seem to have central roles in this scenario: the activity of both channel types is crucial for the specific movement-related physiological functions of SN DA neurons, and thus for the viability of the organism. However, their systemic block or general loss reduces the particularly high vulnerability of SN DA neurons to metabolic stress, degeneration and to PD. The functional expression of Cav1.3 LTCCs and of K-ATP channels and related signaling networks in SN DA but not VTA DA neurons might thus reflect a kind of *antagonistic pleiotropy*: being beneficial and conferring a fitness advantage in youth and young adult life, but might have detrimental effects on SN DA survival later in life during aging, and in PD (Kirkwood and Austad 2000; Lambeth 2007; Parsons 2007; Surmeier *et al.* 2012).

Given the promising results from mouse models and epidemiological studies, pharmacological inhibition of these

channels consequently offers novel therapeutic avenues for neuroprotective PD-therapies – which need to start *before* PD-motor symptoms manifest and most SN DA neurons are already lost. The use of well-established LTCC blockers (phase III isradipine study in early PD, clinicaltrials.gov identifier: NCT02168842), and the development of more specific Cav1.3 LTCC blockers (Kang *et al.* 2012), are the most advanced strategies. Noteworthy in a therapeutic context is that LTCCs, as well as K-ATP and other here discussed ion channels, are not only expressed in SN DA neurons, but in a variety of other neuronal and non-neuronal cells. Given the emerging complex, interdependent, and bidirectional functions of these ion channels that are not yet fully understood, and their intricate signaling network in SN DA neurons (that might well be even much more complex than outlined here), the pharmacological modulation of just one parameter (i.e. block of one ion channel type) might also have a variety of – unpredictable – consequences on the viability and/or the physiological function of SN DA neurons, and thus unwanted side-effects besides the desired neuroprotection. First safety studies indicate that this seems not to be the case for isradipine (Simuni *et al.* 2010). However, further research is needed to develop specific pharmacological ion channel blockers (or activators), and to further dissect their complex acute and chronic effects – on SN DA neurons as well as on the whole human body – in health and in PD.

Acknowledgments and conflict of interest disclosure

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