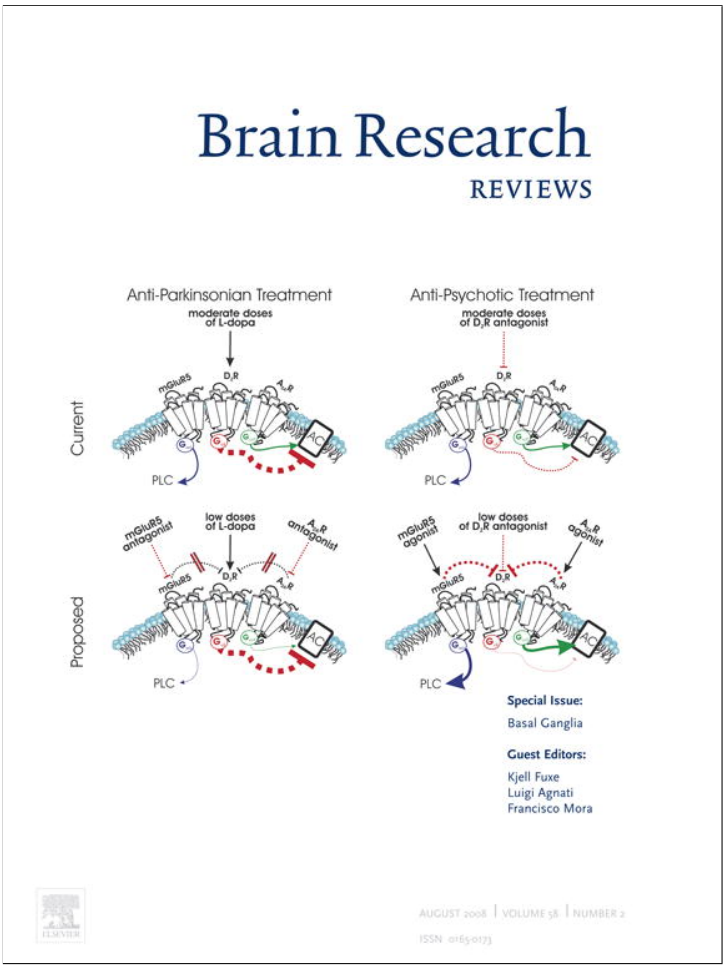

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Review

Individual dopamine midbrain neurons: Functional diversity and flexibility in health and disease

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ABSTRACT

Dopaminergic midbrain neurons are involved in many important brain functions including motor control, as well as emotive and cognitive tasks. They also play critical roles in major disorders likes Parkinson disease, schizophrenia, drug abuse and attention-deficit hyperactivity disorder. This bewildering diversity of distinct dopaminergic functions appears to be in contrast to the routinely assumed functional homogeneity of dopaminergic midbrain neurons at the level of individual cells. If they indeed would conform to a single stereotypical phenotype, the functional diversity of dopaminergic neurons would be predominantly mediated by their involvement in anatomically distinct subcortical and cortical neuronal networks and their distinct postsynaptic targets. However, there is increasing evidence for functional diversity as well as plasticity within the population of dopaminergic midbrain neurons. In addition, dopaminergic midbrain neurons are also not homogeneously affected by disease processes, but instead show large differences in their relative vulnerability, especially their susceptibility to cell death in Parkinson disease. Here, we review recent progress in understanding diversity and flexibility of individual dopaminergic midbrain neurons at molecular and functional levels.

Contents

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Abbreviations: Cav1.3, voltage-gated calcium channel; DA, dopaminergic; D2R, dopamine receptor subtype 2; GIRK2, G-protein-coupled inwardly rectifying potassium channel; HCN, hyperpolarization and cyclic nucleotide gate cation channel; ISI, interspike interval; K-ATP, ATP-sensitive potassium channel; Kir, inwardly rectifying potassium channel; Kv4.3, voltage-gated (A-type) potassium channel; MPTP, methyl-4-phenyl-1,2,3,6-tetrahydropyridin; Nav, voltage-gated sodium channel; PD, Parkinson's disease; SK3, small conductance potassium channel (calcium-sensitive); SN, substantia nigra; SUR, sulfonylurea receptor; TTX, tetrodotoxin; T-type Ca, voltage-gated T-type calcium channel; VTA, ventral tegmental area

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1. Introduction

Ever since the discovery of dopamine, 50 years ago by Arvid Carlsson and colleagues, particular interest has been focused on the dopaminergic (DA) midbrain system (Bjorklund and Dunnett, 2007b). The development of histofluorescence techniques for identification of catecholaminergic neurons also allowed the detailed anatomical mapping of DA neurons in the midbrain and the delineation – initially in the rodent brain – of several nuclei, mainly the substantia nigra pars compacta (SNc, A9), the ventral tegmental area (VTA, A10) and the retrorubral field (A8), which harbor most of the dopaminergic cell bodies (Bjorklund and Dunnett, 2007a) (compare Fig. 1, lower panels). Subsequent tracing studies demonstrated that these DA midbrain neurons projected to a wide variety of subcortical and cortical target areas, most prominently among them the mesostriatal and mesolimbic projections, which are arranged to some degree in topographical order in the midbrain (Bjorklund and Dunnett, 2007a) (see Fig. 1). We now have also evidence – in parallel with the large expansion of the DA midbrain system from rodent to primate and human brains – that the cortical innervation by DA midbrain neurons has also extended to many novel cortical regions beyond those few prefrontal target areas of DA midbrain neurons in the rat (Haber, 2003). Functional analysis of individual DA midbrain neurons was boosted by the now classical studies of Grace and Bunney in the early eighties, who were the first to record electrical activity of neurochemically defined DA midbrain neurons intracellularly *in vivo* (Grace and Bunney, 1980; Grace et al., 2007). They defined a still valid functional fingerprint of DA midbrain neurons: a distinct action-potential waveform associated with two different discharge patterns that spontaneously occurred *in vivo* in the anesthetized rodent brain – either discharge in an irregular single spike mode with a very narrow frequency band (between 1 and 8 Hz) (Grace and Bunney, 1984b), or alternatively short bursts of action potentials at higher frequencies (Grace and Bunney, 1984a) (compare Fig. 2B). Given the nature of their experimental settings, the behavioral significance of these distinct patterns of DA activity had to remain unclear. Schultz and colleagues recorded electrical activity of single DA midbrain neurons, identified by their electrical fingerprints as described above, in awake and behaving primates, which has allowed them in a string of landmark studies to define the behavioral significance of the distinct discharge patterns of DA midbrain neurons in an unprecedented fashion (Schultz, 2007a). It is mainly due to their research efforts over the last 20 years that we now possess a sophisticated and quantitative

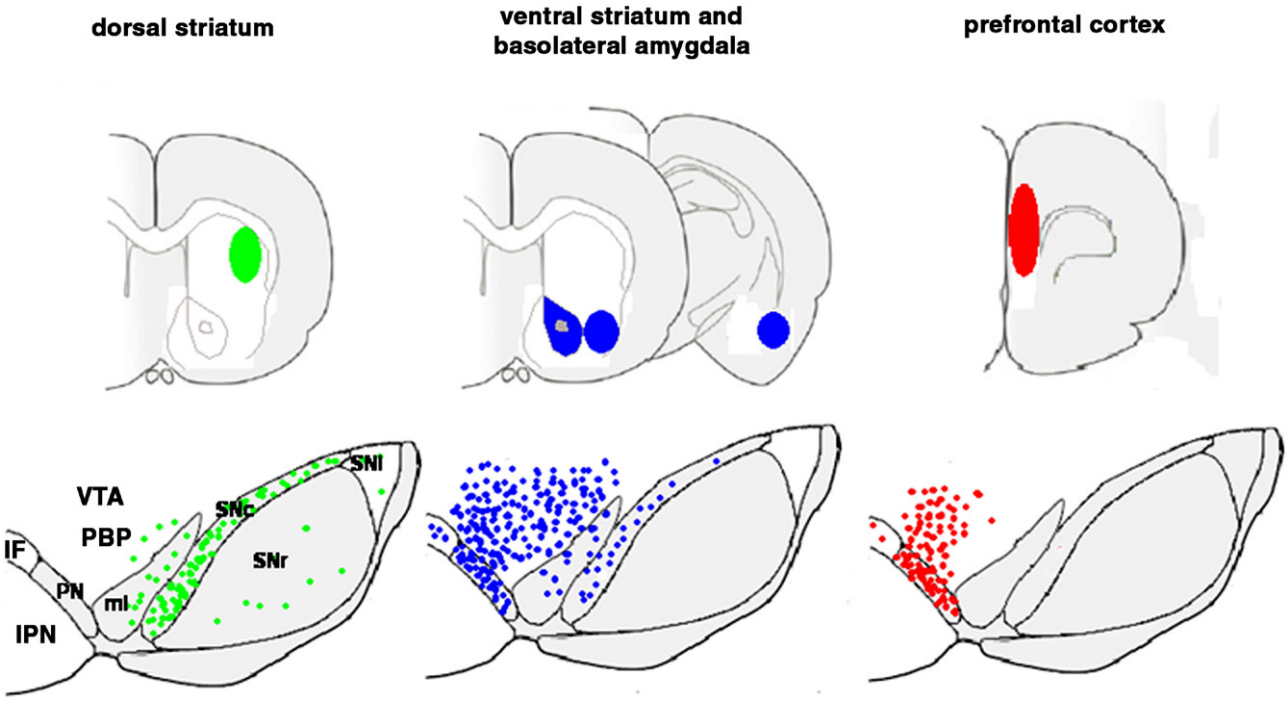

Fig. 1 – Anatomical localisation of dopaminergic midbrain neurons in SN and VTA and their axonal projections into striatal, limbic and cortical areas. Schematic illustration of coronal adult mouse midbrain sections (lower panels) containing dopaminergic neurons (green, blue and red dots), as well as their respective projection areas (upper panels). Left: location of classical SN DA neurons, projecting to the dorsal striatum (green). Middle: location of VTA DA neurons, projecting to limbic areas (blue): ventral striatum (nucleus accumbens shell/core) and basolateral amygdala. Right: location of VTA DA neurons, projecting to cortical areas (red). Abbreviations: IF – interfascicular nucleus, PN – paranigral nucleus, PBP – parabrachial pigmented nucleus, SNl – substantia nigra pars lateralis, SNr – substantia nigra pars reticularis, SNc – substantia nigra pars compacta, ml – lemnicus medialis, IPN – interpeduncular nucleus.

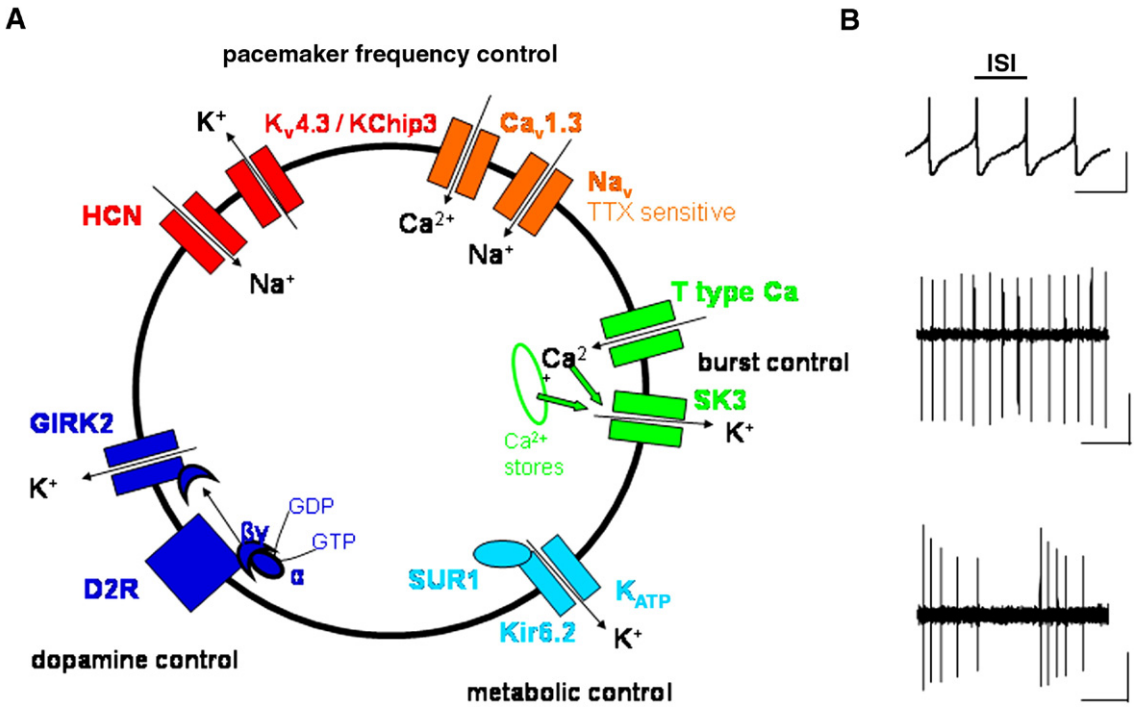

Fig. 2 – Spontaneous activity patterns and related ion channels expressed in dopaminergic midbrain neurons. (A) Schematic illustration of distinct ion channels expressed in dopaminergic midbrain neurons, modulating their spontaneous activity (pacemaker, burst as well as metabolic and dopamine control, for details, see text). (B) Examples of activity patterns of three individual dopaminergic midbrain neurons from adult mouse: *In vitro* (upper, current-clamp, perforated-patch recording) and *in vivo* (middle, extracellular recording, sampling rate 12.5 kHz) pacemaker activity, as well as *in vivo* burst activity (lower panel). Scale bars: upper 0.5 s, 25 mV, middle/lower 1 s, 0.5 mV. Abbreviations: ISI – interspike interval, HCN – hyperpolarization and cyclic nucleotide gate cation channel, Kv4.3 – voltage-gated (A-type) potassium channel, Cav1.3 – voltage-gated calcium channel, Nav – voltage-gated sodium channel, TTX – tetrodotoxin, T-type Ca – voltage-gated T-type calcium channel, SK3 – small conductance potassium channel (calcium-sensitive), K-ATP – ATP-sensitive potassium channel; Kir – inwardly rectifying potassium channel, SUR – sulfonylurea receptor, D2R – dopamine receptor subtype 2, GIRK2 – G-protein-coupled inwardly rectifying potassium channel.

theory of the behavioral roles of the distinct DA discharge patterns, which prominently appear to signal reward prediction errors in context of temporal-difference learning paradigms (Schultz, 2007b). In parallel, the development of reduced brain preparations and patch-clamp techniques enabled the study of biophysical mechanisms and ion channels that generate and control electrical activity in DA neurons (Puopolo et al., 2007). Most recently, the combined analysis of function and gene-expression at the level of individual DA midbrain neurons enabled their distinct molecular definition (Liss and Roeper, 2004). These complementary levels of analysis enable us to readdress open and still controversial topics of DA midbrain neurons – in particular the question regarding their diversity and plasticity. In line with the variety of proposed DA functions including motor control, emotive and cognitive key functions, and their distinct roles in major disorders like Parkinson diseases, schizophrenia, drug abuse and attention-deficit hyperactivity disorder (ADHD), DA midbrain neurons might also be more diverse at a cellular and eventually a molecular level (Korotkova et al., 2004; Neuhoff et al., 2002). In addition to dopaminergic diversity according to their distinct anatomical or functional identities under physiological control conditions, other functional differences might only beome apparent during disease-related challenges thereby revealing distinct "*pathophysiological identities*" among DA midbrain neurons (Liss et al., 2005). Indeed, the phenomenon of differential vulnerability of DA neurons to degeneration in PD is well documented and reflects the fact that some of the DA cell groups in the midbrain are particularly affected by the neurodegenerative process of PD while others are relatively spared (Damier et al., 1999). This review focuses on recent progress to delineate these two variants of diversity of DA midbrain neurons: their physiological and pathophysiological differences. We highlight functional and molecular single-cell studies, as they enable an unbiased approach towards the question of diversity in the DA system. We focus on the key phenotypic features of DA midbrain neurons and their plasticity and discuss underlying mechanisms.

2. Defining dopaminergic subpopulations: Distinct identities of dopaminergic midbrain neurons

As recently discussed by Bjorklund and Dunnett (2007a), many researchers have relied on useful but simplified heuristic definitions of subpopulations of DA midbrain neurons. DA midbrain neurons were identified and differentiated either by

their distinct somatic localization in different nuclei, thus defining the DA substantia nigra (SN) neurons and the DA neurons of the ventral tegmental area (VTA). Alternatively, DA midbrain neurons were defined by their axonal projections leading to distinct mesostriatal and mesocorticolimbic DA systems (compare Fig. 1). These definitions presume rather than demonstrate certain degrees of diversity within the DA system. In a complementary classical concept, DA midbrain neurons have been defined as those nerve cells positioned in the mesencephalon, which possess the ability for synthesis, packaging, release, and reuptake of the neurotransmitter dopamine. These key features are easily captured by qualitatively probing for the expression of a number of marker genes like tyrosine-hydroxylase (TH), AADC (aromatic amino acid decarboxylase) or the vesicular monoamine transporter (VMAT2) (Bjorklund and Dunnett, 2007a). The absence of other catecholamine-containing neurons in the midbrain allows using TH expression as a consistent and sufficient marker for demonstrating dopaminergic identity within the midbrain that has been used in many studies. While the definition of the neurochemical dopaminergic identity per se is simple, defining consistent and meaningful molecular markers for distinct DA subpopulations is far from trivial. For unbiased definition of subpopulations of dopaminergic neurons at the molecular level, global (microarray-based) gene-expression profiling and cluster analysis at the level of the individual dopaminergic midbrain neurons would be necessary (Kamme and Erlander, 2003). However, up to now, microarray-based expression profiling of dopaminergic midbrain neurons has still only been published for the level of microdissected tissue or large pools of dopaminergic neurons (Chung et al., 2005; Greene et al., 2005; Grimm et al., 2004).

This general problem of defining neuronal subtypes, might also be discussed in the light of homeostatic neuronal plasticity (present in DA midbrain neurons, see below) (Davis, 2006). Studies suggest that neurons when perturbed possess an astonishing flexibility to regain their cell-specific functional properties – even in the absence of key components of their biophysical machinery. This homeostatic plasticity might argue for a conceptional preference of "*functional identity*" rather than the use of a rigid set of molecular markers defining a "*molecular identity*", when aiming to define neuronal subtypes (Schulz et al., 2006). Thus, one alternative approach for functional and molecular definition of DA subtypes is to focus on their intrinsic excitability in addition to the expression of basic marker-gene-sets (*phenotype–genotype correlation*). Intrinsic excitability defines the input–output relations of specific neurons within synaptic networks thus endowing it with a "functional identity" (Schulz et al., 2006). However, this functional identity is not simply fixed by the co-expression of a certain set of marker genes and ion channels but it actively adapts (functionally as well as molecularly) during perturbations to defend a specific type of intrinsic excitability that gives rise to their typical cell-specific neuronal activity (as discussed below in more detail).

3. Dopaminergic midbrain neurons generate and maintain spontaneous pacemaker activity

In their landmark studies, Grace and Bunney showed that DA midbrain neurons *in vivo* discharged in two distinct modes of electrical activity in the anaesthetized rodent brain: one of the two modes was characterized by a slow irregular single-spike pattern between 0.2 and 10.8 Hz with an average of 4.5 Hz but with most DA neurons firing in the narrow frequency range between 1 and 8 Hz (Grace and Bunney, 1984b). Later *in vitro* studies by Grace and others demonstrated that DA neurons are spontaneous pacemakers that generate regularly spaced action potentials in frequencies between 1 and 10 Hz even in complete synaptic isolation (Grace et al., 2007) (compare Fig. 2B, upper and middle). Even with electrical stimulation, DA midbrain neurons do not manage to fire at much higher frequencies than those generated spontaneously, indicating that intrinsic conductances determine this narrow frequency bandwidth of their pacemaker (Kuznetsov et al., 2006). As summarized in Fig. 2A, functional and molecular single-cell studies have identified several ion channels and their putative subunit compositions that generate this spontaneous pacemaker and control its limited frequency range. Several studies have highlighted the important role of voltage-gated L-type calcium channels for creating the basic subthreshold membrane potential oscillations that underlie pacemaker activity (Puopolo et al., 2007). However, the calcium dependence of the spontaneous pacemaker is not a homogenous property of all DA midbrain neurons (Chan et al., 2007; Puopolo et al., 2007). While DA neurons of the SN pars compacta completely stopped firing when calcium was replaced by equimolar concentrations of cobalt, or when L-type Ca^{2+} channels were pharmacologically blocked, the inhibition of calcium channels did not prevent firing in DA neurons recorded in the neighboring VTA. A recent study points to a key role of L-type calcium channel Cav1.3, which is activated at more negative potentials compared to other members of the L-type calcium channel family, also expressed in DA midbrain neurons (Chan et al., 2007). In SN DA neurons, Cav1.3 channels carry the bulk of calcium inward currents during the interspike interval (ISI, see Fig. 2B, upper) (Puopolo et al., 2007). This calcium component is far more dominant in SN DA neurons compared to those of other pacemaker neurons in the brain, which mainly rely on interspike sodium influx by TTX (tetrodotoxin)-sensitive sodium channels or hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels (Bean, 2007).

However, DA midbrain neurons seem to possess robust defense mechanisms that retain their individual functional identity. Thus, DA midbrain neurons retain their typical electrical activity even in the complete absence of dopamine in dopamine-deficient mice (DD KO-mice, lacking the enzyme needed to convert tyrosine into levodopa) (Robinson et al., 2004). This indicates that they completely compensate (by unknown mechanisms) the loss of the well-described dopamine-mediated inhibition of their spontaneous electrical activity, mediated by D_2-autoreceptor activation of G-protein-coupled potassium (GIRK2) channels (see Fig. 2A) (Beckstead, et al., 2004). The second – even more stunning example for their homeostatic plasticity – is the finding that SN DA neurons from Cav1.3 KO mice show also normal pacemaker activity, due to compensation for the genetic inactivation of their key pacemaker channel, by upregulating alternative sodium-based ion channels for pacemaker activity (Chan et al., 2007). This flexibility to switch pacemaker mechanisms appears to preclude the use of differences in pacemaker activity as a

robust criterion to discriminate DA subpopulations. However, distinct DA populations might differ in the degree of flexibility of pacemaker switching, in particular as switching appears to be related to the presence of HCN channels. These channels, which are composed of single DA midbrain neurons by slow-gating HCN2, HCN3, and HCN4 channel variants (Franz et al., 2000), show large differences in HCN channel density among different DA subpopulations (Neuhoff et al., 2002). Several studies (both in early postnatal and in 3-month-old adults) clearly suggest that under control conditions only a subpopulation of DA midbrain neurons (within the SN) actively use HCN channels for pacemaker frequency control (Neuhoff et al., 2002; Seutin et al., 2001; Zolles et al., 2006). Only in these SN DA neurons are HCN channels sufficiently activated during the interspike interval, thereby accelerating the depolarization to threshold and thus increasing their discharge rate. In the medial posterior VTA, immunohistochemically defined DA neurons have been identified that possessed almost no functional HCN channels, indicating that their electrophysiological phenotypes might be very distinct from that of the conventional "classic" SN DA type (Neuhoff et al., 2002). Nevertheless, the presence of a HCN-mediated so-called "sag component" (i.e. a depolarizing sag of membrane potential in response to injection of hyperpolarizing currents due to activation of HCN channels) has been used in many studies as a functional criterion to identify and define DA midbrain neurons (Grace et al., 2007). However, given the large variability of HCN channel expression among DA midbrain neurons, as well as the presence of HCN currents in neighboring non-DA midbrain neurons, this sag component alone is not a valid criterion to identify DA neurons.

The interspike depolarization towards threshold, driven by calcium and sodium and HCN channels is opposed by fast-inactivating A-type potassium channels in SN DA neurons. These A-type K^+-channels are composed of the pore-forming alpha-subunits Kv4.3L (long splice variant) and the auxiliary beta-subunits Kchip3.1 (Liss et al., 2001). A quantitative single-cell PCR analysis demonstrated strong correlations between the rate of discharge and the density of Kv4.3/Kchip3.1 K channels as well as between their density and the abundance of the respective Kv4.3 and Kchip3.1 mRNAs in individual DA neurons (Liss et al., 2001) (see Fig. 2A). In contrast to the fast switching between calcium- and sodium-based pacemaker mechanisms which relies on the allosteric regulation of existing HCN channel proteins (Chan et al., 2007), negative frequency control by A-type Kv channels might be predominately controlled on the transcriptional level on a slower time scale e.g. as shown when adapting to chronic challenges of antipsychotic substances (Hahn et al., 2006; Hahn et al., 2003). Again, the large variations of A-type currents and its correlated distinct Kv4.3 and Kchip3 mRNA expression might rather be reflecting different regulatory states of DA neurons than a reliable marker to discriminate distinct subtypes of DA midbrain neurons (Liss et al., 2001).

In essence, pacemaking of dopaminergic neurons has been shown to be highly flexible with varying contributions of sodium, calcium and potassium channels. Faced with this astonishing flexibility, however, we do not yet have sufficient data to define distinct DA midbrain neurons based on differences in pacemaker mechanisms alone. Furthermore, as discussed above, DA neurons are flexible in defending their functional identities, thus the large quantitative differences in channel expression (at protein or mRNA levels) might simply reflect ongoing homeostatic plasticity, than define distinct types of dopaminergic neurons. Thus, more quantitative biophysical and molecular studies are needed to better understand the diversity and flexibility of calcium- and sodium-based pacemaker channels in individual DA midbrain neurons. However, if the controversial finding that DA midbrain neurons projecting to cortical targets consistently fire at higher frequencies above those of conventional DA midbrain neurons (Chiodo et al., 1984; Gariano et al., 1989) would be confirmed, these potential differences in pacemaker frequencies could indeed be used to discriminate two functionally distinct DA subpopulations irrespective of the underlying biophysical mechanisms.

4. Dopaminergic midbrain neurons reversibly switch to phasic burst firing

Activity patterns of dopaminergic neurons are tightly correlated to their presynaptic dopamine release patterns (Cragg, 2003). Ever since the first extensive description of electrical activity of DA neurons *in vivo* by Grace and Bunney in the rodent, and by the pioneering work by Schultz and colleagues in the awake primate, it was apparent that DA neurons not only discharge in a single-spike pacemaker mode but also switch to firing in short, high-frequency bursts that, on average, contain about three action potentials (Grace et al., 2007; Schultz, 2007b) (compare Fig. 2B, lower panel). The critical behavioral importance of this burst discharge was elegantly defined by Schultz in the behaving primate, where this subsecond phasic DA signal was shown to be quantitatively related to the value functions of reward-predicting stimuli or actions as well as to the extent of prediction errors upon expected reward delivery (Schultz, 2007a). In contrast to pacemaker activity, bursting was – in most cases – not observed spontaneously in DA midbrain neurons in reduced *in vitro* preparations (Grace et al., 2007). This indicated that burst-firing of DA neurons depends at least in part on the interplay between patterned synaptic input and intrinsic conductances. In the absence of synaptic input, apamin-sensitive small-conductance calcium-activated potassium (SK) channels and their selective upstream calcium sources play a key role in suppressing spontaneous burst discharges in DA midbrain neurons (Ji and Shepard, 2006; Wolfart et al., 2001) (see Fig. 2A). Similar to the large differences of HCN channel expression among individual DA midbrain neurons (discussed in Section 3), large differences in SK channel-mediated action potential after-hyperpolarizations and membrane currents exist among different DA midbrain neurons (Wolfart et al., 2001). It is interesting to note that those DA neurons in the medial VTA that possessed almost no HCN conductances also had the smallest SK channel-mediated after-hyperpolarizations – further highlighting this DA population as most distinct from the "conventional" SN DA phenotype. In case of SK channels, the biophysical data were well correlated with differences in SK3 channel protein expression, indicating that differential SK3 expression might be responsible for the observed differences in after-hyperpolarizations and stability of pacemaker discharge (Wolfart et al., 2001). However, the

large variations of SK channel expression and function might again indicate homeostatic plasticity of DA neurons, which in this case could stabilize a distinct threshold for burst-firing in the presence of variable synaptic inputs. Alternatively, the combined near absence of SK3 and HCN channel function within DA neurons in the medial VTA might signify their distinct functional identity associated, compared to the classic SN DA neurons, with a different functional role *in vivo*. These two possibilities are both valid given the actual experimental data.

However, there is strong evidence that dopamine is released in different temporal patterns (Schultz, 2007b). In prefrontal cortex, for example, sustained tonic dopamine release over many minutes is described, in behavioral situations that require physical or cognitive effort like working memory (Stefani and Moghaddam, 2006). These sustained types of DA release are not easily captured in the context of reward-based signaling of prediction errors and uncertainty that is predominantly coded by phasic dopamine release. The above-described robust defense of phenotypic properties might indicate its vital significance for DA release and signaling. Indeed, even small differences in tonic or phasic activity of DA neurons might have important behavioral consequences. For instance, White and colleagues reported that increased impulsivity and increased drug taking in high responder rats was correlated with increased firing rates of DA midbrain neurons predominately in the VTA (Marinelli and White, 2000). It should be noted in this context that, while the strength of excitatory and inhibitory synapses of DA midbrain neurons are modified by *in vivo* challenges by drugs of abuse, their intrinsic electrical properties (although not studied in great detail in this context) appear at the same time to be unaltered (Liu et al., 2005; Ungless et al., 2001). This could indicate that homeostatic plasticity of DA neurons has a blind spot concerning synaptic changes associated with different phases of drug abuse. On the other hand, even transient changes in striatal D_2 receptor signaling have been shown to chronically change the properties of mesocortical DA neurons illustrating the limits of preserving the functional identity of DA midbrain neurons (Kellendonk et al., 2006).

5. Differential vulnerability of dopaminergic midbrain neurons to degeneration – A tale of two channels

In Parkinson disease, certain DA midbrain populations are significantly more vulnerable compared to others (Damier et al., 1999). DA neurons in the SN that project to the dorsolateral striatum (nigrostriatal pathway) are substantially more vulnerable in PD and its chronic animals models compared to those that constitute the mesolimbic or mesocortical DA pathways. The latter might even display hyperactivity at early stages of PD (Williams-Gray et al., 2007). Mitochondrial dysfunction appears to be at the heart of pathogenesis of idiopathic and drug-induced cases of PD as well as for a number of monogenic familial forms (Sulzer, 2007). Acute challenges of DA midbrain neurons with toxins (rotenone, MPTP) that perturb mitochondrial function and induce DA neurodegeneration and parkinsonism revealed two types of acute responses: while the spontaneous electrical activity of less vulnerable mesolimbic VTA DA neurons is not affected by toxin concentrations sufficient to induce neurodegeneration *in vivo*, the electrical activity of highly vulnerable SN DA neurons is dramatically altered (Liss et al., 2005). Selective activation of ATP-sensitive potassium (K-ATP) channels in these DA neurons (build by Kir6.2 and SUR1 subunits) hyperpolarized the membrane potential and completely prevented action potential generation in response to PD toxins *in vitro* in adult mice (see Fig. 2A). Studies in K-ATP channels knockout mice demonstrated that functional K-ATP channels were indeed necessary mediators of this response (Liss et al., 2005). The significance of selective K-ATP channel activation *in vivo* was revealed by chronic MPTP PD-mouse models comparing K-ATP knockout and wild-type mice, where a complete and selective rescue of highly vulnerable SN DA neurons in K-ATP KO mice was identified, while the mild loss of VTA DA neurons *in vivo* was not affected (Liss et al., 2005). A similarly selective, but in this case only partial rescue, was obtained in a mechanistically independent genetic model of DA neurodegeneration, the *weaver* mouse. Quantitative single-cell analysis demonstrated that both K-ATP channel mRNAs, SUR1 and Kir6.2, were expressed at about 2-fold higher levels in vulnerable SN DA neurons compared to more resistant VTA DA neurons. The selective activation of K-ATP channels, however, appeared to be controlled by upstream mechanisms including different degrees of uncoupling of the mitochondrial membrane potential and the differential expression of uncoupling proteins (Liss et al., 2005).

In essence, these data revealed fundamentally different "*pathophysiological identities*" between distinct DA midbrain neurons, and identified selective K-ATP channel activation as a first functionally defined candidate mechanism for the differential vulnerability of DA midbrain neurons in PD. Very recently, Chan and colleagues elegantly identified a second, also channel-based, mechanisms for differential vulnerability among DA midbrain neurons. Based on their above-described finding that SN DA neurons continued to generate spontaneous pacemaker activity in a Cav1.3 knockout mouse, due to a switch from calcium-to-sodium-based pacemaking, they demonstrated that a corresponding drug-induced pacemaker-switching ("rejuvenation") of SN DA neurons significantly reduced their vulnerability in a chronic MPTP PD-mouse model (Chan et al., 2007). Whether the neuroprotection induced by pharmacological L-type channel inhibition was also selective for the highly vulnerable SN DA neurons has not yet been analyzed, but might be likely, as VTA DA neurons possess, as described above, a less calcium-dependent pacemaker mechanism (Chan et al., 2007).

These two complementary findings might provide an essential reason why DA neurons invest to defend their functional pacemaker identity; given that electrical silencing or reduction of pacemaker activity by K-ATP channel activation appears to be a prerequisite for neurodegeneration in PD. Indeed, a previous study has demonstrated that electrical activity and its associated influx of sodium and calcium ions was necessary for the survival of DA neurons, at least *in vitro* (Salthun-Lassalle et al., 2004). On the other hand, inducing the SN DA neurons to maintain their activity by switching them to a metabolically less demanding sodium-based pacemaker (Bean, 2007; Chan et al., 2007) would manage to keep their K-ATP channels closed and thus promises to keep them alive longer throughout the PD disease process (Deutch and Winder, 2006; Michel et al., 2006; Sulzer and Schmitz, 2007).

Both, blockers of K-ATP channels (sulfonylureas) and blockers of L-type calcium channels (dihydropyridines) are clinically established and widely used drugs for treating, e.g. diabetes mellitus type II or hypertension, respectively (Deutch and Winder, 2006; Michel et al., 2006; Sulzer and Schmitz, 2007). Given the PD mouse model findings summarized here, it will be very interesting to study whether the use of these drugs in PD patients has effects on the progression rate of their clinical symptoms or the rate of their progressive loss of DA innervation.

In conclusion, dopaminergic midbrain neurons continue to surprise us in physiology and pathophysiology, and we yet have to find objective means to reliably distinguish between compensatory modes of homeostatic flexibility and genuine functional and molecular subtypes within this group of fascinating dopaminergic neurons.

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