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Simulating Idiopathic Parkinson's Disease by *In Vitro* and Computational Models

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1. Introduction

1.1 Short history

The motor disorder of Parkinson's disease (PD) results from injury of the basal ganglia (BG). Understanding of the pathophysiology came late, especially with regard to the involvement of the substantia nigra. The description of 'shaking palsy' or 'paralysis agitans' (Parkinson, 1817) did not bring forward the recognition of its pathological origin. Lewy (1912, 1913) put emphasis on the globus pallidus and putamen. Von Economo's emphasis (1917) on the substantia nigra (SN) in encephalitis lethargica, with analogous clinical appearance, prompted others to pay attention to this nucleus. Tetriakoff (1919) was the first to describe SN involvement in paralysis agitans. Hassler (1937, 1938, 1939), by studying the normal cytoarchitecture of the SN, discovered a differential damage in the pars compacta using the collection of Cecile and Oscar Vogt. Moreover, he described the damage in the locus coeruleus. It was Friede, already in 1953 (1953, 1966), using histochemical techniques, who proposed a relation with catecholaminergic systems. Although the SN-catecholamine doctrine was regularly scrutinized in the early days of catecholamine research (see e.g. Mettler, 1964), it has not been overthrown up till now.

1.2 Idiopathic Parkinson's disease

Parkinson's disease is nowadays subdivided in idiopathic Parkinson's disease and Parkinson plus syndromes (see Usunoff et al., 2002). Parkinson plus syndromes counts for 15% of all Parkinsonism, although in large autopsy series the percentage augmented to 20-25% (Hughes et al., 1992), thus leaving idiopathic Parkinson's disease as the most frequently occurring form (Jellinger, 1987). Nevertheless contested (see e.g. Gibb, 1988; Kingsburry et al., 1999), the suggestion that the vulnerability of SN neurons is related to the neuromelanin/tyrosine hydroxylase content is favored in idiopathic Parkinsonism (Hirsch et al. 1988). Idiopathic Parkinson's disease is characterized by neuromelanin-containing cell

loss and by the presence of Lewy inclusion bodies in surviving neurons in the SN and other areas (for an overview see Usunoff et al., 2002). "Lewy bodies in the SN are considered the pathological hallmark of Parkinson's disease, which means that if they cannot be found, the diagnosis is not Parkinson's disease" (Usunoff et al., 2002). The conclusion that idiopathic Parkinson's disease involves degeneration of pigmented neurons of the brain stem is inevitable (Greenfield & Bosanquet, 1953). This conclusion is also based on the distribution of Lewy bodies in other brainstem areas. However, within the human SN not all subareas degenerate (for an overview see Usunoff et al., 2002). Since topography is present in the human SN, circuits of these sparse unharmed areas do survive.

1.3 Models of idiopathic Parkinson's disease

Animal research profoundly increased by the detection of MPTP. In 1982, a young male, age 29, in northern California, used a new synthetic heroin, which brought him and his also addicted brother and several others, profound and unremitting Parkinsonism (Langston et al. 1999). In this case Meperidine (Demerol, Pethidine) was used. MPPP, the 'Designer heroin' (1-methyl-4-phenyl-4-propionoxypiperidine) contained not only MPPP but also 2.5 to 2.9% of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) by weight, a byproduct in the synthesis of MPPP. Biotransformation produces from MPTP the 1-methyl-4-phenylpyridinium ion (MPP^+), which is taken up by the dopamine transporter of the SN neurons, where it blocks the mitochondrial respiratory chain (see Langston et al., 1999 and references herein). An experimental monkey model was developed (see Vitale et al., 2009, for ethical criticism on this monkey model). MPTP was quickly shown experimentally to selectively destroy nerve cells in the SN after systemic administration. The resulting striatal dopamine depletion explained most, if not all of the clinical features of Parkinson's disease (for an extensive summary see Langston et al., 1983; Langston et al., 1999, and a report of an earlier case by Davis et al., 1979). MPTP works in dogs, cats and pigs, but is less effective in rats and guinea pigs, while the MPTP effect is strain dependent in mice. The overview of monkey MPTP results (Israel and Bergman, 2008) shows that these studies demonstrated the importance of the inactivation of the STN, inducing Parkinson symptoms and that the onset of synchronized bursts and high frequency oscillations interfered with normal function of the spatio-temporal function of the basal ganglia.

"However, although an experimental animal model is present and enormous efforts have been carried out to detect the cause of Parkinsonism, what initiates the disease is still unknown. Moreover, human studies have an ethical drawback and a case as described above is seldom found in literature. Therefore, experimental results from animals, often not possible to translate to the human situation, especially rat and mouse results, is what scientists have to relay on. Consequently model studies, using systemic, neuroanatomically developed, models, are of the utmost importance in the study of Parkinson's disease and are significant in their contribution to the understanding of Deep Brain Stimulation (DBS), nowadays mainly carried out in the subthalamic nucleus (STN)" (Heida et al., 2008; see also Toulouse & Sullivan, 2008).

It is, therefore, the gathering and the correct transformation of experimental animal results into newly developed PD models that determine the success of such a model in the contribution to the understanding of idiopathic Parkinson's disease. This review paper will first give an overview of the (classic) connection scheme of the basal ganglia-corticothalamic

circuit. Then we will concentrate on rat SN and STN experimental results as obtained in our group. On the one hand anatomical data of the afferent and efferent connections of the SN and STN are presented, while on the other hand electrophysiological data of the neuronal activity patterns as observed in dissociated STN cell cultures and brain slices is discussed. Finally, a selection of computational models developed in our Applied Analysis and Mathematical Physics, and Biomedical Signals and Systems groups, related to different aspects of idiopathic Parkinson's disease are summarized. One of the aims of these computational models is to understand the mechanism of DBS. This review relies also on earlier publications, congress abstracts and presented posters (Cagnan et al., 2009; Heida et al., 2008, 2009, 2010a,b,c,d; Lourens et al., 2009, 2011; Marani et al., 2008, 2010; Meijer et al., in press; Moroney et al., 2008; Stegenga et al., 2009, 2010a,b,c).

2. Which connections are involved in idiopathic Parkinson's disease?

2.1 Classic connectivity diagram of the corticothalamic-basal ganglia network

The major pathways within the basal ganglia-thalamocortical circuit, which are known to be involved in the execution of voluntary movement, are illustrated in Figure 1 (see Gerfen & Wilson, 1996; and Groenewegen & Van Dongen, 2008). Albin et al. (1989) and De Long (1990) first proposed these pathways through the basal ganglia (BG). Two major connections link the BG input nucleus (striatum) to the output nuclei (globus pallidus interna (GPi)/substantia nigra pars reticulata (SNr)), namely the 'direct' and 'indirect' pathways. The critical balance between these two pathways determines normal motor behavior. The BG output nuclei have a high rate of spontaneous discharge, and thus exert a tonic, GABA-mediated, inhibitory effect on their target nuclei in the thalamus. The inhibitory outflow is differentially modulated by the direct and indirect pathways, which have opposing effects on the BG output nuclei, and thus on the thalamic targets of these nuclei.

The 'direct' pathway arises from inhibitory striatal efferents that contain both GABA and substance P and projects directly to the output nuclei. It is transiently activated by increased phasic excitatory input from the SNc (substantia nigra pars compacta) to the striatum. Activation of the direct pathway briefly suppresses the tonically active inhibitory neurons of the output nuclei, disinhibiting the thalamus, and thus increasing thalamocortical activity. The 'indirect' pathway begins with inhibitory striatal efferents that contain both GABA and enkephalin. These striatal neurons project to the GPe (globus pallidus externus). The GPe projects to the STN, via a purely GABAergic pathway, which finally projects to the output nuclei via an excitatory, glutamatergic projection. There is also a direct projection from the GPe to the output nuclei. The indirect pathway is phasically activated by decreased inhibitory input from the SNc to the striatum, causing an increase in striatal output along its pathway. Normally the high spontaneous discharge rate of GPe neurons exerts a tonic inhibitory influence on the STN. Activation of the 'indirect' pathway tends to suppress the activity of GPe neurons, disinhibiting the STN, and increasing the excitatory drive on the output nuclei. The decreased GPe activity also directly disinhibits the output nuclei. The resulting increase in activity of the output nuclei inhibits the thalamus further, decreasing thalamocortical activity. Activation of the direct pathway thus *facilitates* movement, whereas activation of the indirect pathway *inhibits* movement (see McIntyre and Hahn, 2010, for an extended overview).

The cortico-STN-GPi 'hyperdirect' pathway (Nambu et al., 2000, 2002; Nambu, 2005; Brown, 2003; BarGad et al., 2003; Squire et al., 2003) conveys powerful excitatory effects from the motor-related cortical areas to the globus pallidus, bypassing the striatum. The hyperdirect pathway is therefore an alternative direct cortical link to the BG, possibly as important to motor control as the direct pathway, which is typically considered to be the main cortical relay in the BG.

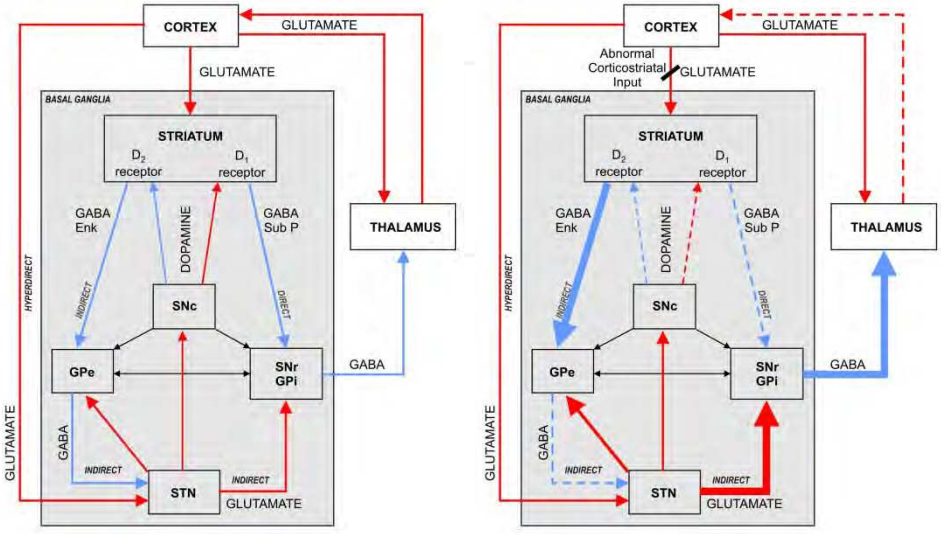


Fig. 1. Connection diagram of the basal ganglia-thalamocortical motor circuit. The relative connection strengths are indicated for Left: the normal healthy brain, and Right: the parkinsonian brain. Blue lines indicate inhibitory pathways; red lines indicate excitatory pathways. GPi: globus pallidus internus; GPe: globus pallidus externus; SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; STN: subthalamic nucleus; GABA: gamma amino butyric acid.

Nigrostriatal dopamine projections exert contrasting effects on the direct and indirect pathways (see Figure 1). Striatal neurons projecting in the direct pathway have D1 dopamine type receptors (D_1 and D_5) which cause excitatory post-synaptic potentials, thereby producing a net excitatory effect on striatal neurons of the direct pathway. Those projecting in the indirect pathway have D2 type receptors (D_2, D_3 and D_4), which cause inhibitory post-synaptic potentials, thereby producing a net inhibitory effect on striatal neurons of the indirect pathway. The facilitation of transmission along the direct pathway and suppression of transmission along the indirect pathway leads to the same effect – reducing inhibition of the thalamocortical neurons and thus facilitating movements initiated in the cortex. Thus, the overall influence of dopamine within the striatum may be to reinforce the activation of the particular basal ganglia-thalamocortical circuit which has been initiated by the cortex (Gerfen, 1992; Gerfen & Wilson, 1996; see also Hurley & Jenner, 2006). Due to the differential effects of dopamine on the D1 and D2 dopamine receptors of the striatum, a loss of striatal dopamine results in a reduction in transmission through the direct

pathway and an increase in transmission through the indirect pathway, causing an imbalance between the two pathways.

The depletion of striatal dopamine changes neuronal firing rates in basal ganglia nuclei. Increased firing rates are found in the striatum, GPi and STN and a minimally decreased discharge in the GPe. A summary of tonic firing rates of BG nuclei in the normal and parkinsonian situation can be found in Heida et al. (2008). However, the pattern of discharge of basal ganglia neurons is thought to be equally as important as the rate of discharge in the execution of smooth movements. Several alterations in the discharge pattern have been observed in neurons of the BG in PD subjects. These alterations include a tendency of neurons to discharge in bursts, increased correlation and synchronisation of discharge between neighbouring neurons, rhythmic and oscillatory behaviour (Brown, 2003). Coherence between STN and GPi activity has been confirmed at tremor frequencies (3-10 Hz) (Brown et al., 2001). These oscillatory patterns are projected to GPi's thalamic projection site, the nucleus ventralis anterior thalami, and the cortex. In addition, STN and GPi demonstrate a tendency to synchronization at 11-30 Hz, which is likely to be driven from the motor areas of the cortex (Brown, 2003). In this circuit, the thalamus is in a key position as it receives the convergent afferent input from the GPi, the cortex, and the peripheral system, which it then projects back to the cortex, including motor areas (Smith et al., 1998).

2.2 Pedunculopontine and cholinergic connections

Gait is related to the pedunculopontine nucleus (Piallat et al., 2009), and therefore this nucleus plays an important role in gait and balance disorders that are common in Parkinson's disease. Topographically a pars compacta and a pars dissipatus of the PPN is discerned (Jacobsohn, 1909; Olszewski and Baxter, 1954). The pars dissipatus is supposed to contain glutamatergic neurons. Both parts contain cholinergic neurons. In humans the pars compacta exists of 90% cholinergic cells and the pars dissipatus of 25-75% (Mesulam et al., 1989). It should be noted, that intra-striatal pathways and PPN connections towards the SNc are mainly cholinergic. Cholinergic systems are also degenerated in Parkinson's disease. Nevertheless, next to a dopamine receptor balance a dopamine-acetylcholine balance also plays a role and "a cholinergic overactivity has been used to explain the improvement of some motor signs such as tremor, reported after muscarinic receptor blockade" (Calabresi et al., 2006), although a more cooperative role is supported for acetylcholine and dopamine in human cognitive performance. Cholinergic drugs do also have an improving effect on cognitive behavior in Parkinson's patients (Calabresi et al., 2006).

Physiological studies subdivided PPN neurons in three types (I, II and III) identified on the basis of their electrophysiological membrane properties obtained by intracellular recording. Type I neurons are characterized by low threshold calcium spikes (LTS), which give rise to a burst of fast action potentials after the offset of a hyperpolarizing current. The neurons also fire bursts of spikes when a depolarized stimulus is given during hyperpolarization. According to Takakusaki and Kitai (1997) Type I neurons are glutamatergic. Type II neurons do not burst. Instead they fire single action potentials with large after-hyperpolarizations in response to injections of depolarizing current. This type is thus suited to generate a relatively slow tonic repetitive firing pattern. About 50% of Type II neurons are cholinergic. Type III neurons miss characteristics of Type I and Type II PPN neurons, thus lacking low-threshold spikes. The PPN neuron receives pallidal information, gathers information from the STN-SN network with strong SN influence, also from the cortex, from the limbic system

and hypothalamus, from the cerebellum and from the brainstem and can transmit it towards nearly all nuclei of the thalamus (both the cholinergic and non-cholinergic ones), and weakly towards the cortex and basal ganglia (Usunoff et al., 2003).

PPN plays a role in the control of muscle tone by means of its excitatory projections to the muscle tone inhibitory system in the brainstem. The PPN is also thought to produce the main influence on the parafascicular thalamic nucleus in cases of SN degeneration; the parafascicular nucleus being involved in motor control (Yan et al., 2008). In Parkinson's disease the increased inhibitory basal ganglia output, together with a decrease in cortical excitation of the PPN, may increase the level of muscle tone causing rigidity (Takusaki et al., 2004).

2.3 Subthalamic nucleus connections

The STN projection neurons are glutamatergic, excitatory, and heavily innervated by widely branching axons of the substantia nigra (SN), the internal pallidal segment (GPi), followed by the external pallidal segment (GPe) and the pedunculopontine tegmental nucleus (PPN). The most well-known afferent connections of the STN arise in the GPe. The STN is also innervated by glutamatergic corticosubthalamic axons. A substantial, bilateral cholinergic/glutamatergic projection arises in the PPN, while the thalamic centromedian-parafascicular complex also innervates the STN (see Table I). Finally, serotoninergic fibers from the raphe nuclei terminate profusely within the STN. The nigrosubthalamic connection was demonstrated by axonal transport techniques, and transmitter immunocytochemistry. The nigrosubthalamic connection arises from the dopaminergic (DA) neurons of the SN pars compacta (SNc). In addition, a moderate projection was described from the parvalbumin immunoreactive, presumably GABAergic neurons of the SN pars reticulata (SNr) to the STN. The nigrosubthalamic connection has always been described as ipsilateral, but rat tracer studies now oppose this view (see below and Marani et al., 2008). STN-pallidal fibers arborize more widely and terminate on more proximal neuronal elements of the pallidum than striato-pallidal fibers. Thus, the striatal and STN inputs to GPi form a pattern of fast, widespread, divergent excitation from the STN, and a slower, focused, convergent inhibition from the striatum (Squire et al., 2003). Furthermore, cortico-STN neurons and cortico-striatal neurons belong to distinct populations. Thus, signals through the hyperdirect pathway may broadly inhibit motor programs; then signals through the direct pathway may adjust the selected motor program according to the situation (the 'center-surround' model of Nambu, 2005). STN neurons can discharge continuously and repetitively at low frequencies (10-30 Hz) and can fire with bursts of high frequency spikes. STN neurons are physiologically subdivided in non-plateau neurons (neurons that react with low threshold spikes) and plateau generating neurons (those that can react with bursts, low threshold spikes or plateau potentials). The neuron has to be in a hyperpolarized state, for depolarizing or hyperpolarizing current pulses to induce plateau behavior (Beurrier et al., 1999). The tonic discharges are sodium-dependent, while its hyperpolarizing phases are calcium dependent. Bursts are calcium-dependent phenomena.

3. Rat experimental results: Anatomy and electrophysiology

3.1 Anatomy: Outline of SN connections in the rat

The afferent and efferent connections of the rat SN, studied with degenerative and tracer techniques, are restricted to the cortex, brainstem and cerebellum (the cerebellum and its

connections are not treated in this review). All thalamic connections are SN efferent, except for the parafascicular thalamic nucleus. The afferent SN connections of this nucleus are ipsilateral. All efferents of the SN to the other thalamic nuclei are also ipsilateral, with the exception of the connections to medial dorsal-, ventral medial- and central lateral thalamic nucleus, which are both ipsi- and contralateral, like those of the pedunculopontine nuclei and superior and inferior colliculus connections. Ipsi- and contralateral afferents to the SN are found for the hypothalamus, laterodorsal tegmental nucleus and the parabrachial nuclei. All other connections, including cortex, caudate-putamen and pallidum connections are afferent and/or efferent and always ipsilateral. It means that SN influence is mainly ipsilateral and only a few pathways can also steer the contralateral side of certain thalamic nuclei, lateral habenular nucleus, superior and inferior colliculus, periaquaductal gray, and the pedunculopontine nucleus (see Table I and Marani et al., 2008).

Afferents to substantia nigra				**Efferents from substantia nigra**		
SNr	SNc	SNl		SNr	SNc	SNl
ipsi con	ipsi con	ipsi con		ipsi con	ipsi con	ipsi con
x	x	x	Cortex	x	x	x
x	x	x	Caudate-Putamen	x	x	x
x	x		Pallidum	x	x	
x	x		Accumbens		x	
			Hippocampus		x	
	x	x	Amygdala		x	x
			Lateral dorsal thalamic nucleus	x		
			Medial dorsal thalamic nucleus	x x	x	
			Ventral medial thalamic nucleus	x x	x	
			Central medial thalamic nucleus	x	x	
			Central lateral thalamic nucleus	x	x	
x	x	x	Parafascicular thalamic nucleus	x	x	
			Paracentral thalamic nucleus	x		
			Lateral posterior thalamic nucleus	x		
x	x	x	Lateral habenular nucleus		x x	
			Dorsal lateral geniculate nucleus	x	x	x

SNr		SNc		SNl			SNr		SNc		SNl	
ipsi	con	ipsi	con	ipsi	con		ipsi	con	ipsi	con	ipsi	con
						Zona incerta	x					
x		x		x		Subthalamic nucleus			x			
x	x	x	x	x	x	Hypothalamus	x		x		x	
						Superior colliculus	x	x				
						Red nucleus	x					
x		x				Entopeduncular nucleus						
						Inferior colliculus					x	x
						Periaqueductal gray			x	x		
x		x				Raphe dorsalis			x			
						Cuneiform nucleus	x					
						Mesencephalic reticular nucleus	x		x			
		x	x			Pedunculopontine tegmental nucleus	x	x	x			
x	x	x	x	x	x	Laterodorsal tegmental nucleus	x					
x	x	x	x	x	x	Parabrachial nuclei	x		x			
						Locus coeruleus	x		x		x	
						Parvocellular pontine reticular nucleus	x		x			
			x			Cerebellum		x		x		x

Table 1. Overview of afferent and efferent connections of the SN. SNr: substantia nigra pars reticularis; SNc: substantia nigra pars compacta; SNl: substantia nigra pars lateralis; ispi: ipsilateral; con: contralateral; x: existing connection.

3.1.1 Nigro-subthalamic connections in the rat

The outline of the nigro-subthalamic connections is shown by large injection sites with the anterograde BDA (biotinylated dextran amine) tracer that was injected into the lateral SNr (reticulata) and SNc (compacta). The axons running towards the brainstem and the mounting axons to the forebrain take at first a medial way towards the prerubral area. Few nigro-thalamic axons course dorsally towards the tegmentum. Most of the axons directed to the brainstem and forebrain progress immediately dorsal to SN, and some axons pass lateromedially of the SNc. Few axons bend ventromedially and travel along the border

between SNr and the cerebral peduncle. Reaching the caudal pole of the STN, the labeled axons pierce into the nucleus through its lateral wedge, but also into its ventral border and enter also from the medially running bundle, dorsal to the STN. Within the STN, especially in the lateral half of the nucleus, along with passing fibers oriented mediolaterally, a large amount of terminal labeling is present. In the medial part of the STN mainly discrete bursts of labeled endings are noted. Midline crossing of SN axons occurs at several places. The most substantial component of crossed axons runs in the mesencephalic tegmentum ventral to the periaqueductal gray (PAG). Such bundles are found through the entire rostrocaudal extent of the mesencephalon. Some fibers in the rostral mesencephalon in fact come into the STN through its dorsal border. The midline is also crossed in the commissure of the superior colliculus and in the posterior commissure. The efferent SN axons cross the midline (crossed nigro-thalamic axons) rostral to the SN, and the last component of crossing axons runs in the supraoptic decussation, immediately above the optic tract. Some of these axons take a dorsomedial course towards the contralateral STN. In the contralateral STN a lower amount of labeled axons are noted. Nevertheless, they form very distinct mediolaterally extended patches. Most of these discrete fields of terminal labeling are in the central and lateral portions of the STN, but also medially some terminal 'whorls' are seen.

These results provide data for the existence of a substantial nigrosubthalamic connection in the rat, which emits also a moderate component to the contralateral STN (Figure 2). Ipsilaterally the efferent SN axons terminate in large, profuse terminal fields, whilst contralaterally they terminate in discrete, sharply circumscribed patches. Although the crossed nigrosubthalamic connection is moderate, exactly by its topical distribution, its 'point to point' connection is especially evident. The medial SNc projects to the contralateral medial STN, and the lateral SNc also projects mainly to the lateral half of the contralateral STN (see Marani et al., 2008).

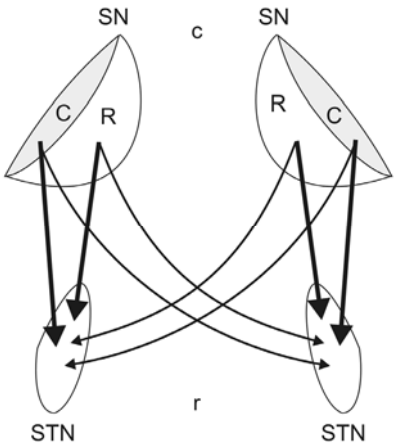


Fig. 2. Ipsilateral and contralateral nigro-subthalamic connections. SN: substantia nigra; STN: subthalamic nucleus; C: SN pars compacta; R: SN pars reticulata; c: caudal; r: rostral.

3.1.2 Nigro-trigeminal connections in the rat

It is generally accepted that also in the rat, the SN is involved in oral movements and orofacial dyskinesias. Until now, is has been believed that the SN influences the trigeminal motoneurons via a multisynaptic pathway (see Usunoff et al., 1997; Lazarov, 2002). Histopathological changes in this rat model's substantia nigra have been demonstrated in tardive dyskinesias (Andreassen et al., 2003). Direct stimulation by STN DBS improves orofacial dyskinesia in a rat model (Creed et al., 2010). Therefore, a renewed interest in the rat nigro-trigeminal pathway arose.

Specifically, the large BDA injection in the lateral SNc and parts of the adjacent SNr and SNl (lateral) resulted in anterograde labeling throughout the Me5 (mesencephalic trigeminal nucleus, see Usunoff et al., 1997) with a strong ipsilateral predominance, but contralateral labeling was also present. The results for SNc are summarized in Figure 3. Surrounding the injection site many intensely labeled neurons were present. Terminal labeling was observed among the perikarya of pseudo-unipolar neurons in the ipsilateral Me5c (caudal). At this sectional plane, virtually all pseudo-unipolar neurons were at least partially surrounded by varicose fibers, contacting their cell surface. The intensity of anterograde labeling in the Me5r (rostral) decreased almost bilaterally. Moderate terminal labeling was present around but not on pseudo-unipolar neuronal somata, both in the caudal and rostral Me5, on the contralateral side.

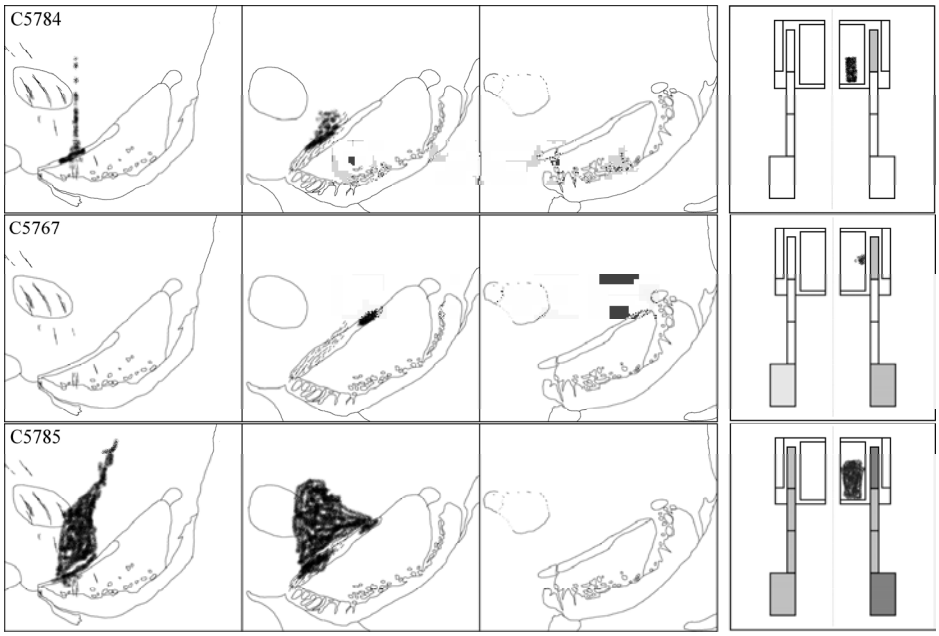


Fig. 3. Overview of the injection sites of three transversal slices of the SNc. The column at the right schematically shows the ipsi- and contralateral labeling in Me5, which is subdivided in a head and three tail areas. Anterograde labeling and injections (summed throughout the nucleus) are indicated in a map of the SN, with SNc, SNr and SNl, and Me5. Darker gray levels indicate a higher intensity of the labeling in Me5.

Selective injections into the medial SNc and lateral SNc produced labeled axons that were followed exclusively to the ipsilateral Me5c while the contralateral Me5c and Me5r on both sides displayed few labeled fibers (Figure 3). Terminal labeling was present in an extensive network in the ipsilateral Me5c, diminishing slightly from medial to lateral. Most of the terminal labeling surrounded the pseudo-unipolar mesencephalic trigeminal neurons. Some perikarya clearly displayed terminal and passing boutons covering their cell exterior. Throughout the neuropil of Me5c a meshwork of fine labeled fibers with varicosities was also present after injection into the lateral SNc. Single pseudo-unipolar neurons containing boutons en passant and boutons termineaux clearly visible on their surface were noted. The terminal labeling extended medially to the Me5c, to include the area of smaller cells in the locus coeruleus. A minute injection focus selectively infiltrated the SNl. In the Me5 area only few varicose fibers and their terminals reached the ipsilateral Me5c, while the rostral portion of this nucleus showed a slightly larger number of labeled fibers. In this case, no anterograde terminal labeling was observed in Me5 contralaterally.

The results of this study provide strong evidence that the SN also directly innervates the proprioceptive trigeminal neurons and thus, both the motor and sensory neurons controlling jaw muscles involved in mastication. Since pseudo-unipolar mesencephalic trigeminal nucleus neurons send axons to the pontine and spinal trigeminal nuclei, it appears that the entire trigeminal nuclear complex (see Usunoff et al., 1997) is profoundly influenced by the SN. Therefore, it can be inferred that inputs from SN possibly modify, modulate or interact with outputs from all these nuclei to control the masticatory behavior (see Marani et al., 2010).

3.2 Electrophysiology: Rat brain slices and dissociated STN cell cultures

We describe two approaches which can be followed to investigate the neuronal properties and network activity patterns *in-vitro*. Both use multi-electrode arrays (MEA's, Figure 4) to measure the extracellular membrane potential of neurons located close to the MEA's 60 electrodes. The first method we describe makes use of acute slices of rat brain, in which some of the structure remains intact but which can only be used for a short time (less than 8 hours). The second is to put neurons from a particular area in culture on top of the MEA, which loses all spatial structure, but can be used for months.

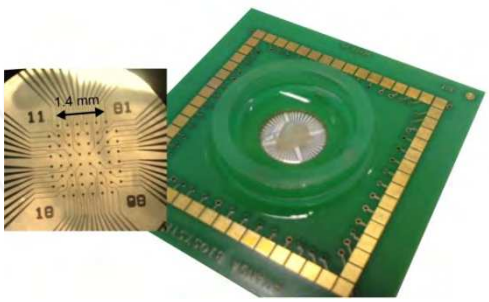


Fig. 4. View from above on a MEA (Ayanda Biosystems) used in slice research. The round culture chamber has an inner diameter of 2.4 mm. The 60 electrodes are spaced 200 µm apart in an 8 by 8 grid (the inset shows the electrodes covered by a slice). The electrodes are conically shaped, with the tips protruding 50-70 µm from the glass surface.

Horizontal slices of the STN-containing midbrain produced from rats, aged between 16-52 days, were placed such that the electrode matrix of the MEA was underneath the STN. Signals of spontaneous STN activity recorded in continuously perfused and carboxygenated artificial cerebro-spinal fluid (ACSF) were amplified, bandpass filtered (10 Hz-10 kHz) and digitized using a measurement system of MCS (MultiChannelSystems GmbH, Reutlingen, Germany). After software filtering (50-350 Hz), threshold crossings exceeding 5 times standard deviation were stored. Results show average firing rates of four neurons firing at mean frequencies of 0.1, 0.1, 0.06 and 1.2 Hz, respectively (Figure 5). In this example, the most active fourth neuron is the only neuron localized within (the motor cortex innervated area of) the STN. Neurons 2 and 4 were classified as 'bursty', neuron 1 was termed 'random' and neuron 3 was labeled 'regular' (for definitions of random, regular and bursty see Kaneoke and Vitek, 1996). On further inspection, many spikes were part of *doublets* (two spikes in close succession), while bursts of longer duration were rare. Such bursting STN activity was noted in many rat slices and the method used was able to discriminate several spiking patterns (Figure 6). Here, properties of random, regular and bursty spikes were allocated to the electrodes with their position within STN. Network connections may be detected by cross correlation analysis of spontaneous activity (Le Feber et al., 2007) or post-stimulus histograms (see Stegenga et al., 2010b). Moreover, the extranuclear network can also be studied (Figure 7, right panel).

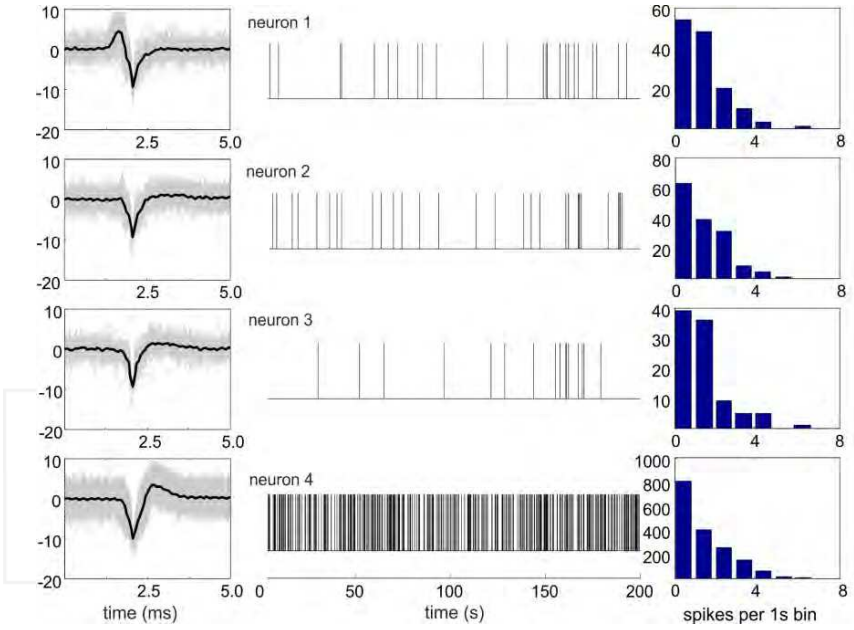


Fig. 5. Action potential waveforms and spike train analysis of 4 simultaneously recorded neurons in a horizontal slice of the rat brain including STN. Left: aligned action potential waveforms. Middle: spike trains of the 4 neurons. Right: density histograms of the spike trains, i.e. the number of occurrences that an interval of 1 s contained a certain number of spikes . The histograms were compared to a Poisson distribution for classification. Neuron 1: 'random'; neuron 3: 'regular'; neuron 2 and 4: 'bursty'.

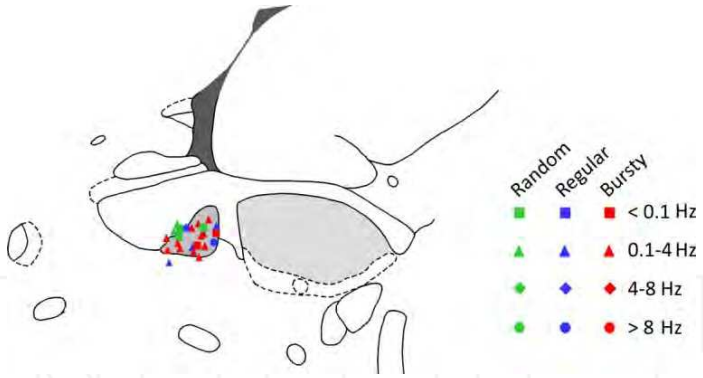


Fig. 6. Localization of activity in horizontal slices of STN (-8.1 mm from Bregma, figure 187 in Paxinos & Watson, 2007) classified according to mean frequency and spike train properties. STN neurons predominantly displayed low mean firing frequencies (<4 Hz) while spike trains were generally 'bursty'.

The addition of dopamine to acute slice preparations shows its effect on the firing rate and patterns of STN neurons, but also on SN neurons, depending on the placing of the MEA. Increasing concentrations of dopamine added to slices shows an increase in the spontaneous activity of the STN neurons, while a decrease of the spontaneous activity in SN was noted for a short period, before the increase started. Based on an analogous approach as done for intra-nuclear connections, mapping of extra-nuclear connections is also possible, here between STN and SN (Figure 7).

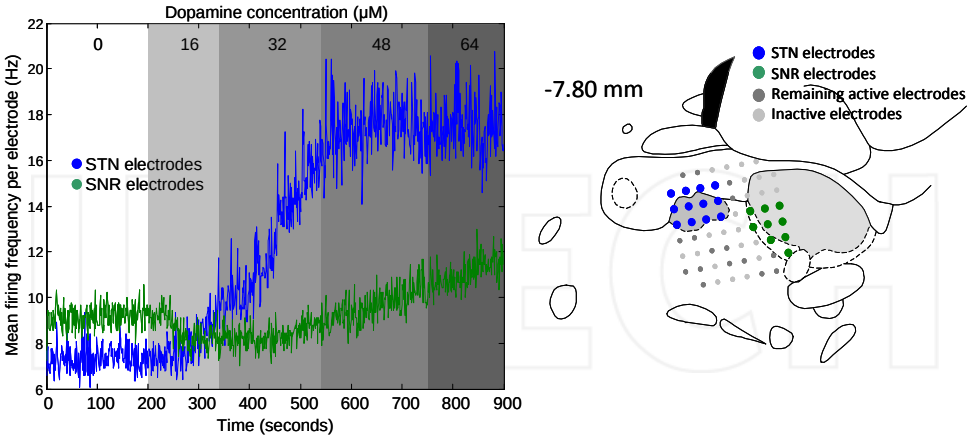


Fig. 7. Response of neurons located in STN and SN to dopamine application. Left: mean firing rate of 12 electrodes located in STN (blue) and 9 electrodes located in SN (green). The dopamine concentration was increased from 0 to 64 µM in steps of 16 µM (shaded gray background). Right: corresponding figure (188, Paxinos & Watson, 2007) with the position of the MEA electrodes included.

The rat neuroanatomical studies show a refined connection pattern between SN, Me5 and STN, which is new and hardly incorporated into models. Moreover rat dissociated STN neurons in culture and rat horizontal brain slices, containing the STN, provide the possibility to study artificial and original STN networks (in part), respectively. Herewith the mechanisms underlying bursting and oscillation of the rat STN neurons can be investigated, since the reaction of whole intranuclear, but also extranuclear networks can be studied together with single neuron reactions on artificial modulation of activity or by ablation of connections or by adding agonists and antagonists of neurotransmitters or receptors.

3.2.1 Mimicking the cholinergic PPN-STN connection in dissociated cultures

The pedunculopontine nucleus (PPN) is used as a new therapeutic target for DBS in patients suffering from Parkinson's disease with severe gait and postural impairment (Plaha & Gill, 2005). DBS of the PPN is only effective, if carried out at low frequencies (~20 Hz), while STN-DBS requires high frequencies (~130 Hz) to be successful. This is hardly comprehended. The projections from the PPN are reciprocal both towards the SN and the STN (see Usunoff, 2003). This nucleus contains cholinergic neurons (mainly Ch5 in the rat), that project onto the STN. There exists a direct relation between the severity of Parkinson's disease and the loss of cholinergic neurons in the PPN (Rinne et al., 2008), which provided the incentive to look into cholinergic effects on STN neurons.

To this end, rat STN areas from one day old rat pups were dissociated and cultured on the coated glass surface of a MEA similar to the example shown in Figure 4, in chemically defined, serum free, medium. Acetylcholine (ACh) was added in steps of 10 µM and extracelluar action potential activity of a maximum of 60 neurons was recorded. Addition of ACh reduced the spontaneous activity immediately and substantially for 50-100 seconds (Figure 8).

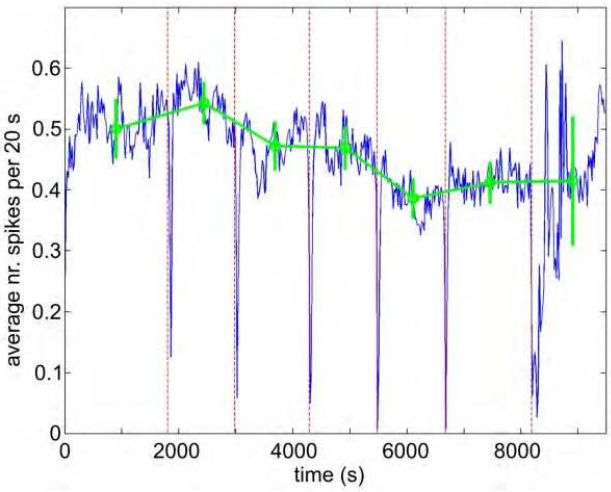


Fig. 8. Normalized spike activity in dissociated STN cultures after administration of ACh. Each red striped line marks the time at which the concentration ACh was increased by 10 µM. At the last line ACh was washed out. The action-potential activity of all recorded neurons was averaged and binned in 20 s bins. The green dots show the average activity for the different concentrations of ACh.

The total spike activity after the entire period of 1.5 hours (the period starting at the moment the first 10 µM was added till the moment ACh was washed out) was reduced by almost 25%. If synchronized bursting activity was present in the cultures, this activity was not reduced by addition of ACh. This suggests that cholinergic PPN input in the STN has a decreasing effect on the spontaneous activity of the STN. Loss of cholinergic input, on its turn, increases STN spontaneous activity (see Heida et al., 2008).

It should be noted that the symptoms of Parkinson's disease are a result of alterations in the network activity of the motor cortex, the thalamus and the basal ganglia. The alterations ultimately originate from a shortage of dopamine as a consequence of degeneration of (a.o.) the dopaminergic cells in the substantia nigra pars compacta. However, compensatory mechanisms effectively combat the effect of dopamine-shortage until roughly 80% of dopaminergic SNc cells have died. At any stage, we study not only the disease, but also the mechanisms nature uses to combat its effects (and maintain function). To model the disease and possible interventions (L-DOPA; DBS), fundamental research about the electrophysiology of all of the involved nuclei is needed.

Experiments with acute slices can provide us with detailed data, but only with respect to one moment in time and only by damaging a large part of the network that is involved in PD. The short-term effect of chemicals (drugs) and electrical stimulation (DBS) can be studied in tremendous detail and may lead to a better understanding of particularly the latter (and ultimately to better interventions). The progress made in MEA technology and slice preparation allows even more complex systems to be studied. For mice and rats, the maximum thickness of slices (~400 um) is enough to preserve much of the connectivity of the basal ganglia. The preservation of connections between distant nuclei (motor cortex, thalamus, substantia nigra) may well be possible, even though they may not be within a single slice. For now, these techniques allow us to check the models that have been created to study basal ganglia (dys)function such as the reciprocal coupling between STN and globus pallidus, which is claimed to result in bursting activity at low concentrations of dopamine. We can also test whether there is feedback from STN to the SN, thus ameliorating the effect of shortage of dopamine, but possibly speeding up PD progression (Blandini et al., 2000). We have already observed a marked increase in firing rate in both STN and SN with rising dopamine concentration. We expect that compensatory mechanisms (i.e. adaptation) will counter large changes in firing rate on the longer term, possibly by changing the firing patterns (i.e. neuronal and synaptic properties). Medication by L-DOPA may have the same effect in PD patients; enabling neurons to fire in patterns that, at least, do not interfere with normal function. The question of which patterns do not interfere with normal function, may be answered by simulating DBS in-vitro, since the effects of DBS are visible almost instantly.

For longitudinal studies, cultures of dissociated neurons, or even (non-dissociated) co-cultures can be used. Even though these will create networks that differ from those in the intact brain, they can be used to study basic mechanisms and how they evolve over longer periods of time. Here, more fundamental questions about neuronal adaptation to various inputs and chemical additions can be answered. From cultures of cortical neurons we know that basic properties of these networks (e.g. percentage of excitatory/inhibitory connections) develop in a similar way compared to in-vivo counterparts. This even allows the study of age-related effects.

4. Computational models of PD and DBS treatment

Computational studies are useful in investigating how pathological conditions and DBS induced activity may find their way through the basal ganglia-thalamocortical circuit. High-frequency stimulation leads to somatic inhibition of neurons that are close to the electrical field while simultaneously afferent and efferent axons may be excited. Both cellular and network effects may contribute to the overall clinical effects of DBS. McIntyre and Hahn (2010) claim that: "changes in the underlying dynamics of the stimulated brain networks may represent the core mechanisms of DBS and that those basic dynamical changes can be achieved via activation, inhibition, or lesion". Stimulation does not necessarily has to restore the network to a pre-pathological/normal state, but should allow improvement in Parkinson's symptoms.

Normally a parametric approach based on investigations of the biological system and network or molecular/channel characteristics, is preferred. Since not all systems are studied in detail, a non-parametric based model may be used, in which only input and output are considered, leaving the system a black box. Due to the extreme data gathering on Parkinson's disease non-parametric approaches are uncommon and the models brought forward can be classified as parametric. Here we will concentrate on the models for the thalamocortical relay neuron and the PPN neuron, thus directing towards an efferent thalamo-projecting model and an efferent spinal-projecting model.

4.1 Model of the thalamic relay neuron

The output of the basal ganglia network is directed towards the thalamic nuclei (Figure 1), which influences the motor cortex and its output is relayed via the pyramidal tract towards the secondary motor neurons. In two recent studies we have investigated how DBS can affect the functioning of the thalamus as a relay station (Cagnan et al., 2009; Meijer et al., 2011). Although this is a simplication, it is presumed that this relay should retransmit incoming information from cortex and sensory systems back to cortex. This extends earlier work by Rubin and Terman who showed that the mechanism of DBS may be regularizing the output of thalamus (Rubin & Terman, 2004). They demonstrated that under pathological conditions STN-GPe networks can show a pacemaker rhythm at tremor frequency (Terman et al., 2002). These phasic signals from basal ganglia may impair the transmission of thalamocortical information. When replacing the pathological oscillations by regular DBS input thalamocortical relay may be restored (Rubin & Terman, 2004; Guo et al., 2008).

We started to model a simpler situation by first focussing on rest tremor. A GPi-spike train obtained from a human PD patient during DBS surgery with characteristic patterns of rest tremor was used to generate GPi input to the thalamus. Without relay of cortical input (rest situation), the thalamic model response consisted of rebounds at the same tremor frequency (Figure 9).

By including excitatory input the combined effects of relay, PD and DBS could be examined. The pathological input was partially replaced by DBS pulses reflecting a limited volume of tissue being activated by the stimulation. For DBS there are two common targets: STN and GPi.

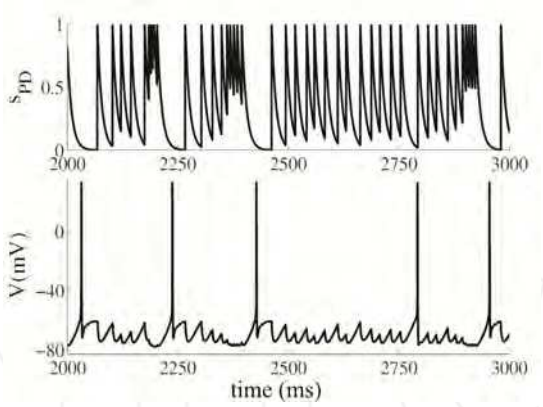


Fig. 9. Top: The model synaptic input reflects the burstiness of the activity of the measured GPi neuron. Bottom: The thalamic relay cell exhibits post-inhibitory rebound action potentials, i.e., during the pause after the GPi burst an action potential is generated.

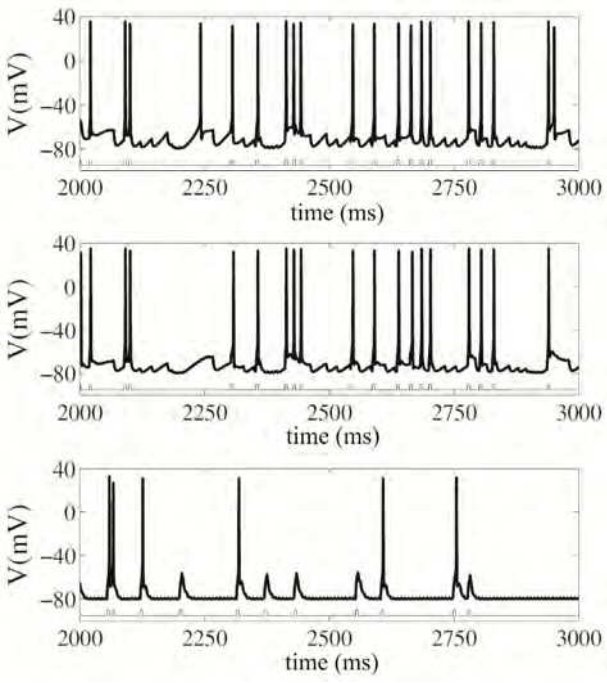


Fig. 10. The effect of overwriting the pathological GPi input by increasing DBS amplitude. The upper traces represent the membrane voltage (V) of the TC cell. The precise timing of the excitatory input (mean rate 16.5 Hz) is displayed beneath each voltage trace. Top: If the stimulation amplitude is too weak, rebounds and an incorrect signal relay occur. Middle: With moderate DBS amplitude, rebounds are quenched and relay is correct. Bottom: With high DBS amplitude, relay of sensory information is impaired.

Stimulation of the STN may recruit efferent fibers that excite GPi. In both cases it is therefore plausible that DBS leads to additional downstream GPi output. At the thalamus, the input from the basal ganglia comes from the GPi and is therefore inhibitory. A key property of thalamic relay cells is their low-threshold T-type calcium current. When the thalamic relay cell is inhibited long enough, it fires rebound action potentials (Janssen & Llinàs, 1984). The effect of such phasic pathological inhibition is that the thalamic output activity does not reflect the original input. This stems from two sources of errors. Long periods of inhibition diminish the responsiveness of the TC cell and rebound spikes are mixed with successful relays.

In the model we found that this extra inhibition can jam the transmission of pathological oscillations around the loop. Additionally we investigated the frequency dependence of this therapeutic regime and showed that in the model it persists to frequencies as low as 60 Hz, although the plateau starts at 100 Hz. In Cagnan et al. (2009) we considered a similar setup but considered the frequency content of the output. In particular, we showed that DBS can diminish the power at pathological frequencies in the spectrum of the thalamic output. Finally, we also found that if the frequency of the relay input is sufficiently high, and the variance low enough, this can also block the transmission of pathological low-frequency oscillations. This may be interpreted as a suppression of rest tremor.

4.2 Model of the PPN neuron

Due to its location in the brainstem and its function in locomotion and postural control, the pedunculopontine nucleus (PPN) has been suggested as a target for DBS to improve gait and postural instability. The glutamatergic PPN neurons are reciprocally connected with the basal ganglia and these neurons provide the descending PPN output to the spinal cord. Therefore, PPN has a pivot role in regulation of the basal ganglia and spinal cord, and providing indirect pathway for the basal ganglia to regulate the initiation of gait (Pahapill & Lozano, 2000; Hamani et al., 2007).

In Lourens et al. (2011) we have developed a computational conductance based model for the glutamatergic PPN type I to investigate the response of the PPN cell to various basal ganglia inputs. The specific characteristics of PPN currents are described by Takakusaki and Kitai (1997). A persistent sodium current is responsible for subthreshold membrane oscillations in PPN type I neurons, which underlies spontaneous repetitive firing. Moreover the LTS property is mediated by the T-type calcium current. The model is based on neurophysiological data of the thalamocortical relay neuron, and the pre-Bötzinger neuron. The PPN Type I is modelled as a single compartment model using the Hodgkin-Huxley formalism, except for the calcium current which is described by the Goldman-Hodgkin-Katz ion current equation. We used the basal ganglia model as proposed by Rubin and Terman (2004) to generate input to the PPN type I model. Moreover, we include the projection from the PPN back to the basal ganglia.

The model of an isolated type I PPN neuron shows the experimental behaviour as described in literature (Takakusaki & Kitai, 1997). The PPN neuron model shows spontaneous firing at 8 Hz, and bursting behaviour after the release of a hyperpolarizing input. In the network model, including 8 cells of STN, GPe and GPi and 1 PPN cell, we found that under PD conditions the firing rate of the PPN cell decreases and its firing pattern regularizes. In

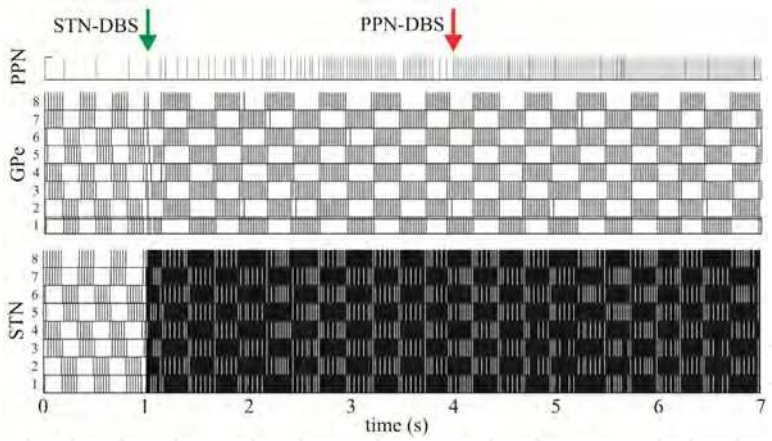
addition, we investigated the effect of DBS in PPN and STN on the behaviour of the PPN-basal ganglia network and on the relay property of the PPN cell. DBS is modeled as a train of positive current pulses, injected directly into the target cells. PPN-DBS is applied with high amplitude (100 μA cm^{-2}) at 40 Hz and STN-DBS is applied at 130 Hz with an amplitude of 400 μA cm^{-2}; both stimuli have a pulse width of 0.15 ms. For the relay property it turns out that combined high-frequency stimulation of STN and low frequency stimulation of PPN hardly improves the effect of exclusive STN stimulation. PPN-DBS eliminates the pathological firing pattern of STN and GPe cells, whereas STN-DBS and combined STN- and PPN-DBS eliminate the pathological firing pattern only from STN cells, see Figure 11.

5. Discussion

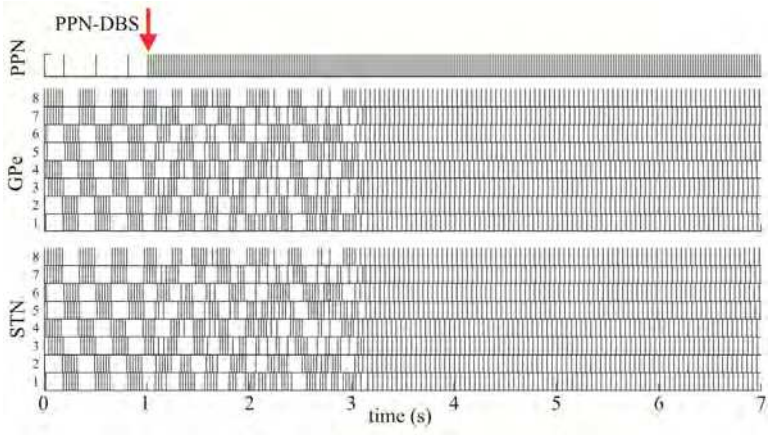
In general there is a wide gap between experimental animal results, especially with respect to neuroanatomical data, and computational modeling. In order to be able to investigate the anatomical and functional properties of afferent and efferent connections between the different nuclei of the basal ganglia, similar studies need to be performed as described for the substantia nigra. These studies, though very time-consuming, are essential to decide which pathways play important roles in normal functioning and therefore need to be included in modeling studies. In addition, it should be known what neuroanatomical changes take place resulting from the neurodegeneration associated with Parkinson's disease and how they affect network behavior. For instance, the direct effects of DBS on motor control are of interest, but since DBS has a low threshold to side effects, additional non-motor pathways are expected to be involved. Including these pathways in network models may shed light on the extent and effect of stimulation. Similarly, as PPN stimulation may have a beneficial influence on gait and balance, different pathways are important regarding the different motor symptoms of Parkinson's disease.

The classic diagram of the basal ganglia as presented in Figure 1 not only leaves out a number of connections that are discussed in this review, it also is based on average firing rates while currently it is known that firing patterns change under parkinsonian conditions. The irregular firing characteristics within the nuclei of the basal ganglia are transformed into a more synchronous and bursting activity pattern. Functionality of neuronal networks is dependent of neurotransmitters and their receptors, together with the channels present in the cell membrane. Studies on the properties and localization of the receptors and channels are therefore a prerequisite for adequate modeling. However, the enormous amount of receptors and channel types, and their variability in distribution makes it virtually impossible to describe neuronal function for all basal ganglia nuclei (see e.g. the eloquent studies on GABA in the basal ganglia; Tepper et al., 2007).

Dissociated neural cultures as well as brain slices positioned on multi electrode arrays open the possibility to study basal ganglia nuclear functional action and interaction, i.e. the overall result of all cell membrane activities of a neuron or group of neurons. By the addition of neurotransmitters, their agonists or antagonists, PD basal ganglia activity can be mimicked in-vitro. It is expected that this alternative route of studying PD will bring up the most needed extra information to support fine-tuning of neuron and neuronal network models and will as a consequence incorporate the more subtle connections nowadays described in neuroanatomical studies.



A)



B)

Fig. 11. Effect of PPN-DBS and STN-DBS on the activity of GPe and STN cells. Stimulation starts at 1 s and 4 s, as indicated by the arrows. With STN-DBS stimulation clusters remain and the time period of the clusters gets longer in GPe, while STN cells are locked to the STN-DBS frequency. Addition of PPN-DBS shows no change (A). With only PPN-DBS (B) the STN and GPe clusters are disrupted after some transients.

Essential in modeling is to formulate reduced models that still capture essential properties of the dynamics but are able to include even these subtle connections. Models need verification by experiments to demonstrate that the model has reality value. With the increasing amount of in-vitro and in-vivo experimental data computational models may become applicable in human research and health care problems. The therapeutic stimulation parameters for DBS (polarity, pulse amplitude, pulse width, frequency) will in the near future rely more on the predictions made by model simulations (Cutsuridis et al., 2011).

6. Future direction

"Despite numerous diverse- and at times frankly bizarre- etiologic speculations over a considerable period of time, the cause or causes of Parkinson's disease remain unknown" (Stern, 1996). This statement still holds and therefore the efforts in Parkinson´s research are focused on investigating ´what goes wrong in the parkinsonian brain, and how can we reverse this pathological behaviour´. Medication and deep brain stimulation are meant to restore direct causes of the disease symptoms: compensating the loss of dopamine, and desynchronizing the pathological oscillations, respectively. A lot of attention is drawn to the basal ganglia and the causes and effects of its dysfunction. Individual neuronal receptor studies on basal ganglia or SNc cells hardly can give the overall outcome of the typical neuronal dysfunction. Dissociated neuronal cultures and brain slices presumably will be more successful.

Due to the neuronal plasticity of the brain, several mechanisms are involved in functional compensation for the progressive loss of dopamine. PD symptoms do not become clinically manifest until neuronal death exceeds a critical threshold: about 70–80% of striatal nerve terminals and 50–60% of SNc dopamine neurons (Bezard et al., 2003). While initially it was suggested that the preclinical state reflected the ability of the affected neuronal system to actively compensate for the loss of dopamine (Bezard et al., 2003), we now know that compensatory mechanisms outside that basal ganglia exist. Although the basal ganglia may be in a parkinsonian state, these mechanisms may prevent the appearance of symptoms. The overactivation of lateral premotor areas as found from PET and fMRI studies may express compensation processes (Samuel et al. 1997; Berardelli et al. 2001; Bezard et al., 2003). The cerebellothalamocortical circuit is proposed to play an important role in these processes since the cerebellum has strong connections with the lateral premotor areas. It is concerned with externally triggered movement, which may explain the beneficial effect PD patients experience from external cues in guiding movements. In contrast, the cerebellothalamic circuit was also hypothesized to play a role in tremor generation (Helmich et al., 2011). A prerequisite for the development of novel therapeutic methods in-vitro and computational models of Parkinson's disease is to include those circuits that are involved in compensating for the parkinsonian symptoms, based on cellular and connective studies.

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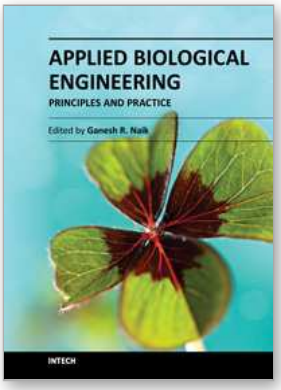

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Biological engineering is a field of engineering in which the emphasis is on life and life-sustaining systems. Biological engineering is an emerging discipline that encompasses engineering theory and practice connected to and derived from the science of biology. The most important trend in biological engineering is the dynamic range of scales at which biotechnology is now able to integrate with biological processes. An explosion in micro/nanoscale technology is allowing the manufacture of nanoparticles for drug delivery into cells, miniaturized implantable microsensors for medical diagnostics, and micro-engineered robots for on-board tissue repairs. This book aims to provide an updated overview of the recent developments in biological engineering from diverse aspects and various applications in clinical and experimental research.

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