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Selective degeneration of dopamine neurons in Parkinson's disease: emerging roles of altered calcium homeostasis and nucleolar function

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease. Its classic major motor-symptoms are caused by the progressive loss of dopamine in the striatum, and of dopamine (DA) releasing neurons in the midbrain, particularly within the Substantia nigra (SN). The cause for PD is still unclear, hampering the development of curative therapies. However multiple genetic and environmental PD trigger factors have been identified, pointing to a common, mutually interdependent pathomechanism of cell-specific metabolic dysfunction and altered gene expression. Here, we summarize and discuss these emerging PD-pathomechanisms, that could provide novel potential therapeutic targets, with a focus on altered Ca^{2+} homeostasis and nucleolar function. We discuss how animal models with impaired nucleolar ribosomal RNA synthesis can enable identification of novel cell-specific vulnerability factors, and how complex homeostatic adaptation of SN DA neurons could enable a flexible adjustment of their functional activity to metabolic needs, but also might render them particularly vulnerable to degenerative triggers and cell-death in PD.

Keywords: Parkinson's disease; dopamine; ion channels; rRNA; cell metabolism

Introduction

Parkinson's disease (PD) is besides Alzheimer's disease the second most common neurodegenerative disorder, affecting about 300,000 patients in Germany alone. The number of patients is continuously increasing with ageing (http://www.epda.eu.com). The characteristic motor-symptoms of PD that are reflected by its alternative name as "Schüttellähmung", are slowness of movement (bradykinesia, akinesia), muscle rigidity, postural instability, and a resting tremor. These so-called cardinal symptoms are caused by a progressive loss of dopaminergic (DA) midbrain neurons, particularly within the *Substantia nigra* (SN), accompanied by a respective progressive loss of dopamine, particularly in the dorsal striatum, the axonal projection area of SN DA neurons. For unclear cause, neighboring more medial DA midbrain neurons in the ventral tegmental area (VTA), with projections to corticolimbic areas are much more resistant to PD-triggers. However, it should be noted that other neurons besides SN DA neurons are also degenerating in PD, particularly noradrenergic neurons within the *Locus coeruleus*, and neurons e.g. within the pedunculopontine nucleus, or the dorsal motor nucleus (Surmeier et al., 2017). The causes for the differential vulnerability of DA midbrain neurons, as well as the causes for most PD cases, are still unclear. However while age is the most prominent risk factor for PD, a variety of different genetic and environmental trigger-factors seem to contribute to disease progression. The identification of genetic mutations (PARK-genes and of lower risk variants) that are linked to rare familial forms of PD (about up to 30% of all cases), helped to identify PD trigger-factors and patho-mechanisms. Among them are accumulation of protein aggregates, mitochondrial dysfunction and elevated levels of metabolic and oxidative stress, altered calcium-homeostasis, changes in electrical activity, and transcriptional and translational dysregulation of SN DA neurons (Duda et al., 2016).

In a clinical-therapeutic view, as the molecular mechanisms of PD pathology are still unclear, there are currently no curative but only symptomatic, dopamine-mimetic therapies available. L-DOPA (the blood-brain-barrier permissive precursor of dopamine) together with dopamine-receptor agonists are still the gold standard in drug-based PD-therapy (Oertel and Schulz, 2016). Furthermore, the major motor-symptoms manifest not before the majority (about 70%) of SN DA neurons are already lost. Hence, even if we would fully understand PD-pathology, it would be too late for a neuroprotective therapy, once these motor-symptoms manifest, but only symptomatic or novel

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neurorestorative therapy strategies could be applied. However the latter (aiming to replace lost DA neurons) like stem-cell-based approaches, are still if anything but experimental. In essence, the prerequisite for a successful neuroprotective PD-therapy, aiming to slow down or even halt the degenerative process, is the identification of early pre-clinical disease markers as well as a molecular understanding of the complex PD-pathomechanisms.

Although PD is a multifactorial disease, a variety of interdependent genetic and environmental trigger-factors have been identified, pointing to a common downstream pathomechanism that affects preferentially SN DA neurons, and leads to pathophysiological changes in their functional activity, followed by their progressive degeneration and ultimately PD-symptoms. There is accumulating evidence that altered, activity-dependent Ca^{2+} homeostasis and Ca^{2+} signaling, as well as altered gene-expression and protein synthesis in SN DA neurons are central processes for this downstream pathomechanism (Duda et al., 2016; Parlato and Liss, 2014; Surmeier et al., 2017).

In this review, we focus on discussing how cell-specific dysregulation of Ca^{2+} homeostasis and transcription, in particular ribosomal RNA (rRNA) synthesis in the nucleolus, could allow adaptation of SN DA neuron function to metabolic needs, but also render these neurons particularly vulnerable to degeneration and to PD-triggers.

The specific electrical activity of SN DA neurons causes Ca^{2+} related metabolic stress

Intracellular free Ca^{2+} levels, Ca^{2+} microdomains, Ca^{2+} buffering, and the mode of Ca^{2+} entry are tightly controlled in neurons, as Ca^{2+} modulates and controls a variety of cellular functions, like excitability, neurotransmitter release, ATP-production, apoptosis, as well as general enzyme activity and gene expression. Ca^{2+} regulates mitochondrial motility, stimulates the mitochondrial enzyme nitric oxide synthase, enzymes of the tricarboxylic acid cycle and the mitochondrial electron transport chain (ETC), and thus Ca^{2+} stimulates ATP-production (Duda et al., 2016). However, Ca^{2+} can also increase metabolic and oxidative stress levels, and associated detrimental processes. SN DA neurons might be particularly vulnerable to these harmful Ca^{2+} induced processes, as their resting Ca^{2+} levels appear to be higher compared to e.g. VTA DA neurons (J. Surmeier, personal communication).

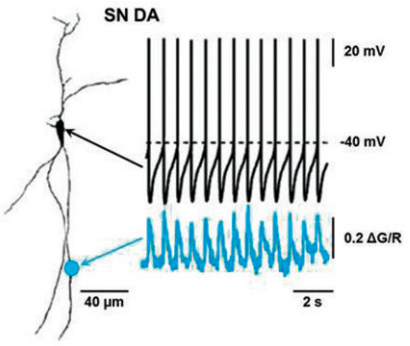

Fig. 1: Illustration of the pacemaker activity of a SN DA neuron. Upper black trace based on whole cell current clamp recording of a SN DA neuron (shown left as a projection image), illustrating the typical low-frequency pacemaker activity (mV). Lower blue trace depicts parallel 2-photon laser scanning fluo-4 Ca^{2+} imaging of the same neuron, illustrating the dendritic Ca^{2+} oscillations (ΔG/R). These oscillations are fully blocked in the proximal dendrites by the L-type Ca^{2+} channel blocker isradipine, while activity of SN DA neurons remains largely unaffected (figure adapted from (Duda et al., 2016).

It is important to have in mind that the main feature of SN DA neurons is their electrical activity. As illustrated in figure 1, this activity is intrinsically generated and accompanied by Ca^{2+} oscillations in the dendrites. Figure 2 shows that the electrical activity of SN DA neurons is modulated by a complex and intricate interplay of distinct ion channels, transporters, and receptors, and it is crucial for presynaptic and somatodendritic dopamine release, and hence for all dopamine-mediated functions (Duda et al., 2016). The activity of SN DA neurons is also modulated by dopamine itself, in a negative feedback loop, by activation of G-protein coupled K^+ channels (GIRK2) via dopamine autoreceptors of the D2-type (D2-AR) (internalized at the membrane by the protein β-arrestin). However, a variety of other ion channels, signaling molecules and pathways can modulate the functional, dopamine-dependent activity of SN DA neurons as illustrated in figure 2. The ion channels include: **a)** ATP-sensitive K^+ (K-ATP) channels (sensors of metabolic stress, build up by Kir6.2 and SUR2 subunits in SN DA neurons), **b)** Ca^{2+} and voltage sensitive A-type K^+ channels (build up by Kv4.3 and KChip3.1 – a member of the neuronal calcium sensor family – in SN DA neurons) and **c)** voltage gated Ca^{2+} channels (VGCCs) (Duda et al., 2016). Typically, A-type K^+ channels rapidly generate inactivating currents that regulate neuronal excitability and firing frequency. Opening of the voltage gated Ca^{2+} channels results in the increase of intracellular calcium levels that, if protracted, are cytotoxic. We could show that VGCCs not only facilitate spontaneous activity

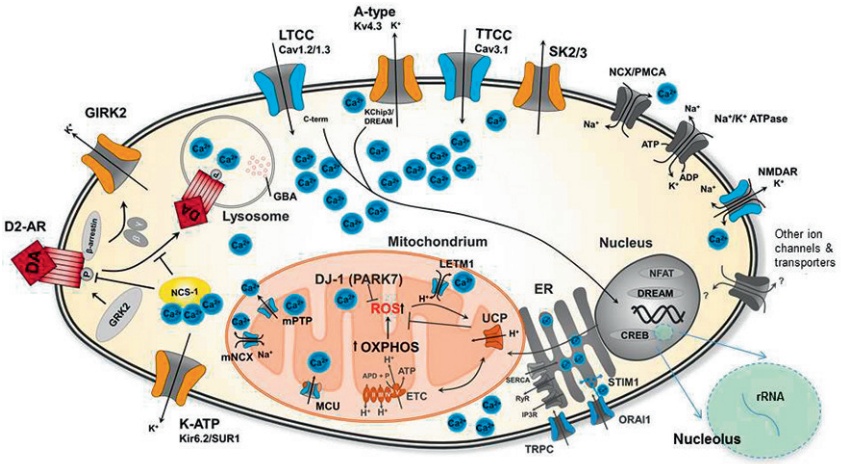

Fig. 2: Converging pathways of ion channel activities, Ca^{2+} homeostasis and metabolic stress in substantia nigra dopaminergic neurons in health and Parkinson's disease. Cartoon illustrating distinct ion channels, receptors and transporters that generate or modulate the activity-patterns of SN DA neurons in vivo and in vitro, and that are associated with oscillating Ca^{2+} levels. Signaling pathways linked to oscillating Ca^{2+} levels and affecting mitochondrial and lysosomal function as well as gene-expression in health and in Parkinson's disease (PD) are also included (see text for details). The nucleolus, as the sub-nuclear compartment in which rRNA synthesis takes place, is also shown. 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, and have both been particularly linked to SN DA degeneration and PD (modified from (Duda et al., 2016)).

of SN DA neurons, but in a negative feedback loop, they also inhibit SN DA activity via stimulation of the neuronal calcium sensor NCS-1 that modulates the D2-AR function activating GIRK2 (Dragicevic et al., 2014; Duda et al., 2016; Poetschke et al., 2015).

Neuronal activity per se implies high-energy demand and metabolic stress, mainly due to stimulation of the Na^+/K^+ ATPase that is necessary to maintain the asymmetric ion distribution after action potentials and that is consuming about 50% and more ATP in active neurons. SN DA neurons seem to be particularly dependent on proper Na^+/K^+ ATPase activity. In this context, it is important to note that the metabolic cost of SN DA neuron activity is particularly high, compared to VTA DA and other neurons. Indeed specific subtypes of voltage-gated Ca^{2+} channels are active, causing an activity-related, oscillatory increase in intracellular Ca^{2+} levels (see Figure 1 and 2). L-type voltage-gated calcium channels (LTCCs) are high voltage activated, they show slow gating and in neurons include members of the Cav1 family. Oscillatory Ca^{2+} changes – assumed to be particularly caused by Cav1.3 L-type VGCCs – cause related oscillatory changes of mitochondrial membrane potentials, ROS-levels, and of Ca^{2+} transporter activity (Figure 2). As shown in Figure 2, voltage-gated Ca^{2+} channels regulate intracellular Ca^{2+} levels, but also mitochondria, the endoplasmic reticulum (ER) and lysosomes contribute to maintain calcium homeostasis in SN DA neurons via specific membrane proteins, for example exchangers (mNCX, LETM1), uniporters (MCU) or enzymes (e.g. SERCA at the ER and the glucocerebrosidase GBA at the lysosomes).

This specific mode of electrical activity of SN DA neurons not only generates periodically elevated Ca^{2+} and metabolic stress levels, but also likely renders them particularly vulnerable to additional metabolic stressors and PD-triggers (like mitochondrial, proteasomal, lysosomal, and PARK-gene dysfunction), and thus could explain their preferential degeneration in PD. Thus, inhibition of the long lasting activation of L-type calcium channels in SN DA neurons should have neuroprotective effects and it offers a novel therapeutic PD-strategy. Indeed, epidemiological studies indicate that blood-brain-barrier permissive LTCC blockers of the dihydropyridine (DHP) class, used in the treatment of hypertension, reduce the risk for PD by about 30%, and the DHP isradipine is currently already in a phase III clinical trial (ClinicalTrials.gov Identifier: NCT02168842) for neuroprotective PD-therapy (Duda et al., 2016; Surmeier et al., 2017).

Given its high metabolic costs, the oscillatory VGCC activity and the associated Ca^{2+} signal in SN DA neurons must be crucial for their specific physiological functions. Mechanisms that maintain or stimulate electrical activity and Ca^{2+} mediated dopamine-release, and thus the ability for voluntary movement, could be beneficial or even life-saving for the organism – particularly under metabolic demand situations (e.g. food deprivation, or fight-or-flight situations). Indeed, LTCCs stabilize the ongoing

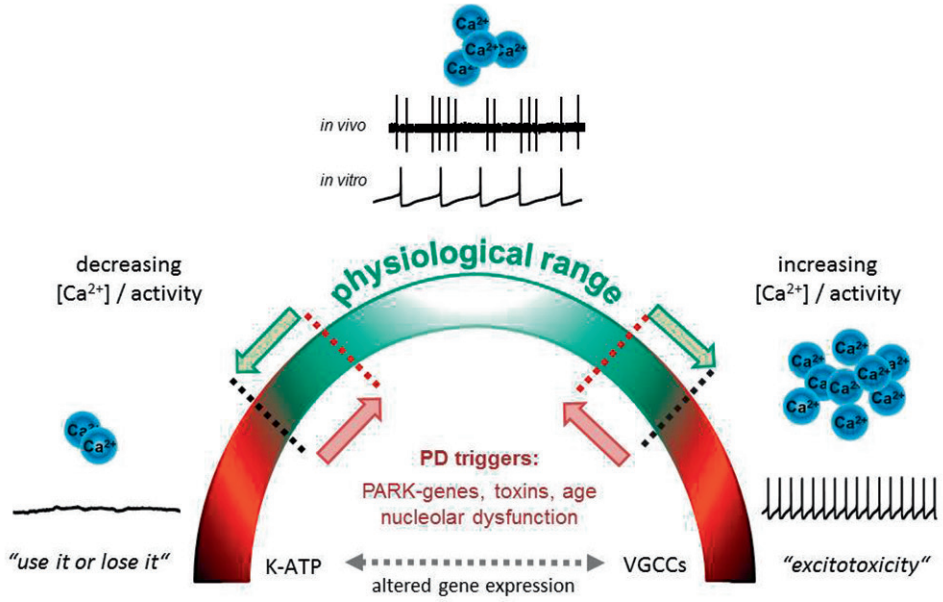

Fig. 3: Double-edged roles of activity and free intracellular Ca2+ levels for physiological functions of SN DA neurons and their vulnerability to PD-triggers. Shown are typical *in vivo* and *in vitro* single spike activity-patterns of SN DA neurons from adult mice (methods detailed in (Dragicevic et al., 2014; Schiemann et al., 2012). The cartoon summarizes that SN DA neurons possess several intrinsic feedback and feed-forward mechanisms to protect and to adapt their activity-pattern. In addition SN DA neurons show 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 the "*use it or lose it*" principle by which inactive neurons are more prone to death and by "*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 and K-ATP channels are of particular importance, as they have bidirectional physiological functions, can stimulate each other's activity (indicated by dotted grey double-arrow), and they both can trigger selective SN DA degeneration and PD (for details see text; modified from (Duda et al., 2016).

activity of SN DA neurons (reviewed in (Duda et al., 2016)). Furthermore, the associated oscillatory increase in intracellular Ca^{2+} stimulates the tricarboxylic acid cycle and the mitochondrial electron transport chain, and thus ATP-production. In this view, in a feed-forward cycle, LTCC activity would ensure electrical activity, ATP production, and dopamine release of SN DA neurons, and thus movement, particularly under metabolic demand situation. However as a drawback, the ongoing stimulated activity of SN DA neurons and associated high metabolic stress levels will render SN DA neurons more vulnerable to excitotoxicity and PD-triggers (Figure 3). On the other hand, mechanisms that reduce SN DA activity, should protect SN DA neurons from excitotoxic events, but would impair voluntary movement, and thus could be detrimental for the organism, particularly under situations were immediate and ongoing motion is required for survival (food deprivation, fight-or-flight situations).

This (and figure 3) illustrates a general "dilemma" of SN DA neurons: on the one hand they ensure and adjust electrical activity and Ca^{2+} signaling to metabolic needs, while on the other hand they prevent their own death (Duda et al., 2016). Given these thoughts, we reason that SN DA neurons display a context-dependent, flexible bandwidth of activity-patterns and associated Ca^{2+} levels and they can adapt them to physiological needs. In line, there is accumulating evidence that SN DA neurons possess several intrinsic feedback and feed-forward mechanisms to protect and to adjust their activity-pattern as well as their calcium-homeostasis in both directions within a physiological range. Both ends of this spectrum can trigger cell death and PD trigger factors could narrow the physiological bandwidth of SN DA neurons and facilitate detrimental processes (Figure 2 & 3) detailed in ((Duda et al., 2016)).

More precisely as summarized in Figures 2 & 3 and as summarized in (Duda et al., 2016), we propose a scenario, in which VGCC activity stabilizes and stimulates SN DA activity and their ATP-production in a positive feed-forward mechanism: the more active the neuron is, the more dopamine gets released, the more ATP is needed and it would be produced due to VGCC activity and Ca^{2+} stimulation of

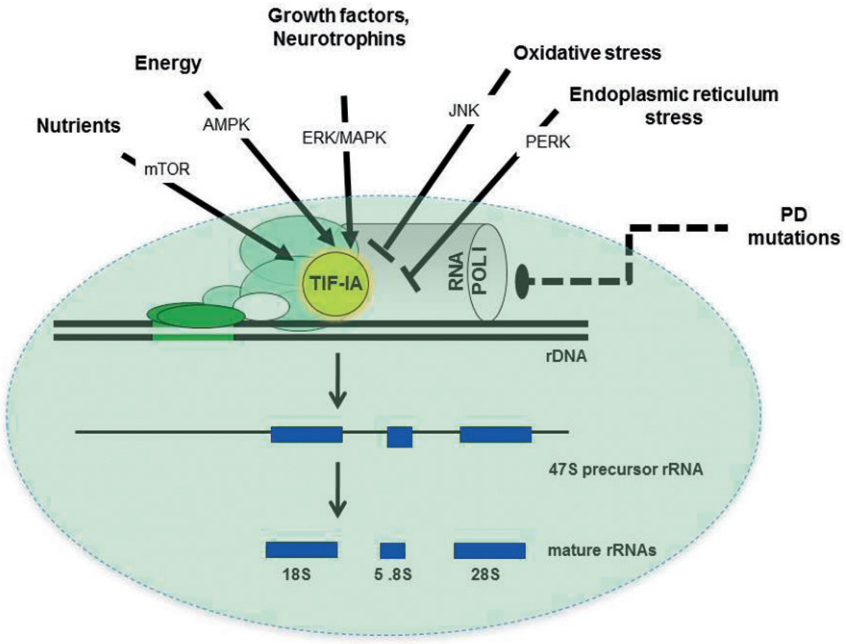

Fig. 4: The nucleolus is a stress sensor and its activity is responsive to environmental changes. Shown is a schematic representation of a nucleolus (dashed area) and of a typical rDNA promoter occupied by the transcriptional machinery. This transcribes the 47S precursor rRNA further processed to produce mature rRNAs. Depicted are also conditions regulating the activity of the nucleolar transcription factor TIF-IA important for recruiting the RNA polymerase I to the transcriptional machinery. Different protein kinases activate or inhibit TIF-IA in response to permissive or detrimental stimuli, moreover the potential role of known mutant proteins in Parkinson's disease has been suggested in the inhibition of rRNA synthesis, although the mechanisms are not completely understood (dashed arrow) (for details see text, and (Parlato and Bierhoff, 2015)).

enzymes. However, in indirect negative feedback-loops, VGCCs and Ca^{2+} can also stimulate inhibitory responses that reduce SN DA activity and Ca^{2+} levels e.g. via Ca^{2+} mediated stimulation of NCS-1/ dopamine D2 autoreceptors/ GIRK2 activity, or Ca^{2+} mediated sensitization of the A-type Kv4.3/KChip3 channel, or metabolic stress activated K-ATP channels (Dragicevic et al., 2014; Duda et al., 2016; Poetschke et al., 2015; Schiemann et al., 2012). Membrane hyperpolarization and reduced SN DA activity resulting from activated K-ATP channels represent an intrinsic control mechanism to prevent overexcitability but may also lead to neuronal death (based on the "use or lose it" principle by which inactive neurons are more prone to death). Furthermore, on a more permanent level, VGCCs can homeostatically adapt SN DA neuron function to physiological needs via alterations of Ca^{2+} dependent gene-expression as they are particularly effective in activating Ca^{2+} dependent transcription factors, like the cAMP-response element binding protein CREB, the nuclear factor of activated T cells NFAT, and the downstream regulatory element antagonistic modulator DREAM. Moreover, the C-termini of Cav1.3 and Cav1.2 LTCCs can be cleaved and translocate from the plasma membrane to the nucleus in a Ca^{2+} dependent fashion, where the C-termini act as transcription factors. Likewise, the A-type K+ channel subunit KChip3 is indeed the transcriptional repressor DREAM, and can shuttle to the nucleus in inverse correlation with cellular Ca^{2+} levels (Figure 2). Altogether, these short- and long-term bidirectional functions of VGCCs and Ca^{2+} in SN DA neurons would ensure their adaptive electrical activity, dopamine release, and thus context-specific movement control, while preventing – to a certain degree – cell death. However, due to their intrinsic high metabolic burden, SN DA neurons are "living on the edge", and thus are particularly vulnerable to trigger factors, that narrow the "points of no return" and cell death (Figure 3).

Dysregulation of rRNA synthesis in the nucleolus of DA neurons as a regulator of the "points of no return" in PD

Within the nucleus, the nucleolus – a nuclear non-membrane bound compartment, traditionally known as the site of rRNA synthesis and ribosome assembly represents an important hub for complex homeostatic networks (Boulon et al., 2010). The nucleolus adapts the transcriptional status of ribosomal DNA (rDNA) genes to coordinate ribosome production with metabolic needs and in response to environmental changes. In general, conditions permissive to cell growth and survival, positively activate rDNA transcription and rRNA synthesis, while harmful conditions result in the opposite effect (Figure 4) (Parlato and Bierhoff, 2015). In light of this flexibility the nucleolus is considered a "stress sensor" and a vast amount of work has been dedicated to a better understanding of the genetic and epigenetic factors that regulate rDNA transcription and nucleolar integrity, as recently reviewed by (Parlato and Bierhoff, 2015).

Given the high metabolic demand of DA neurons as previously explained, the critical role of the nucleolus in the regulation of this "critical life-death decision" is very likely. In fact, it is important to emphasize that PD-triggers such as increased DNA damage, reduced neurotrophin levels, reduced level of ATP, impaired proteostasis, and elevated oxidative stress all seem to impair rDNA transcription, disrupt nucleolar integrity and result in a condition defined as "nucleolar stress" (Figure 4). Ca^{2+} stimulation induces a reorganization of subnuclear structures however the impact on rRNA synthesis has not been investigated.

Nucleolar integrity in general is tightly linked to rDNA transcription: if stress conditions are protracted, loss of rRNA synthesis results in loss of nucleolar integrity and nucleolar stress. This condition is identified by the inhibition of rRNA synthesis and the release of nucleolar proteins – that are usually shuttled between the nucleolus and the nucleoplasm – in the nucleoplasm. In particular, the release of ribosomal proteins affects the proteasomal degradation of the transcription factor p53 resulting in its increased stability. Consequently, p53 plays a major role in the cellular stress defense by the activation of DNA repair, antioxidant enzymes, and autophagy. In view of this effect on p53 function, the nucleolus is also considered a mediator of the cellular response to stress conditions (Boulon et al., 2010).

A link between activity-dependent membrane-to-nucleus gene expression and rDNA transcription has been further supported by studies showing that long-term neuronal stimulation results in an increase in nucleolar numbers and protein synthesis.

Given the multifactorial basis of PD, and the strong metabolic burden sustained by DA neurons, we have been among the first groups addressing whether nucleolar stress might play a role in PD (Parlato and Liss, 2014). We have shown that rDNA transcription and nucleolar integrity are disrupted in DA neurons (Rieker et al., 2011), by monitoring and quantifying rRNA synthesis and the mislocalization of the nucleolar protein nucleophosmin in DA neurons in post-mortem PD brains. Indeed, altered expression of nucleolar and ribosomal proteins in human PD brains at different disease stages has been found, indicating that the protein synthesis machinery is strongly impaired in PD (Garcia-Esparcia et al., 2015). Furthermore, the expression of the PARK7 (DJ-1 L166P) mutation that leads to a familiar form of PD, alters rRNA synthesis most likely by interfering with pre-rRNA processing and maturation of rRNA (reviewed in (Parlato and Liss, 2014)). Moreover, pre-rRNA levels are reduced in the SN of conditional PARK2 (parkin) knockout mice, and also in patients with sporadic PD (associated with increased p53 levels), indicating that PARK-mutations and their altered signaling pathways also affect nucleolar activity and integrity (Parlato and Bierhoff, 2015).

The limited possibility to analyze presymptomatic stages in human PD can be bypassed using animal models. We have shown in neurotoxin based PD-mouse models evidence of impaired rRNA synthesis and altered nucleolar integrity (Rieker et al., 2011).

To investigate the impact of nucleolar stress in DA neurons, we have generated a mouse model mimicking nucleolar stress in specific neuronal-types. The system is based on the deletion of the gene encoding the nucleolar transcription factor TIF-IA by genetic engineering in mice. This gene deletion results in the specific loss of TIF-IA only in DA neurons, leading to inhibition of rDNA transcription and disruption of nucleolar integrity, enabling us to identify the sequence of molecular and cellular events dependent on nucleolar stress at different stages. TIF-IA is a transcription factor essential for the transcription of the rDNA genes because it recruits the RNA Polymerase I (Pol I) to the rDNA promoter (Figure 4). TIF-IA activity is regulated by different kinases: mammalian/mechanistic target of rapamycin (mTOR), AMP-activated protein kinase (AMPK), extracellular signal–regulated kinases (ERKs) or classical mitogen-activated kinases (MAPK), the stress-dependent c-Jun N-terminal kinase (JNK), or protein kinase

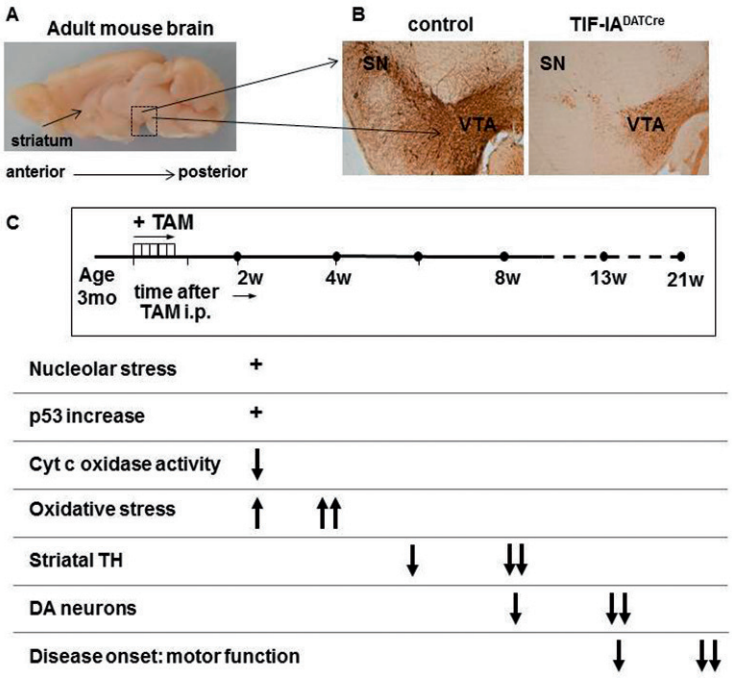

Fig. 5: The nucleolus is a mediator of the stress response and leads to progressive loss of SN DA neurons. A: Sagittal view of an adult mouse brain shows the ventral midbrain region (dashed area) in which SN and VTA DA neurons are located. B: Selective vulnerability of SN DA neurons in the conditional DA-specific TIF-IA knock-out mice (TIF-IA^{DATCre}) by immunohistochemistry with tyrosine hydroxylase (TH) antibody, an enzyme involved in the synthesis of dopamine and used to visualize DA neurons (Rieker et al., 2011). C: Schematic representation of the procedure followed to dissect the sequence of events following induction of nucleolar stress by the use of a inducible conditional DA-specific TIF-IA knock-out mice (TIF-IA^{DATCreERT2}) based on the intraperitoneal injection (i.p.) of tamoxifen (TAM) in adult mice (3 months, mo) causing the ablation of TIF-IA in adult mice (Rieker et al., 2011). The scheme also summarizes the major events downstream of nucleolar stress over time (in weeks, w) (for details see text).

R-like endoplasmic reticulum kinase (PERK). These kinase activities result in specific phosphorylation patterns that can either activate or inactivate TIF-IA function. In response to ATP, neurotrophins, growth factors TIF-IA is active and rRNA synthesis takes place. Inactivation of TIF-IA leads to inhibition of rRNA synthesis in response to alteration of the endoplasmic reticulum (ER) function (also known as ER stress), oxidative stress, or DNA damage (Figure 4). Thus, deletion of TIF-IA gene can be used to inhibit rRNA synthesis and mimic a condition of nucleolar stress.

To our surprise, nucleolar stress, although equally induced in all DA neurons, resulted in the preferential loss of SN neurons, while VTA neurons appeared more resistant to nucleolar stress, recapitulating one of the most typical phenotypic alterations of PD (Rieker et al., 2011). Other PD-related alterations included p53 increase, impaired mitochondrial activity, loss of dopamine in the striatum, impaired motor coordination, here assessed by rotarod test (Figure 5).

The signaling cascades triggered by nucleolar stress and the molecular mechanisms underlying this Parkinsonian phenotype are current object of our investigation. The "TIF-IA models" may be instrumental for the identification of early neuroprotective strategies adopted in the very beginning of the response to impaired rRNA synthesis. In fact, we should point out that despite a strong impact on neuronal survival, there is a time window in which rRNA synthesis is altered but the neurons are just "sensing" this condition and try to cope with it. Interestingly, medium spiny neurons of the striatum, when lacking TIF-IA can survive up to three months in mice, while SN DA neurons only for a couple of weeks (reviewed in (Parlato and Bierhoff, 2015)).

However, our studies identified in both neuronal types a negative feedback inhibiting the activity of the mTOR pathway, essential for regulation of protein synthesis and regulation of autophagy. Interestingly, we could also prove the potential relevance of the "TIF-IA models" for testing therapeutic strategies. In fact, along with being able to improve mouse lifespan upon the use of the classical L-DOPA treatment, we have also genetically manipulated the mTOR pathway by generating double mutant mice lacking both TIF-IA and the phosphatase PTEN, a major regulator of mTOR. Loss of TIF-IA leads to downregulation of mTOR

activity. Nevertheless, the specific ablation of the mTOR repressor PTEN in adult mouse DA neurons leads to activation of mTOR pathway and it is neuroprotective restoring striatal dopamine in TIF-IA knockout mice, and rescuing locomotor impairments (Domanskyi et al., 2011).

In summary, these TIF-IA based models are extremely useful in dissecting the events triggered by nucleolar stress. It is important to mention that these are early events prior to any effect on protein synthesis, at a stage when neurons are activating strategies to cope with stress conditions. Another important aspect underscored by our models, is that it takes time for the neurons to die and there is a differential response depending on the neuronal contexts. Based on these premises, our vision is to employ these models as a reference to "isolate" similar processes and responses in pathological conditions at a preclinical phase.

Conclusions and Perspectives

The "high calcium, high activity, high metabolism" phenotype of SN DA neurons means that they are energetically "living on the edge." Hence, any factor that perturbs their delicate metabolic balance (e.g. PD-triggers) might "tip them over the edge." Meaning that all their immediate and gene-expression based 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. In this view, PD-trigger factors (environmental factors or PARK-genes) 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". 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 excitotoxicity or apoptosis. To make things worse, once the intricate steady-state of SN DA neurons gets out of balance, the players that enable and maintain their physiological flexibility, could now – not at least due to 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.

While Ca^{2+} dependent regulation of gene-expression is well-established, a direct link between altered Ca^{2+} homeostasis and regulation of rRNA synthesis is still missing for SN DA neurons. Yet, maintenance of Ca^{2+} homeostasis and transcriptional adaptive mechanisms adopted by the nucleolus might represent major strategies to homeostatically adapt SN DA activity to metabolic needs, and/or to compensate for metabolic stress and PD-trigger factors. However, in a self-accelerating spiral, mitochondrial dysfunction, altered Ca^{2+} homeostasis and altered nucleolar function, caused by PARK-genes or environmental factors, would particularly lead to further mitochondrial and nucleolar and cellular stress specifically in SN DA neurons, until a point of no return. Consequently, drugs that could disrupt this vicious cycle could provide novel therapeutic strategies for neuroprotective PD-therapy beyond the currently evaluated LTCC-inhibitors.

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Abbreviations

A-type Kv/KChip	A-type voltage-gated K^+ channel. The open/closed (active/inactive) status depends on changes in the electrical potential of the membrane
AMPK	AMP activated protein kinase. AMP is produced upon use of ATP and it is an indicator of energy availability
CREB	cAMP response element-binding protein, transcription factor
DA	dopaminergic
D2-AR	dopamine D2 autoreceptor
DJ-1	PARK7 gene product
DREAM	downstream regulatory element antagonistic modulator, also known as KChip3 or calselinin
ER	endoplasmatic reticulum
ERK	extracellular signal-regulated kinases
ETC	electron transport chain
GBA	glucocerebrosidase
GIRK	G-protein coupled inwardly rectifying K^+ channel
GRK2	G-protein-coupled receptor kinase 2
IP3R	inositol-3-phosphate receptor, it leads to release Ca^{2+} from the endoplasmic reticulum
JNK	c-Jun N-terminal kinase, stress-activated kinase
K-ATP	ATP-sensitive K^+ channel
LETM1	high Ca^{2+} affine leucine zipper EF-hand containing transmembrane protein 1, mitochondrial Ca^{2+}/H^+ exchanger
LTCC (Cav1.3)	Cav1.3 L-type voltage-gated Ca^{2+} channel

mCU	mitochondrial Ca^{2+} uniporter
MAPK	mitogen-activated protein kinases, originally called ERK, extracellular signal-regulated kinases
MNCX	mitochondrial Na^{+}/Ca^{2+} exchanger
mPTP	mitochondrial permeability transition pore
mTOR	mammalian/mechanistic target of rapamycin
NCS-1	neuronal Ca^{2+} sensor 1
NCX	Na^{+}/Ca^{2+} exchanger
NFAT	nuclear factor of activated T cells
NMDA-R	N-methyl-D-aspartate glutamate receptor
ORAI1	store operated calcium channels, activated by the depletion of internal calcium stores
OXPHOS	oxidative phosphorylation
PARK-gene	Parkinson's disease associated gene
PERK	protein kinase R (PKR)-like endoplasmic reticulum kinase, transmembrane protein kinase resident in the endoplasmic reticulum. It is induced by ER stress that is caused by misfolded proteins.
P	phosphate
PD	Parkinson's disease
PMCA	plasma membrane Ca^{2+} ATPase
ROS	reactive oxygen species
rRNA	ribosomal RNA
RyR	ryanodine receptor, intracellular Ca^{2+} channel that senses intracellular Ca^{2+} levels.
SERCA	sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase
SK	small conductance Ca^{2+} sensitive K^{+} channel, activated by an increase in the concentration of Ca^{2+} in the cell
SN	Substantia nigra
STIM	stromal interaction molecule, it detects lower Ca^{2+} in the endoplasmic reticulum, activator of ORAI1.
TIF-IA	transcription initiation factor-IA
TCA	tricarboxylic acid cycle
TRPC	transient receptor potential channel, non selective ion channels
TTCC	T-type voltage-gated Ca^{2+} channel
UCP	uncoupling protein
VGCCs	voltage-gated calcium channels
VTA	ventral tegmental area

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