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A new paradigm for Deep Brain Stimulation in hemiparkinsonian rat model

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Titel: Ein neues Paradigma für die Tiefe Hirnstimulation im Hemi-Parkinson-Rattenmodell

Stichwörter: Tiefe Hirnstimulation; Amphetamine / Apomorphin-induzierte Rotation;

Parkinson-Erkrankung (Morbus Parkinson); Ratte

Zusammenfassung:

Tiefe Hirnstimulation (DBS) des Nucleus subthalamicus (STN) gilt mittlerweile als wirksame Behandlungsform von Bewegungsstörungen wie der Parkinson-Erkrankung, sowie zunehmend auch für andere neuropsychiatrische Störungen. In der vorliegenden Doktorarbeit habe ich die Auswirkungen der STN-DBS auf dem Apomorphin / Amphetamine- induzierte Drehverhalten im Hemi-Parkinson-Rattenmodell untersucht.

Es wurden männliche Wistar-Ratten verwendet, die zu Beginn jedes Experiments zwischen 280 und 290 g wogen. Das Hemi-Parkinson-Rattenmodell wurde durch einseitige Injektion von 6-Hydroxydopamin (6-OHDA) in das mittlere Vorderhirnbündel (MFB) induziert. Darüber hinaus wurde den Ratten einseitig eine Elektrode für die tiefe Hirnstimulation im ipsilateralen STN implantiertAlle Tiere konnten sich nach der Operation drei Wochen lang erholen. Anschließend wurde jeweils das Apomorphin- und Amphetamin-induzierte Drehverhalten getestet, um zu bestätigen, dass das Hemi-Parkinson-Rattenmodell erfolgreich induziert werden konnte. Jeder Ratte wurde entweder Apomorphin (0,05 mg/kg, subkutane Injektion (s.c.)) oder Amphethamine (2,5 mg/kg; intraperitoneale Injektion (i.p.)) injiziert, die Ratte anschließend in einen selbstmachten Rotations-Setup gesetzt und dann das Verhalten für entweder eine (Apomorhin) oder zwei Stunden (Amphetamin) aufgezeichnet. Der Setup wurde von LED-Leuchten beleuchtet, um den Kontrast und damit die Auflösung zu verbessern. Nachdem die Ratten die minimale Anzahl von Rotationen pro Minute (6 Umdrehung / Minute) erreicht hatten, wurde die STN-DBS für die Dauer von jeweils 1 Minute in 4-Minuten-Intervallen angewandt. Über die gesamte Stimulationsperiode hinweg wurde die Frequenz auf 130 Hz und die Pulsbreite auf 60 µs eingestellt – dies entspricht klinisch zugelassenen Werten. Die Amplitude der elektrischen Impulse lag konstant bei 0-450 µA. Das Drehverhalten der Hemi-Parkinson-Ratte wurde vor,

während und nach der STN-DBS gemessen und ein Rotationsindex durch Division der Anzahl der Rotationen während der Stimulation und der durchschnittlichen der Anzahl der Rotationen vor und nach der Stimulation ermittelt.

Unsere Daten zeigten, dass STN-DBS die Anzahl der Rotationen in einer amplitudenabhängigen Weise vermindert. Außerdem zeigten unsere Ergebnisse, dass ein pseudorandomisiertes Weglassen von 10, 20 oder 30 Prozent der Impulse keine wesentlichen Auswirkungen auf den STN-DBS-Effekt hat.

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Title: A new paradigm for Deep Brain Stimulation in hemiparkinsonian rat model

Keywords: Deep Brain Stimulation; Amphetamine/Apomorphine-induced rotation; Parkinson's disease; Rat

Abstract

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is now established as an effective treatment of movement disorders such as Parkinson disease, and increasingly for other neuropsychiatric disorders. In this study, we tried to investigate the effects of STN-DBS on the turning behavior induced by apomorphine/amphetamine injection in a hemiparkinsonian rat model.

Male Wistar rats weighing 280-290 g at the beginning of each experiment were used. The hemiparkinsonian rat model was induced by unilateral injection of 6-hydroxydopamine (6-OHDA) into median forebrain bundle (MFB). In addition, rats were unilaterally implanted with one electrode for deep brain stimulation in the ipsilateral STN. The electrode was implanted so that it was covering the STN extent in depth. The animals were allowed to recover from surgery for three weeks. Afterwards, apomorphine or amphetamine-induced turning behavior was tested to confirm whether the hemiparkinsonian rat model was successfully induced. Each rat was injected with apomorphine (0.05 mg/kg, subcutaneous injection (s.c.)) or amphetamine (2.5 mg/kg; intraperitoneal injection (i.p.)), put in a homemade rotation setup and recorded over a period of 1 or 2 hours for apomorphine and amphetamine, respectively. The setup was illuminated by LED lights in order to enhance the resolution. When the number of rotations increased stably beyond a criterion value (more than 6 rotations/minute), STN–DBS was applied for a duration of 1 minute with 4 minute intervals. The frequency was set at 130 Hz and the pulse width at 60 μs -which are clinically approved- over the stimulation period for all of the stimulated animals. Continuous electrical biphasic rectangular pulses (with negative pulse leading) were delivered to the STN electrodes (0–450 μA). The turning behavior of the hemiparkinsonian rat before, during, and after STN-DBS was measured and rotation index was

calculated by dividing the number of rotations during the stimulation to the average number of rotations before and after stimulation.

Our data revealed that STN-DBS reduced the number of rotations in an amplitude dependent manner. Moreover, our findings indicated that randomly dropping of 10, 20, and 30 percent of the pulses does not cause any significant effect on STN-DBS efficacy.

Liste der verwendeten Abkürzungen

ANOVA	analysis of variance
BG	Basal Ganglia
DA	dopamine
DBS	Deep Brain Stimulation
GABA	gamma-aminobutyric acid
GP	globus pallidus
GPe	globus pallidus external
GPi	globus pallidus internal
i.p.	Intraperitoneal injection
IgG	Immunoglobulin G
KPBS	potassium phosphate buffered saline
MFB	medial forebrain bundle
MSNs	medium spiny neurons
PD	Parkinson's disease
PBS	phosphate Buffered Saline
Pf	parafascicular
PFA	paraformaldehyde
PFC	prefrontal cortex
CM	centromedian nuclei
PPN	pedunculopontine nucleus
s.c.	Subcutaneous
SEM	Standard Error of Mean

SN	substantia nigra
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
STN	subthalamic nucleus
TH	tyrosine hydroxylase
Thal	thalamus
VIM	ventral intermediate nucleus
VTA	ventral tegmental area
e.g.	Exempli gratia (for example)
6-OHDA	6-hydroxydopamine

Contents

1. Chapter one: Introduction

1.1. Parkinson's disease

Parkinson's disease (PD) is a devastating neurodegenerative disorder affecting a tremendous number of people worldwide (Blesa et al., 2012; Sutton et al., 2013; Pienaar et al., 2012; Coelho & Ferreira, 2012; Ribeiro et al., 2013). Although PD is not a terminal disease by its very nature, its advanced consequences might lead to eventual demise (Pienaar et al., 2012). The quality of life of the PD patients is highly affected by the motor and cognitive disorders, especially in the late stages of the disease (Dauer & Przedborski, 2003). An immense progressive neurodegeneration caused by PD is followed by extensive clinical symptoms: from movement disorders to specific mood and cognitive impairments (Krack et al. , 2010). Even though an inherited form of PD has been declared, most of the PD cases (95%) are categorized as sporadic (without any pronounced genetic linkage) PD (Dauer & Przedborski, 2003). From a neuropathological point of view, PD results from the degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). This neuro-depletion will consequently cause burst activities in the basal ganglia especially in the subthalamic nucleus (STN) and internal part of globus pallidus (Duty & Jenner, 2011; Li et al., 2012). It has been suggested that brain oscillations are altered as a result of these unusual burst firings (Li et al., 2012; Wilson, 2014). Ultimately, widespread movement impairments occur due to these disrupted basal ganglia activities (Dauer & Przedborski, 2003; Fang et al., 2006; Li et al., 2012). In recent years, studies of PD have received a remarkable attention. The reasons for this popularity are threefold: first of all, the prevalence of PD is dreadfully high, as it has been reported as the second most common neurodegenerative disease (Dauer & Przedborski, 2003; Blesa et al., 2012; Sutton et al., 2013; Pienaar et al., 2012; Coelho & Ferreira, 2012; Ribeiro et al., 2013). Moreover, the exact underlying mechanism of DA neurodegeneration is yet a matter of intense debate. More

importantly, the current clinical knowledge still suffers from the lack of an adequate treatment that can retard the degeneration and permanently alleviate the clinical signs.

1.2. History of Parkinson's disease

The early reports of PD trace back to approximately 1000 BC, when traditional Indian texts and ancient Chinese medicine suggested the existence of Parkinson's disease (Goetz, 2011; Zhang etal., 2006). However, it was not until the mid-17th century that the first diagnostic manifestations such as resting tremor and festination were initially described by Sylvius de la Boë and Sauvages, respectively (Goetz, 2011). Eventually, James Parkinson pioneered the first clear clinical description of the disease in 1817 (Betarbet et al., 2002; Garcia Ruiz, 2004; Goetz, 2011). In a monograph entitled "Essay on the Shaking Palsy," he explained the core clinical features. This discovery would go unheeded for nearly 50 years until, Jean-Martin Charcot, elucidated further and reported a complete list of clinical signs for PD including bradykinesia as a separate feature (Goetz, 2011). Moreover, in honor of James Parkinson, Charcot suggested the renaming of the disease to Parkinson's disease. He also rejected the paralysis agitans or shaking palsy as the main diagnostic criterion for PD, demonstrating that PD patients do not necessarily show tremor (Goetz, 2011). Subsequently, in 1912, "Lewy bodies" were introduced by Frederic Lewy (Forman et al., 2005) and a few years later the substantia nigra was reported as the main affected cerebral structure (Parent & Parent, 2010). In 1953, with the advancement of pathological techniques, Greenfield and Bosanquet reported a complete pathological analysis of PD (Goetz, 2011). However, it was in the 20th century that translational PD studies truly began. Further investigations led to proposing dopamine as a neurotransmitter and its dominant role in PD (Goetz, 2011). Consequent to this discovery, the first effective medical treatment of PD was

introduced. A groundbreaking study, in the 1960s, pioneered Levodopa (L-Dopa) application as a possible treatment and opened a new avenue in the PD treatments especially for early stages of the disease (Goetz, 2011). Influenced by the progressive development of the genetic studies, 20th century ended by introducing the inheritor form of the PD. In 1997 the protein synuclein was reported as the main component of Lewy bodies which is the most common pathological hallmark of PD (Forman et al., 2005). Following that, the mutations that cause PD were discovered in the late 1990s. Since then the trend of the PD research has hitherto developed in two major fields. First, molecular biology has been evolving elaborate progress in the underlying mechanisms of the disease and new alternative treatments by manipulating the intra-cellular machinery. As an example, neural stem cell transplantations were introduced and gained much attraction in the recent years (Borlongan & Sanberg, 2002; Carvey et al., 2001; Yasuhara et al., 2006; Torres et al., 2008). On the other hand, system physiology has mainly focused on the altered brain activity during task execution and behavioral tests, in both animal models of PD and parkinsonian patient cases. Consequently, DBS was emerged to mitigate PD symptoms. In the late 80s, the first systemic DBS method was conducted in combination with Thalamotomy (Benabid et al., 1987), although the DBS that is applied in current days was reported in 1993 (Benabid et al., 1993). Animal models, behavioral experiments as well as recent optical studies, have deepened our understanding of the principles of PD and its intrinsic neurodegeneration mechanism. Eventually, although much has been learned about Parkinson's disease yet much is a mystery.

1.3. The affected neural circuits in Parkinson's disease

PD primarily results from the death of dopaminergic neurons which originate from the substantia nigra and project to the dorsolateral parts (caudate and putamen) of the striatum (Dauer & Przedborski, 2003; Blesa et al., 2012). Since the naive striatal (Dauer & Przedborski, 2003) and nigral (Forman et al., 2005) dopamine cells express neuro-melanin, the pattern of cell loss can be anatomically confirmed by measuring the magnitude of depigmented neurons (Dauer & Przedborski, 2003; Marsden, 1983). Today it is known that the degeneration is more prevalent in the putamen rather than the SNc neurons (Pienaar et al., 2012). This, consequently, justifies the dominance of the movement abnormalities in PD (Dauer & Przedborski, 2003; Pienaar et al., 2012). The dopaminergic neurons of the ventral tegmental area (VTA) and caudal parts of the dorsal striatum are much less affected (Dauer & Przedborski, 2003) which is compatible with fewer changes in reward-related behavior. More recent evidence highlights that neurodegeneration is more profound in dopaminergic neurons in basal ganglia, however, it is not restricted to the dopaminergic cells and can progressively spread far further than motor-related areas in the brain (Dauer & Przedborski, 2003). In fact, the neurodegeneration in locus coeruleus noradrenergic (Beal, 2010), serotonergic (Pienaar et al., 2012; Ohno et al., 2015; Politis & Niccolini, 2015), and cholinergic (Pienaar et al., 2012; Karachi et al., 2010) systems have been well reported. Moreover, the cell demise has been found in the cerebral cortex, olfactory bulb (Höglinger et al., 2004; Jankovic, 2008), hippocampal structures (Pienaar et al., 2012), and autonomic nervous system (Cersosimo & Benarroch, 2013), mainly in more severe or late-stage disease. This widespread degeneration causes cognitive abnormalities— such as depression and speech problems— and might lead to eventual demise (Dauer & Przedborski, 2003; Pienaar et al., 2012). For a better understanding of the main dopamine-rich brain pathways which are

highly affected in PD, a brief introduction into the main motor pathways and affected nuclei is given below.

The basal ganglia (BG) are the mainly affected brain areas in PD. The BG are located in the forebrain and consist of several nuclei including striatum (caudate nucleus, putamen), the globus pallidus (GP), subthalamic nucleus (STN), substantia nigra (SN), and olfactory tubercle (Hélie et al., 2015). Based on anatomical and electrophysiological studies, the BG are divided into three functional areas: sensorimotor, associative or cognitive, and limbic regions (Parent & Hazrati, 1995; Krack et al., 2010). The BG have two input nuclei. The main input structure is striatum which receives glutamatergic afferents from almost the entire cortex (except V1) and the second one is STN (Nambu et al., 2002; Utter & Basso, 2008; Kreitzer & Malenka, 2008; Hélie et al., 2015). The primary output structures consist of the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). As the output target, they send their projections to cortex through thalamus (Fig.1). This connection provides an explanation for the involvement of BG in the motor, sensory, and cognitive processes (Middleton & Strick, 1994; Krack et al., 2010). Furthermore, they innervate the brain stem areas including the superior colliculus, and the pedunculopontine nucleus (PPN). Via PPN projection, BG controls the spinal cord processing and postural control (Utter & Basso, 2008). Moreover, BG is involved in movements of the head and eyes through the superior colliculus projection (Utter & Basso, 2008; Hikosaka et al., 2000). Interestingly, apart from the afferent and efferent projections, the BG, per se, have an enormous internal innervation (Utter & Basso, 2008; Kreitzer & Malenka, 2008; Hélie et al., 2015). Indeed, the intrinsic projection of the BG neurons among themselves makes it an extraordinary subcortical network (Utter & Basso, 2008). Consequently, based on its strategic position as well as its unique intra-innervation network, the BG are involved in a wide variety of brain higher

functions. This large innervation system and BG immense involvement in several information processing pathways make this structure a prevalent part of the brain motor-cognitive system whose chaos can cause a widespread disorder (Krack et al., 2010). In the parkinsonian brain, the BG neural activity is not only hyperactivated, but also disrupted and therefore it cannot accurately accomplish its function (Benabid, 2003; Hess et al., 2013; Wilson, 2014). In the following sections, the reason of this abnormal BG activity is explained.

1.3.1. The main input structure of the basal ganglia: Striatum

The striatum is the main input structure of the BG and consists of two nuclei, the caudate and putamen which have a similar function, but the different neuroanatomical structure (Kreitzer & Malenka, 2008; Hélie et al., 2015). The caudate is caudally separated from putamen by internal capsule (Utter & Basso, 2008). Three different interneurons, including the large spiny cholinergic neurons, medium spiny gamma-aminobutyric acid (GABA) ergic neurons, and somatostatin spiny neurons have made a large innervation inside striatum (Utter & Basso, 2008; Kreitzer & Malenka, 2008). Striatal neurons may also contain neuropeptide Y (Kreitzer & Malenka, 2008). Although the striatum does not have any glutamatergic neurons (Kreitzer & Malenka, 2008), the projections to the striatum are intrinsically excitatory, including glutamatergic input from almost the entire cortex (Hélie et al., 2015) and thalamic nuclei (Smith et al., 2004; Utter & Basso, 2008), and dopaminergic afferents from the brain stem (Kreitzer & Malenka, 2008). It is remarkable to note that different cortical areas project to distinct regions of the caudate and putamen (Utter & Basso, 2008). Accordingly, anatomical and physiological studies have proposed five parallel information processing circuits including a motor circuit, an oculomotor circuit, a dorsolateral prefrontal circuit, a lateral orbitofrontal circuit, and an anterior

cingulate circuit (Alexander & DeLong, 1986; Utter & Basso, 2008). On the other hand, the output is exclusively inhibitory from GABAergic medium spiny neurons (MSNs) which express D1 (Kreitzer & Malenka, 2008). The Striatum inhibitory output reaches the globus pallidus pars externa (GPe), which in turn sends GABAergic projections to the STN (Kreitzer & Malenka, 2008; Hélie et al., 2015; Utter & Basso, 2008). The striatum also receives a major input from dopaminergic neurons located in the ventral midbrain including the substantia nigra pars compacta (SNc) and the ventral tegmental area (Kreitzer & Malenka, 2008; Hélie et al., 2015; Utter & Basso, 2008). The dopaminergic projections originating from the VTA, innervate the caudate, putamen, and the ventral striatum (nucleus accumbens). The mesolimbic dopamine (DA) system is mainly involved in mechanisms of reward and addiction (Kalivas & Nakamura, 1999; Kalivas & Volkow, 2005; Kelley et al., 2005; Koob & Volkow, 2010; Di Chiara & Bassareo, 2007). Like other neurotransmitters in the brain, DA exerts its effect by binding to its receptors. Striatal output neurons express two types of DA receptors; D1 and D2 (Kreitzer & Malenka, 2008; Hélie et al., 2015; Utter & Basso, 2008). It has been demonstrated that DA receptor subtypes are dissimilarly distributed on the dendrites of striatal neurons (Kreitzer & Malenka, 2008; Hélie et al., 2015; Utter & Basso, 2008). The direct and indirect BG output projections express D1 and D2 receptors, respectively. Activation of D1 receptors facilitates the cortical-striatal activity while activation of D2 receptors has inhibitory effects on it (Kreitzer & Malenka, 2008). Taking such spatial and functional divergence into account, the classic theory for BG function came into existence. The classic approach states that the indirect pathway has inhibitory effects on movement, whereas the direct pathway facilitates movement (Kreitzer & Malenka, 2008; Utter & Basso, 2008). Although this approach is interesting, it failed to be subsequently confirmed (Cui et al., 2013). Anatomical evidence such as the existence of a direct

projection from the GPe to the GPi (Parent & Hazrati, 1995)— which later was called hyper-direct pathway— clearly opposes the validation of the classic view. In the parkinsonian brain, the firing rate of the striatal neurons is higher and the pattern of the firing shows more burst activity compared to the normal state (Chen et al., 2001; Deutch, 2006). The increased MSN neural activity is presumably to compensate for the dramatic reduction in striatal dopamine (Deutch, 2006).

1.3.2. The subthalamic nucleus

The STN is positioned ventral to the thalamus, within the indirect pathway of the BG (Utter & Basso, 2008) and surrounded by several nuclei (Hamani et al., 2004). The posterior limit of STN is the red nucleus. In the dorsal part, STN is separated from the ventral thalamus by a part of the fasciculus lenticularis and the zona incerta. The cerebral peduncle and the substantia nigra are located in the STN ventral border (Hamani et al., 2004). The STN receives afferents from Globus pallidus, cortex, thalamus, as well as the brain stem. Among them, the major STN input comes from Pallido-subthalamic projections. The GPe sends the projection to STN, and reciprocally, STN sends afferents to the GPe, GPi, and SNr. There are various spatial distributions of different species. In rodents, the lateral and medial parts of the STN are innervated by lateral and ventromedial pallidum, respectively (Parent & Hazrati, 1995; Hamani et al., 2004). Like striatum, STN receives direct glutamatergic input from different areas of the cerebral cortex (Nambu et al., 2002; Hamani et al., 2004; Devergnas & Wichmann, 2011). These cortical projections mainly innervate the dorsal parts of STN which consist part of basal ganglia motor loop (Hamani et al., 2004; Krack et al., 2010). Based on the origin of these afferents in the cortex, several homunculi in the STN have been reported (Hamani et al., 2004). The thalamo-subthalamic is another glutamatergic innervation which originates from the parafascicular (Pf)

and centromedian nuclei (CM) in the thalamus. However, in rodents, they are undistinguished from each other and referred to as Pf-CM complex (Hamani et al., 2004). STN receives direct brain stem projections consist of the dopaminergic afferents from substantia nigra (Hamani et al., 2004), mainly cholinergic input from the pedunculopontine nucleus (PPN) and laterodorsal tegmental nuclei (Hamani et al., 2004), and serotoninergic pathway from the dorsal raphe nucleus, just in rodents (Canteras et al., 1988; Hamani et al., 2004). It is notable that, in rodents, besides the aforementioned important pathways, STN receives input from several other brain structures, as well (Canteras et al., 1988; Hamani et al., 2004). Reciprocally, STN sends efferent to several brain structures through three main pathways: subthalamo-pallidal, subthalamo-nigral, and subthalamo-striatal projections (Hamani et al., 2004). Moreover, the presence of a direct subthalamo-cortical loop circuit in the rat has been proposed (Degos et al., 2008; Eusebio et al., 2009). In rodents and non-human primates, STN also sends outputs to the PPN and VTA (Jackson & Crossman, 1981; Hamani et al., 2004). However, the role of STN projections is still poorly understood and future work on its function will benefit better understanding of BG diseases. In the Parkinson disease the STN is hyperactive and like striatum, its pattern of discharge has changed. In addition, the low-frequencies activity in the LFP of the STN neurons has been reported. It has been proposed that the network between the STN and the external globus pallidus (GPe) has a moderating role in such synchronies (Detorakis et al., 2015). The recent investigations revealed a positive correlation between the pathological motor symptoms of PD and these newly developed oscillations (Detorakis et al., 2015; Wilson, 2014).

1.3.3. The output structures of the basal ganglia

The internal part of globus pallidus and the reticulata segment of substantia nigra provide the main output projections from the basal ganglia (Kreitzer & Malenka, 2008; Hélie et al., 2015; Utter & Basso, 2008).

The globus pallidus (GP) is comprised of two nuclei which are segregated by small fibers of passage and pallidal border cells. The internal segment (GPi) is located in the middle part, whereas the external division (GPe) is positioned more laterally (Utter & Basso, 2008). In rodents, GPi is often referred to as the entopeduncular nucleus (Utter & Basso, 2008; Jaeger & Kita, 2011). GPi receives afferents from the striatum, GPe, and STN, and sends its projections to the targets outside the basal ganglia, including the thalamus, the lateral habenula, and the brainstem (Jaeger & Kita, 2011). In contrast, the GPe sends its GABAergic inhibitory output restrictively to GPi and STN (Jaeger & Kita, 2011). Both internal and external parts of GP receive large input from the cerebral cortex (Jaeger & Kita, 2011) and major GABAergic projections via striatum (Utter & Basso, 2008). Cortical input reaches GP via two major pathways which distinctively pass through either striatum (direct pathway, striatal-GPi) or STN (indirect pathway, striatal–GPe–STN–GPi). The GP input is mainly GABAergic, however, depending on the origin of the striatal medium spiny neurons, it may contain other neurotransmitters. The medium spiny neurons originating from the direct pathway (striatal-GPi) contain substance P as well as GABA, whereas in the synapses from the indirect pathway (striatal–GPe–STN–GPi) along with GABA, dynorphin, and enkephalin are also secreted (Utter & Basso, 2008; Jaeger & Kita, 2011). On the other hand, the GP input which passes through STN is solely glutamatergic (Jaeger & Kita, 2011) and has an excitatory effect on the BG network.

The substantia nigra (SN) is one of the most prevalent dopaminergic structures in the brain. Anatomical evidence certifies its location in the ventral midbrain. Like GP, SN is divided into two different neuronal clusters both of which are dorsal to the cerebral peduncle (Utter & Basso, 2008). The SN pars compacta (SNc) sends large dopaminergic input to the striatum (the nigrostriatal DA system) and in turn, receives afferents. Nevertheless, the physiological function of this reciprocal connection has remained unclear (Utter & Basso, 2008). The other part is called substantia nigra pars reticulata (SNr) and is completely distinguished from SNc. Like the internal part of GP, SNr contains GABAergic neurons (Chevalier et al., 1981), and receives inhibitory input from the striatum (Kreitzer & Malenka, 2007). SNr plays a key role particularly in the physiological mechanism of the eye and voluntary movement (Hikosaka et al., 2000; Utter & Basso, 2008), as well as cognitive processes (Boehler et al., 2011; Mukhin et al., 2004; Utter & Basso, 2008).

In the parkinsonian state, basal ganglia output nuclei are hyperactive, with a disrupted pattern of firing and increased burst activity (Benabid, 2003; Miocinovic et al., 2013). This hyperactivity and changes in the firing pattern exert an inhibitory effect on the thalamus and the brain stem structures (Benabid, 2003; Miocinovic et al., 2013). On the other hand, as it was mentioned in the above sections, an oscillation occurs in the low-frequency beta band between the GPe and the STN (Benabid, 2003; Miocinovic et al., 2013; Wilson, 2014).

1.4. Molecular mechanism of Parkinson's disease

Neuroanatomical studies have confirmed that the cell demise in PD is not a simple aging process (Dauer & Przedborski, 2003). However, we have not yet reached a scientific conclusion on what

initially triggers the neurodegeneration in the first place. It has been suggested that the dopamine loss occurs as a result of a pathological mechanism. Anatomical, morphological, and molecular evidence have determined the precise pathological differences between the dopamine depletion in BG during Parkinson's disease and natural aging process in the other brain areas. As an example, despite normal aging, the location of PD neural degeneration is in the caudal parts of the SNc, and the termination process is done via dopamine transporter (Dauer & Przedborski, 2003).

Among the biological markers of the PD, the presence of "Lewy Bodies" (LBs) is believed to be one of the principle features of the disease (Barzilai & Melamed, 2003; Betarbet et al., 2002; Blandini & Armentero, 2012; Dauer & Przedborski, 2003; Orth & Tabrizi, 2003), even though it is yet a matter of debate whether the formation of LBs has protective or toxic effects (Dauer & Przedborski, 2003). LBs were first discovered in 1912 by Fredric Lewy. They are reported as abnormal aggregates of numerous ubiquitinated proteins such as a-synuclein, which appear as spherical masses (Barzilai & Melamed, 2003; Blandini & Armentero, 2012; Dauer & Przedborski, 2003; Forman et al., 2005). Once created, they accumulate inside the cell causing disarrangement in cell structure. Studies indicate that LBs are associated with lipofuscin-containing lysosomes (Duty & Jenner, 2011). The major component of LBs is Alpha-Synuclein which has attracted considerable interest in the field of neuropathological studies. The previous investigations have demonstrated that oxidative damage to α-synuclein can enhance its ability to misfold and aggregate (Dauer & Przedborski, 2003; Giasson et al., 2000; Przedborski, 2005). Therefore, the oxidative stress was proposed as one of the possible triggers of the proteins accumulation. In this hypothesis, the oxidative stress is likely to be the consequence of the mitochondrial dysfunction (Dauer & Przedborski, 2003). The aggregated proteins can indirectly

be made because of the dysfunctional chaperones or proteasome. Oxidative stress through the damages caused by reactive oxygen species (ROS) plays a pivotal role in this process (Dauer & Przedborski, 2003). Impaired complex 1 has been suggested as a possible reason for mitochondrial dysfunction, which can lead to eventual oxidative phosphorylation. The production of the ROS superoxide is increased by complex I suppression. ROS may, in turn, either increase the toxic molecules formation or interact with nitric oxide. Subsequently, these toxic molecules may cause mitochondrial damage by affecting the electron transport chain. These processes reciprocally lead to more ROS production. The more ROS production is associated with more misfolded protein and this vicious circle continues.

The substantia Nigra is especially sensitive to the oxidative stress because of its DA-rich nature. Since the metabolism of DA in dopaminergic neurons produces hydrogen peroxide and superoxide radicals, these neurons are suitable places to produce ROS. Another hypothesis suggests that misfolding and aggregation of proteins, per se, play a crucial role in the dopamine cell death in PD. One possible reason of the aggregated protein toxicity may be their direct or indirect interfering with intracellular trafficking which can alternatively cause cell deformation (Dauer & Przedborski, 2003). Misfolding and abnormal deposition of proteins are in fact a sign of several age-related neurodegenerative diseases, which suggests the toxicity of the protein aggregation. The protein inclusions are made in a rather complicated way, in order to remove the toxic deformed protein and restrict it from the active cytoplasm (Dauer & Przedborski, 2003; Kopito, 2000). Based on this theory, the whole process is a defensive mechanism which is triggered by the attacked cells (Dauer & Przedborski, 2003). Dysregulation of the programmed cell death may contribute to neurodegeneration. There is still a considerable uncertainty with regard to the type of the programmed cell death in PD. A growing body of literature has

suggested that the apoptosis as a classic model of cell death is responsible for degeneration in PD. But, the result of morphological studies says otherwise (Dauer & Przedborski, 2003). On the other hand, we have to take in mind that, since the dead cells disappear immediately and the rate of cell death is low, it makes it difficult to judge just based on morphological data. Many investigations, instead, proposed to measure the residual components of the programmed cell death, for instance, different types of caspase family including caspase-3, caspase-8, and caspase-9 (Dauer & Przedborski, 2003).

1.5. Animal models

The prevalence of neurodegenerative diseases is increasing year by year (Ribeiro et al., 2013). This increase is going to impose a serious socio-economical burden on our public health system. In spite of the high prevalence of these diseases, their exact causal mechanism still suffers from considerable ambiguity (Ribeiro et al., 2013). Our hypothesis for the involved cellular pathways of these diseases fails to explain the exact reason of the neurodegeneration. Another bigger concern is that current possible treatments are not satisfactory and in fact, there is still no absolute treatment for PD. The accurate understanding of the underlying mechanisms is a necessity for the probable eventual treatment strategies (Ribeiro et al., 2013). Currently, animal models are a beneficial tool for deepening our understanding of the underlying mechanisms of such diseases. Since the last century, PD studies have benefited from using animal models (as translational models) for the basic investigations. Nevertheless, animal models were established, not only for studying PD, but also to investigate an enormous range of biological phenomenon including neurodegenerative diseases (Betarbet et al., 2002; Blandini & Armentero, 2012; Deumens et al., 2002). In particular, last decades have witnessed a huge growth in using such models for ascertaining the concrete pathogenesis of a wide range of diseases (Blandini & Armentero, 2012). A striking feature of the animal models is its possibility for testing novel treatments before clinical application in human patients. Animal models provide a unique opportunity to investigate the brain activity before and after treatment with both invasive and noninvasive methods. They can help further with the possibility of checking the microscopic changes and molecular pathways in neurons. Using animal models for PD was initiated in the fifties with the invention of pharmacological models. Considering the extended amount of dopamine depletion in the parkinsonian brain, the first pharmacological models used the

monoamine-depleting drugs to create Parkinson-like motor symptoms (Ribeiro et al., 2013). The first toxin-based model was introduced in 1968 (Tieu, 2011). Tranzer and Thoenen pioneered using 6-hydroxy-dopamine (6-OHDA) as a chemical for neural degeneration. Until now, this methodology has been the most popular model for induction of hemiparkinsonian features in animals (Tieu, 2011). 6-OHDA is a hydroxylated analog of dopamine which was invented in 1959 and was initially reported as a noradrenaline degenerator (Tieu, 2011). The additional hydroxyl group makes it a toxic compound. In the brain, it is combined with dopamine and adrenalin receptors and is transferred to the monoaminergic neurons. Inside neurons, it activates ROS which leads to Quinones generation and triggers some apoptotic pathways (Blesa et al., 2012). The eventual cell death causes massive motor disorders, cognitive abnormalities, and final demise. Due to probable evolutionary reasons, 6-OHDA cannot cross the blood-brain barrier. There are three different targets for its local injection into the brain. The magnitude of the afterward lesion depends on not only the injected amount but also on the place of 6-OHDA local administration and animal species, as well (Blesa et al., 2012). It has been reported that injection to the median forebrain bundle causes the most severe lesion, while administration into the substantia nigra leads to a more moderate lesion and striatal injection will only cause partial lesion (Yuan et al., 2005; Dowd & Dunnett, 2005; Smith et al., 2002). The injections have to be made unilaterally, because of the widespread involvement of dopamine in the reward pathway and hunger state. In the case of bilateral injection, the animal loses its interest for eating and drinking and would be unable of performing the spontaneous or goal-directed behavior (Dunnett & Torres, 2012). In order to prevent the norepinephrine damage, 6-OHDA has to be administered together with an inhibitor of norepinephrine (NE) transporter, such as desipramine (McConnell et al., 2012; Arai et al., 2008; Gerlach & Riederer, 1996). It is known that the lesion

induced by 6-OHDA does not lead to Lewy Bodies formation and is without apoptotic morphological signs (Dauer & Przedborski, 2003). Nevertheless, the mechanism underlying cell death in this model is still poorly understood.

Even though most of the PD studies focus on 6-OHDA as an adequate model, several other toxin based PD models, including Paraquat, Rotenone, lipopolysaccharide (LPS), epoximycin, and 1-methyl-4-phenyl-4-propion- oxypiperidine (MPTP) model have been illustrated. All of these toxins make parkinsonian-like syndrome in animals, although they exert their effect through different mechanisms. 6-OHDA acts through oxidative stress, MPTP and rotenone inhibit the mitochondrial complex I, epoximycin simulates the inhibition of the proteasomes, and LPS conducts its effect through glial cell activation (Duty & Jenner, 2011). Among them, MPTP is one of the most common toxins especially for the primate models of PD. In monkeys, a range of severe parkinsonian hallmarks including tremor, rigidity, freezing, postural instability, and slowness of movement is produced by MPTP. These symptoms are irreversible and very similar to the side effects of this drug in human (Dauer & Przedborski, 2003). Moreover, like human PD, the susceptibility of the MPTP model increases by age (Dauer & Przedborski, 2003). The pattern of cell death in which SN shows a greater amount of lesion compared to the VTA is also similar both in human PD and MPTP-induced PD (Dauer & Przedborski, 2003). Despite the above-mentioned similarities, there are two main differences between human PD and MPTP-induced PD signs. First, controversy exists regarding the formation of classic LBs in the MPTP model, although the presence of some LB-like inclusions has been reported in the MPTP monkeys (Pienaar et al., 2012). Second, consistent degeneration of the monoaminergic neurons, in particular in the locus coeruleus, happens in PD but not in MPTP model (Dauer & Przedborski, 2003).

Even though the toxin-based models have yielded crucial insights into the possible mechanisms involved in PD, none of them to the best of our knowledge has been quite compatible with the human PD. Due to these shortcomings, gene-based PD models were introduced. The first reported mutant gene, which is linked to an inheritory form of PD, was α-synuclein. It was discovered in 1997 and is thought to play a role in the synaptic vesicle recycling. Subsequently, several other genes associated with familial PD were identified, including Parkin, Ubiquitin C-Terminal Hydrolase-L1, DJ-1, PINK1, and LRRK2 (Blesa et al., 2012; Lim & Ng, 2009). The discovery of the involved mutations in the familial PD extended beyond the clinic studies and opened a new avenue for a better understanding of the cellular mechanism and impaired molecular pathways in PD. So far, several mice, fly, and worm models of PD have been generated. Although these non-mammalian PD models cannot relevantly represent the morphology and phenotype of the human PD, they are useful models to study the relationship between PD-linked genes and the dopaminergic depletion (Lim & Ng, 2009). In conclusion, there is no single model which can perfectly recapitulate PD symptoms in detail; but, both toxin- and gene-based models have given us a lot of information to be exploited and interpreted in order to get a better understanding of the underlying mechanism of the parkinsonian state (Lim & Ng, 2009).

1.5.1. Behavioral tests in animals

Different experimental animal models of PD have been developed to achieve comparable clinical criteria of human PD in animals. Various behavioral tests have been accordingly suggested to qualify these animal models. Since in the parkinsonian brain a tremendous amount

of dopaminergic neurons are damaged, a wide range of dopamine-related behaviors is impaired. Considering the profound involvement of dopamine and basal ganglia pathways in behavioral output of the brain (Krack et al., 2010), an immense variety of abnormal functions can be predicted in PD, including problems in general health, motor disorders, olfactory deficits, sleep abnormalities, gastrointestinal dysfunctions, anxiety, depression, and cognitive deficits (Taylor et al., 2010). To simplify the animal research, the behavioral animal experiments on PD, have been categorized in two different type of tests; movement related and cognitive related tests. To evaluate the movement abnormalities, there is a wide range of well-documented behavioral experiments, such as spontaneous sensorimotor asymmetry, sensorimotor integration and neglect, dexterous fine motor control, balance and coordination, spontaneous locomotor activity, and rotational asymmetry. Although these techniques have proven useful for verifying a parkinsonian motor phenotype, they fail to investigate the cognitive deficits in animal behavior. To test the mood and cognitive abnormalities, other behavioral tests have been suggested, including: adhesive removal test for evaluating the disengagement behavior and attentional processes, T-maze and elevated plus-maze tests to check the executive functions, Morris Water Maze (MWM) to test visuospatial navigation, and akinesia paradoxa test for cognitive-related anxiety impairments (Pienaar et al., 2012). Since this dissertation is mainly focused on the motor abnormalities in PD, the following paragraphs will provide a more elaborate introduction about the animal behavioral experiments which test motor deficits in rodents.

Sensorimotor asymmetry can be checked with the "cylinder test" which tests the independent use of forelimbs during rearing (Schallert et al., 2000; Pienaar et al., 2012). "Stepping test" evaluates the sensorimotor integration and lateralized neglect, which is a result of wrong adjusting steps made by the forelimb (Tillerson et al., 2001; Pienaar et al., 2012). Both Stepping test and

cylinder test have been suggested to be equivalent to Akinesia in the human patients, and they are likely to be seen in the mild version of the PD (Fang et al., 2010; Pienaar et al., 2012). Impaired fine motor control in the parkinsonian animal models can be checked by "staircase". Seven different scores of reaching difficulty are represented in the staircase test (Pienaar et al., 2012). "Rotarod test" has been suggested as a suitable behavioral experiment to evaluate the balance and coordination in the parkinsonian rodents, by checking the motor abilities of the animal on a rotating rod with different speeds. Similar to stepping, and cylinder test, rotarod test also can be checked during early stages of the disease (Fang et al., 2010; Pienaar et al., 2012). The spontaneous locomotor activity can be evaluated by the "open field test" which examines the instant distance traveled, the velocity of the movement, the time spent in different zones of the field, freezing episodes, and mobile episodes (Pienaar et al., 2012; li et al., 2012). More importantly for this dissertation, drug-induced rotation assay has been suggested to be a robust method to check rotational asymmetry in the parkinsonian rodents. Studies suggest that rotational asymmetry is equivalent to dyskinesia in human patients which is one of the main motor symptoms in advanced PD, and therefore, is of special importance in particular for translation studies (Pienaar et al., 2012; li et al., 2012, Fang et al., 2010). The rotational assay can be induced by either amphetamine or apomorphine; which both cause rotation behavior. However, the direction of the rotation is different (ipsilateral or contralateral rotation, for amphetamine or apomorphine, respectively). The test was introduced in the 70s by the studies of Arbuthnott and Crow (Arbuthnott & Crow, 1971). The methods section will provide more detailed information about the technique and procedure of drug-induced rotation test.

1.6. Clinical symptoms

"Parkinsonism" is clinically defined as a syndrome characterized by tremor at rest, rigidity, slowness, reduction or absence of voluntary movement (bradykinesia, hypokinesia or akinesia), and postural instability (Gerlach & Riederer, 1996; Dauer & Przedborski, 2003). Although the most common reason of "parkinsonism" is idiopathic PD (around 80% of the cases), almost any disease that includes striatal DA degeneration can cause such syndrome with the aforementioned clinical symptoms (Gerlach & Riederer, 1996; Dauer & Przedborski, 2003).

The most common symptom in PD is a tremor. Nonetheless, as Charcot demonstrated, not all the PD patients show tremor (Goetz, 2011). The clinical term "tremor at rest" is defined by permanent shaking when the limb is at rest. This tremor vanishes away during voluntary movement or sleep (Jankovic, 2008). The shaking occurs more in the distal part of the limb and its frequency is between 4 and 6 hertz.

Hypokinesia is associated with difficulties with planning, initiating and executing the movement, as well as performing sequential and simultaneous tasks (Jankovic, 2008). Initial indications are the problems with fine movements such as writing, sewing or getting dressed (Jankovic, 2008). The level of bradykinesia can be altered depending on the activity and the emotional state of the PD patient. For instance, in consequence of an earthquake that had happened in L'Aquila in Italy, the patients diagnosed with advanced Parkinsonism managed to escape without any assistance and more interestingly even aided other family members (Bonanni et al., 2010; Pienaar et al., 2012). This phenomenon has been described as akinesia paradoxa. Such incredibly fast improvement in movement ability happens presumably by sudden noradrenergic enhancement following the hazardous situation (Pienaar et al., 2012). Another hypothesis is the

compensatory activation of cerebellar circuitry (Goerendt et al., 2004; Pienaar et al., 2012), and activation of basal ganglia (Pienaar et al., 2012).

Rigidity is addressed as stiffness and increased resistance to limb movement (Pienaar et al., 2012). It can be a result of excessive and continuous contraction of muscles. Rigidity has two different forms: uniform or ratchet. The asymmetric rigidity of the whole body is a strong indicator for late stage PD (Reichmann, 2010).

Like rigidity, postural instability occurs in the late stages of the disease and therefore it is not a good indicator for diagnosis of PD in early stages (Reichmann, 2010). Postural instability leads to impaired balance and therefore frequent falls.

Aside from these four characteristic motor manifestations, some other motor signs have been reported such as gait and posture disturbances, freezing, mask-like face expression, neuro-ophthalmological and respiratory abnormalities (Jankovic, 2008). However, a wide range of other motor symptoms may appear in individual PD patients. On the other hand, as PD involves not only the dopaminergic motor-related brain areas, but also glutamatergic, cholinergic, serotonergic, and adrenergic neurons (Reichmann, 2010), and also existence of neural connections between BG and cortical and limbic structures (Krack et al., 2010), a high prevalence of non-motor symptoms may not be too difficult to imagine. Previous studies have shown that 84% of patients manifested cognitive decline (Jankovic, 2008). PD-related dementia, depression, apathy, anxiety, and hallucinations have also been frequently reported (Jankovic, 2008). In addition, obsessive-compulsive and impulsive behaviors (such as craving, compulsive foraging, hypersexuality, pathological gambling, compulsive shopping), sleep disorders and sensory abnormalities are broadly attributed to PD non-motor manifestations (Jankovic, 2008).

1.7. Treatments

Parkinson's disease cannot be cured, but medications can help to alleviate the cardinal manifestations and retrieve the balance of neurotransmitters by increasing dopamine level. There are a variety of available medications which can help to control the symptoms. However, a drug that actually cures PD is yet to be discovered. As it was discussed in the earlier sections, the biggest hurdles to developing treatments for Parkinson disease is the lack of one single accurate model which robustly resembles Parkinson Disease (Smith, 2010).

The gold standard of medical therapy for PD is Levodopa (L-Dopa). L-Dopa is a dopamine precursor and applies its therapeutic effects by increasing the dopamine levels. It was first discovered is the 1950s and has since become the most popular prescription for PD (Goetz, 2011). It is generally taken as a tablet and is often combined with other medication, such as to reduce the side effects. These additional compounds also stop the breakdown of L-Dopa before reaching the brain and therefore enhance its stability. In the brain, L-Dopa is turned into dopamine and, the consequent increase in the levels of dopamine is supposedly associated with improvement in movement abnormalities (Goetz, 2011). But as the PD progresses the efficacy of L-Dopa reduces, because of the increasing loss of dopaminergic neurons over time. As the lack of dopamine plays a crucial role in PD signs, a clever idea was using dopamine agonists as an alternative medication to treat early Parkinson's disease (Muzerengi & Clarke, 2015). Other possible medications are Monoamine oxidase-B (MAO-B) inhibitors. Similar to dopamine agonist compounds, they are also restrictively prescribed for early stages of Parkinson's disease. They block the effects of the dopamine degenerator enzyme (monoamine oxidase-B) (Muzerengi & Clarke, 2015). Compared with L-Dopa, they have much lesser effect; therefore, they are often used along with L-Dopa or dopamine agonists. Catechol-O-methyltransferase

(COMT) inhibitors are another recommended medication for PD. They can be applied for the later stages of the disease. Blocking the COMT enzyme prevents the breakdown of L-Dopa, and therefore, helps L-Dopa to last longer. Nonetheless, sometimes, in particular in late stages of PD, medications would lose their efficacy and the patient has to undergo a surgery. Two surgical approaches, which are generally used for PD, are Pallidotomy and Thalamotomy (Utter & Basso, 2008; Benabid et al., 1987; Miocinovic et al., 2013; Speelman & Bosch, 1998). In Pallidotomy, the hyperactive Globus Pallidus (GP) is taken away by surgical possess. The permanent GP removal helps in returning the balance to brain motor circuit. It eliminates rigidity and significantly reduces tremor, bradykinesia, and balance problems. In Thalamotomy, some parts of the thalamus, which are believed to be involved in passing the impaired motor commands from BG to the cortex, are removed by surgical process. Removing these parts of the thalamus, solely, alleviates the tremor and shaking problem (Duval et al., 2005). Because of the narrowed therapeutic effect on PD (just for tremor) (Duval et al., 2005), Thalamotomy is not the most recommended surgery for PD patients. In addition, it is notable that even though Thalamotomy and Pallidotomy are still applied in clinics, because of the high risk of after surgery side effects, they are the least frequent treatments for PD. However, another type of surgery which is called deep brain stimulation has been found to be much safer and has much fewer side effects. It was first developed in the 1980s and since then has become the most prevalent treatment especially for the late stage PD (Benabid et al., 1987; Benabid, 2003; Benabid & Torres, 2012; Miocinovic et al., 2013).

In addition to surgeries and chemical medications, there are some newly developed techniques which are recently discovered to treat PD. For instance, the embryonic neural stem cell transplantation into the rat striatum has been shown to alleviate motor abnormalities (Martínez-

Cerdeño et al., 2010). Although the neural transplantation technique has been approved extensively effective in rats (Martínez-Cerdeño et al., 2010; Smith et al., 2012), the human patient data are not very convincing (Steiner et al., 2008). Another novel application for treating PD is the administration of conserved dopamine neurotrophic factor (CDNF) which has been shown to protect and rescue midbrain dopamine neurons in rats (Lindholm et al., 2007).

However, as well as aforementioned therapies, several other ways have been put forward to control the PD symptoms. For instance, physiotherapy may improve balance and declines freezing (Tomlinson et al., 2013; Fietzek et al., 2014). Occupational therapy could be useful for dealing with everyday life performances (Sturkenboom et al., 2015). Speech and language therapy may aid improving dysarthria, word production and other speech problems (Herd et al., 2012; Spurgeon et al., 2015; Deane et al., 2001). Moreover, special diet advice (Renoudet et al., 2012; Ulamek-Koziol et al., 2013) and changes in lifestyle, such as ongoing aerobic exercises (Bergen et al., 2002; Carvalho et al., 2015; Duchesne et al., 2015), could also be recommended, in particular, for the early stage Parkinson's disease.

1.7.1. Deep brain stimulation

Deep brain stimulation (DBS) is defined as a therapeutic application of electrical stimulation through an implanted electrode into a certain brain target. It has been mostly used as an essential therapy for Parkinson disease, tremor, and dystonia (Ashkan et al., 2013). It has also been utilized for the treatment of an extended variety of disorders including depression, obsessive-compulsive disorder, Tourette syndrome, epilepsy, addiction, obesity, pain, vegetative state, and dementia (Miocinovic et al., 2013). One remarkable feature of DBS is the reversibility of its effects, which makes DBS a more reliable treatment compared to the therapies such as

Thalamotomy with the permanent lesion and probable severe side effects (Benabid, 2003; Ashkan et al., 2013; Miocinovic et al., 2013).

DBS is applied by stereotactically implanting an electrode into a target area in the brain. The electrical current is provided by a pacemaker which is placed on the chest area beneath the collar bone and is connected to the electrode via subcutaneous wires. Recently, the wireless pulse generators have been developed. Through the pulse generator, high frequency (100-250 Hz) continuous pulse trains at a set amplitude and pulse width are applied to the target area. The traditional pulse generators are voltage controlled which means that the stimulation intensity is set in volts, and therefore, the applied current is dependent on electrode impedance. The resistance of the electrode, however, can change over time, and therefore, the delivered current may not remain constant. Accordingly, current-controlled pulse generators have been developed which give permanently constant stimulation (Miocinovic et al., 2006; Miocinovic et al., 2013; Preda et al., 2016).

The name "Deep Brain Stimulation" comes from the location of the possible targets, which are in rather deep areas of the brain. An implicit criterion of applied DBS for PD is the high frequency of the pulses. Even though in primate studies there are reports that frequency of 80 Hz is effective in reducing the PD symptoms (Baker et al., 2011), in human patients the most effective frequencies are believed to be higher, more than 130 Hz (Moro et al., 2002; Herrington et al., 2016). Interestingly, it has been illustrated that low frequencies (5-10 Hz) have declining effect, and stimulation at 30-100 Hz does not have any significant impact on PD clinical signs (Timmermann et al., 2004; Herrington et al., 2016). Nevertheless, effects of DBS are indeed individual and target dependent. Although the exact rationale behind such varieties in the effective frequency is still unknown, there are several investigations certifying these diversities

(Herrington et al., 2016; Birdno & Grill, 2009). For instance, PPN stimulation does not necessarily need high frequencies, and the clinical effect tends to be seen in empirically lower (25 Hz) frequencies (Herrington et al., 2016; Rauch et al., 2010; Saryyeva et al., 2011). Moreover, some low frequencies have been reported to be peculiarly effective in individual patients (Herrington et al., 2016). It is remarkable to note that the effective low frequencies have been solely reported for the cases with dystonia and dyskinesia, but not for rigidity and tremor (Herrington et al., 2016). The question which can be raised here is that why high frequency is more effective compared to low frequency. The answer can be found based on the hypothesis which suggests that disrupted synchronization in PD and increased the ratio of beta/gamma oscillation is the neurological reason of PD motor symptoms (Li et al., 2012; Herrington et al., 2016). Computational neuroscience proposes that for decreasing the beta dominance and returning the balance to the brain oscillations, the stimulation should decrease the low-frequency pathologic oscillations or accurately align with them. Computational models suggest that only high-frequency pulses adjust the phases of the spiking neuronal subpopulations to the dominant rhythm (Herrington et al., 2016). However, it has been found that high-frequency stimulation of STN induces antidromic action potentials in cortico-subthalamic projections (Li et al., 2007; Santaniello et al., 2012). Since the slow spiking neural subpopulations are more influenced by these antidromic action potentials, they are more disrupted compared to the fast fibers (Herrington et al., 2016). Alleviation of the slow activities reduces the latency of the cortico-basal-ganglia-thalamocortical loop and ultimately suppresses the tremor (Herrington et al., 2016).

1.7.1.1. The grounding of the electrical stimulation

Therapeutical application of nervous system stimulation has a long history. Initially, Scribonius Largus, a Roman physician, applied electric fish as a treatment for headaches. Consequently, in the 10th century, Avicenna, a Persian physician, prescribed stimulation with electric fish as a remedy for muscles rigidity. The investigations were left unheeded over years, although in the meantime, electrical stimulation was applied for a large variety of diseases in human patients including hemorrhoids, gout, and epilepsy (Gionfriddo et al., 2013). However, before the 19th century, the field was suffering from the lack of robust experiments. In 1809, Rolando, in a landmark study, pioneered electrical stimulation of the animal cortex, in order to discover the function of some particular areas of the brain (Sironi, 2011). Subsequently, in 1870 Fritsch and Hitzig, demonstrated that stimulating some specific cortical areas causes muscle contractions in dogs (Sironi, 2011). Moreover, they demonstrated that increasing the stimulation intensity magnifies the contraction response (Gionfriddo et al., 2013). A few years later, in 1874 the American physician Robert Bartholow for the first time conducted cortical stimulation in an awake human (Sironi, 2011). Ultimately, in the 20th century, the systemic and translational application of electrical stimulation started to be taken for granted. In the 1930s, the imaging techniques came into existence and developed a new era in the life science experiments. Inspired by that, in 1937, Wilder Penfield applied the electrical stimulation, combined with visualizing techniques, to localize the origin of seizures in epileptic patients (Gionfriddo et al., 2013; Sironi, 2011). Regardless of quick development of novel techniques, the prospect of a breakthrough in the translational use of the DBS took almost 10 years. Eventually, in the late 1940s, Spiegel and Wycis designed the first stereotaxic frame for human brain surgeries (Gionfriddo et al., 2013; Sironi, 2011). Indeed, their invention shed light on the further DBS investigations (Krack et al.,

2010). By adding more valuable features to it, Lars Leksell completed the design and made it in a form which is currently used (Miocinovic et al., 2013; Gionfriddo et al., 2013). Consequently, a new era in the DBS research began. Intracellular recording of mammalian -and following that on human- brains was pioneered by Albe-Fessard. Moreover, her leading-edge investigation for the first time revealed that stimulation with a frequency of 100-200 Hz mitigated tremor in PD patients. Surprisingly, in the late 1960s, with Levodopa discovery, the brain stimulation fell out of favor (Miocinovic et al., 2013). After almost two decades of silence in DBS studies (due to the revolutionary effect of L-dopa discovery), in 1987, Benabid et al. paved the way for therapeutic use of brain stimulation for PD patients. The translational application of DBS was revitalized again, and by the late 1980s, DBS emerged as a possible treatment for PD. Undoubtedly, the current clinical application of DBS for PD patients is indebted to Benabid state-of-the-art investigation on the effects of thalamic stimulation in PD. Following that, different targets for DBS were introduced. For instance, in 1998 subthalamic nucleus (Miocinovic et al., 2013), and in 2000 the internal part of golobus pallidus (Coubes et al., 2000; Miocinovic et al., 2013) were proposed as possible targets. Subsequently, in 2003, the first long-term follow-up study of thalamic-DBS ended up with almost 50 percent improvement in PD motor symptoms which maintained for more than six years (Miocinovic et al., 2013). Following that, in 2006 the first randomized comparison study approved that STN-DBS has more profound effects compared to L-dopa medication therapy (Deuschl, 2006; Miocinovic et al., 2013). The trend of the DBS studies is still going on, mainly to add insights to the underlying mechanism of DBS.

1.7.1.2. Targets for DBS

Most targets of DBS are located in the deeper regions rather than cortical areas. Localization of the electrode tip is done by MRI, although the precise location is not feasible because of the MRI artifacts (Benabid, 2003). The target discovery has been a result of chance and serendipity, and the most efficient target is yet to be found (Benabid & Torres, 2012). The initial targets were selected based on the prior lesion surgical therapies such as Thalamotomy and Pallidotomy. Due to these clinical observations, the ventral intermediate (VIM) nucleus of thalamus became the first DBS target. VIM thalamus stimulation was reported to be more effective for tremor rather than akinesia and rigidity (Benabid & Torres, 2012). According to the same philosophy, GPi was also suggested as a potential target (Coubes et al., 2000; Miocinovic et al., 2013). GPi-DBS is generally used for dystonia (Benabid & Torres, 2012). The structures which were anatomically adjacent to these areas, such as substantia nigra, were other candidates. However, the results of SNr-DBS are controversial. Although, it was reported that SNr stimulation does not improve parkinsonian symptoms (Benabid, 2003), recent studies have found it effective in improving forelimb akinesia in hemiparkinsonian rats (Sutton et al., 2013). PPN-DBS has been reported to improve gait disturbance and therefore has been suggested as a possible target for DBS (Alam et al., 2012; Benabid & Torres, 2012). Moreover, studies suggest that stimulation of posterior hypothalamus (Young et al., 2009) and center median–arafascicular complex of the thalamus (Jouve et al., 2010) have antiparkinsonian effects. In addition, evidence suggests that the zona incerta (ZI), which is located above the STN or fiber bundles (Forel fields) could also be significant targets for PD (Benabid, 2003). Most authors believe that the subthalamic nucleus is the most clinically efficient target for Parkinsonism. The effects of STN-DBS are immediate, reversible and efficient for reducing dyskinesia (Benabid & Torres, 2012). The optimal

stimulation area inside the STN is thought to be the antero-latero-upper part of STN which in non-human primates is the somatomotor area (Benabid, 2003). The dorsal part of the STN which presumably contains movement-related cells in human (Theodosopoulos et al., 2003), has also been suggested as an ideal target for robust motor improvement and a low rate of morbidity (Starr et al., 2002; Theodosopoulos et al., 2003; Miocinovic et al., 2013).

1.7.1.3. Mechanism of action of DBS

Despite the popularity of DBS research in the recent decades, no explanation about its underlying mechanism has yet not gained general acceptance. Many mechanisms have been proposed and elaborated, but a commonly accepted argument is still missing. The initial studies suggested that DBS acts as a reversible lesion (Benabid, 2003; Miocinovic et al., 2013; Herrington et al., 2016). This hypothesis was proposed based on the equivalent effects of DBS and lesion in the same area (Benabid, 2003; Miocinovic et al., 2013; Herrington et al., 2016). For instance, GPi-DBS and chemical inhibition of the STN or GPi showed the therapeutic effects similar to the Pallidotomy (removal the whole or some parts of pallidus by surgery) for the treatment of PD (Herrington et al., 2016). The initial rationale of the similar cardinal effect of the lesioning and DBS was simple. It was clear by that time that coded messages are transmitted via basal ganglia to the cortex and other output areas. It was proposed that in the parkinsonian brain, these messages are distorted and therefore cannot deliver the precise information through the network. It is likely that both lesioning and DBS prohibit the incorrect meaningless message, and no message is conveyed to the correspondent areas, as Benabid stated: “No message is better than the wrong message! ” (Benabid, 2003).

The lesion hypothesis is also perfectly compatible with the classic model of thalamo-cortico-basal ganglia network, in which direct and indirect pathways are defined (Benabid, 2003; Chang et al., 2008; Krack et al., 2010; Kocabicak et al., 2012; Herrington et al., 2016; Miocinovic et al., 2013). The classic model was elaborately discussed in the earlier sections, but to reconcile it with the lesion theory, here briefly reviews the anatomy of this model again. In the direct pathway, the striatal medium spiny neurons (MSNs) express D1-receptor, and the activation of this pathway facilitates movement. The activation of the indirect pathway, on the other hand, inhibits movement, due to the activation of D2-receptor (Kreitzer & Malenka, 2008; Utter & Basso, 2008). Since D1-receptor activation enhances the neural firing, the dopaminergic input from the SNc to the striatum increases activity in the direct pathway. On the other hand, the same DA efflux decreases activity in the indirect pathway, via D2-receptor of striatal MSNs (Kreitzer & Malenka, 2008; Utter & Basso, 2008). In this simplified model, Parkinson's disease was characterized as a hypokinetic motor abnormality (Herrington et al., 2016), because of the hyperactivation of the indirect pathway in consequence of dopamine absence (Kreitzer & Malenka, 2008; Utter & Basso, 2008). The lesion theory for DBS function hypothesizes that the DBS-induced lesion in the STN decreases basal ganglia activity, and suppresses the hyperactive mode. Several studies revealed that DBS applies an utter suppression of neuronal firing in the GPi (Dostrovsky et al., 2000) and STN (Welter et al., 2004; Benabid, 2003), which supports the hypothesis of neural silencing (Benabid, 2003). Different mechanisms are proposed to be involved in this inhibitory effect, including: sustained depolarization of neural membranes which can cause suppression of the initiation or propagation of action potentials (Herrington et al., 2016), inactivation of voltage-dependent sodium channels (Benazzouz et al., 2000), and elevation of potassium currents (Herrington et al., 2016). Ledonne et al. found that STN-DBS

induces a rapid and input-specific suppression of the synaptic transmission from STN to SNc, which gives sopprt to the theory of the inhibitory effect (Ledonne et al., 2012). The theory of reversible lesion, however, is not anymore matching with the basal ganglia model due to the discovery of another pathway called hyper direct pathway and concurrent activation of striatal direct and indirect pathways (Cui et al., 2013). Moreover, recent studies have challenged the principles of this theory by demonstrating the mixed expression of D1 and D2 receptor in both direct and indirect pathways (Gallo & Javitch, 2015).

In addition to the induction of electrophysiological changes, DBS may exert its therapeutic effect through a synaptic mechanism by induction of inhibitory neurotransmitter release (Herrington et al., 2016). For instance, it has been reported that inhibitory effect of GPi-DBS is applied via GABA release from striatal and pallidal afferents into the internal part of GP (Dostrovsky et al., 2000). Moreover, clinical evidence has demonstrated that STN-DBS enhances extracellular cGMP concentration in the putamen, GPi, and SNr of PD patients, which certifies the increased glutamatergic synaptic input, and accordingly results in hyper active STN (Herrington et al., 2016). Additionally, the pivotal role of adenosine in the suppressed tremor after thalamus-DBS, has been suggested (Ledonne et al., 2012; Bekar et al., 2008). The involvement of dopamine, however, is controversial. Some studies have found an increased level of dopamine in the striatum as a result of STN-DBS (Herrington et al., 2016). In contrast, various investigations have neglected the role of dopamine in the observed clinical effects of DBS (Hilker et al., 2003; Benabid, 2003; Johnson et al., 2008). It has been shown that some symptoms of PD including dyskinesia and tremor, which does not respond to L-dopa therapy, can be alleviated by DBS. Moreover, the clinical effects of DBS and L-dopa are found to be additive, which suggests that the underlying mechanism of DBS maybe dopamine-independent (Herrington et al., 2016).

Recent studies emphesised the involvement of GABA and glutamate in the mechanism of DBS (Melon et al., 2015; Sgambato-Faure & Cenci, 2012).

Taken together, three different mechanisms might be involved in the theory of inhibitory-like effects of DBS: First, a phenomenon which is called "jamming" and is defined by the replacement of the pathologic neural activity pattern to a meaningless and therefore inefficient code. Second, suppression of firing, which can occur based on synaptic inhibition, for example as a consequence of GABA release; and third, the excitation of a sequence of synaptic steps which leads to an inhibitory effect (Benabid, 2003).

Although the initial studies tended to address the local effects of the DBS on the target area, it has been recently suggested that the stimulation can spread outside the anatomical borders of the target (Johnson et al., 2008). Therefore, DBS may apply its therapeutic effects via activation of the neighborhood area (Devergnas & Wichmann, 2011; Johnson et al., 2008; Herrington et al., 2016) such as the anterior and ventral internal capsule adjacent to the striatum (Johnson et al., 2008; Herrington et al., 2016), the fields of Forel and the Zona incerta (ZI) dorsal to the STN (Johnson et al., 2008; Herrington et al., 2016), and the cortex (Li et al., 2012; Devergnas & Wichmann, 2011; Johnson et al., 2008; Herrington et al., 2016). Thalamic fibers such as pallidothalamic and cerebellothalamic, as well as pallidonigral and nigrostriatal fiber tracts, can also be activated after STN-DBS. Direct excitation of cerebellothalamic fiber pathways, which is located posterior and ventral to the STN, is likely to be involved in the DBS-induced suppression of tremor (Johnson et al., 2008). Some studies, on the other hand, have proposed that the inhibition of the neurons adjacent to the stimulation site, and therefore the reduced output from the surrounding area is possibly implicated in the clinical outcome of the DBS (Johnson et al.,

2008; Miocinovic et al., 2013). The activation of the presynaptic inhibitory afferents by DBS was proposed as a presumable mechanism for this theory (Miocinovic et al., 2013).

In contrary to the inhibitory effect of the DBS, some studies have demonstrated that stimulation increases the overall activity of the stimulated area. As an example, thalamic-DBS has been shown to increase the firing rate of thalamic neurons (Miocinovic et al., 2013). Moreover, Hashimoto et al. reported that STN-DBS boosts the neural activity of the downstream nuclei - internal and external parts of GP- and therefore aggregates the output activity (Hashimoto et al., 2003; Miocinovic et al., 2013). Bases on these challenging investigations a controversy was raised regarding the DBS underlying mechanism. Inhibition of STN neurons (Welter et al., 2004; Benabid, 2003) on one hand, and the enhanced firing of the output neurons on the other hand (Hashimoto et al., 2003; Miocinovic et al., 2013), encounters us with a scientific paradox, for which a definitive answer continues to be sought (Miocinovic et al., 2013). Some recent studies have suggested that the different sequence of activation of neural segments is likely to be implicated in this controversy. DBS suppresses cell bodies via activation of the inhibitory synaptic terminals. On the other hand, it directly activates axons. Depending on the distance from the electrode, axon or cell body is affected, which can alternatively lead to the activation or inhibition of the influenced neuronal population. It has been shown thatt the stimulation initiates in axons rather than cell bodies. This order of initiation consequently leads to the regularization of neuronal activity. The replacement of the irregular pathological activity with the organized and regular pattern has been put forward as a possible mechanism of action of DBS (Miocinovic et al., 2013). In addition, in the cortex, DBS reduces ongoing non-stationary features by regularizing the discharge patterns and increases the modulation of the spike. It has been reported that the reason for the enhanced activity of the cortex is the reinforcement mechanisms

which happen following the overlap of feedback antidromic and feed-forward orthodromic responses within the BG-thalamocortical network (Santaniello et al., 2012).

Nonetheless, studies have revealed that high-frequency stimulation does not dramatically change the overall scale of neural firing. Instead, the pattern of firing is considerably altered (Hashimoto et al., 2003; Benabid, 2003) and synchronized with the stimulation. The altered firing pattern is composed of silent periods between bursts of firing, which occur at the time of stimulation (Benabid, 2003). It has been proposed that the specific inactivation of Ca^{2+}- and Na^{+}-voltage-dependent channels, which normally modulate STN neuronal activity, could be responsible for such modified patterns (Benabid, 2003; Surmeier & Bevan, 2003). This interpretation can also explain the inhibitory function of DBS which matches the lesion theory for DBS underlying mechanism. DBS-induced spikes seem to break the oscillatory activity by filling the gaps between the bursts. Therefore, the suppression of the newly generated oscillations leads to alleviation of tremor (Benabid, 2003).

It is remarkable to note that Parkinsonism is also characterized by the development of rhythmic, oscillatory activities within the basal ganglia loop, particularly in the beta (15 to 30 Hz) band (Jenkinson & Brown, 2011; McConnell et al., 2012; Schwab et al., 2013; Detorakis et al., 2015; Wilson, 2014). Oscillations are defined as rhythmic or repetitive neural activity in the brain, and are believed to be substantially conserved during the evolution (Buzsáki et al., 2013; Buzsáki & Watson, 2012; Herrington et al., 2016). The oscillations are pathologically changed in the parkinsonian state (Wilson, 2014), and this alteration persists during the attempted movement (Herrington et al., 2016). These pathologic altered oscillations are dominant in the thalamo-cortico-basal ganglia circuit, and also cerebellum of the parkinsonian brain, although in the normal condition, beta band oscillations can be eminently observed in the sensorimotor cortex

and associated regions of the thalamus, basal ganglia and cerebellum (Takahashi et al., 2011; Herrington et al., 2016). These low-frequency oscillations are profound during the rest or tonic contraction of muscles. However, once the movement starts they are ceased and replaced by high-frequency oscillations (HFO) including gamma (30-100 Hz) or other higher (100-500 Hz) bands (Herrington et al., 2016). Taken together, in a parkinsonian brain, the prominence, coherence and spread of beta oscillations are increased and these low oscillations are not restricted to the rest state of the muscles (Herrington et al., 2016). The dominant beta band appears not only in the STN and GPi (Herrington et al., 2016) but also partially in the motor cortex (de Hemptinne et al., 2015; Herrington et al., 2016). Additionally, in the parkinsonian state, the phase-amplitude coupling (PAC) of beta phase to broadband gamma amplitude is elevated in the cortex, STN and GPi (Shimamoto et al., 2013; de Hemptinne et al., 2015), which is the consequence of the synchronization of the STN neural firing to the phase of network oscillations (Herrington et al., 2016). The STN unit activity is synchronized with the local field potentials of the primary motor cortex (M1) in the gamma (50-200 Hz), theta, alpha, or beta (4-30 Hz) bands. It has been suggested that gamma activity in the primary motor cortex is modulated by the phase of beta oscillation, and therefore, precedes STN spiking (Shimamoto et al., 2013). Although M1 drives the STN unit activity in other motor disorders such as bradykinesia and rigidity, the phase-amplitude coupling between M1 and STN is merely seen in Parkinson's disease (Herrington et al., 2016). It has been demonstrated that the pharmacological blockade or lesion of the connection between STN and cortex or GPe mitigates the pathologic low-frequency oscillations (Wilson, 2014; Tachibana et al., 2011; Herrington et al., 2016). Similarly, treatment with L-dopa has been shown to attenuate the beta-band oscillations in the STN and GPi (Herrington et al., 2016; Chang et al., 2008), and robustly reduces the phase-

amplitude coupling in motor cortex (Herrington et al., 2016). The stimulation of STN seems to exert its therapeutic effect by modifying the low-frequency oscillations of the pallidal and thalamic neural populations to the high-frequency bands (Yang et al., 2014) and reducing oscillatory activity in basal ganglia (Chang et al., 2008), particularly in Gpi (Rosin et al., 2011). STN-DBS also reversibly suppresses the elevated phase-amplitude coupling that accordingly abates the PD motor manifestation (de Hemptinne et al., 2015).

In summary, although the initial studies suggested that DBS prevents the meaningless information to be conveyed in the BG circuit, recent studies believe that DBS seems to trigger a sequence of excitatory and inhibitory effects as a result of increased activity of the stimulated target and the neighboring area (Benabid, 2003). As a consequence, the bursting hyperactive basal ganglia-thalamocortical network is modulated, the neuronal patterns are regularized, and the pathologic oscillations are the desynchronized. The distorted messages are not conveyed through and he sensorimotor information processing is improved which ultimately leads to alleviation of clinical symptoms (Miocinovic et al., 2013). This theory paved the way to understand the mechanisms of DBS, but we have to take in mind that more than one mechanism is likely to be responsible for the therapeutic effects (Benabid, 2003; Miocinovic et al., 2013).

1.7.1.4. DBS Programming

The first step towards efficient DBS is selecting appropriate therapeutic stimulation parameter settings (Miocinovic et al., 2013). The DBS parameters are generally selected based on the observable behavioral responses to the stimuli due to the available guidelines (McIntyre et al., 2014). Stimulation parameters such as electrode contact, stimulus amplitude, pulse width, and

frequency have traditionally been set by a process of trial and error (Miocinovic et al., 2013). "Monopolar review" is a classic guideline for selecting DBS appropriate therapeutic settings. In this method, the stimulation starts at low amplitudes, then the intensity gradually increases until either a therapeutic or an adverse effect is observed. Each electrode contact is individually stimulated as a cathode, and the process is separately repeated for every contact. The contact with the largest "therapeutic window" or stimulus amplitude range between beneficial effect and side effect is chosen for further selection of the stimulation parameters (McIntyre et al., 2014). However, evaluating every single combination of individual stimulation parameter is clinically infeasible. Therefore, the therapeutic efficacy of DBS strongly depends on the electrode location accuracy and the expertise in the process of stimulation parameter selection (McIntyre et al., 2014). Both classic and intensive computational DBS programming techniques aim to select the most appropriate stimulation parameters with the maximum therapeutic benefit and the least side effects (Miocinovic et al., 2013). Modern computational methods have been reported to be more efficient than traditional manuals for selecting the parameters (McIntyre et al., 2014; Frankemolle et al., 2010). Three main factors including the prediction of current transmission in the brain tissue, electrode location, and the precise stimulation target are commonly used to determine optimal stimulation parameters (Miocinovic et al., 2013). The electrode location can be determined using advanced imaging systems, or postoperative computed tomography (Miocinovic et al., 2013). Driving the current flow to the desired direction can be yielded by selecting bipolar or monopolar stimulation and using various combinations of active contacts. In particular, when the therapeutic target sites are close to those causing adverse effects, guiding the current to a special direction, plays a pivotal role in a successful DBS (Miocinovic et al., 2013). In monopolar stimulation a wide electric field is achieved, because of the orientation of the

negative pole, or cathode (active contact) and the positive pole, or anode (the IPG case as a return electrode). Therefore, the stimulation spreads almost equally in all directions. In bipolar stimulation, however, a narrower area is stimulated, since another electrode contact is set as the anode and the spread of current has been reduced (Miocinovic et al., 2013). Stimulation targets can be selected from prior clinical experiences, and then computational models can estimate DBS effect on the adjacent area by measuring the electric field induced by the DBS electrode (Miocinovic et al., 2013). Accordingly, the stimulation parameters with the most activation of the target area and least spread of stimulation are selected based on the above-mentioned predictions (Miocinovic et al., 2013). For instance, McIntyre et al. designed a patient-specific DBS computer model to assist in the clinical programming process. Their model uses the integration of imaging data from surgical targeting and the quantitative prediction of volume of the tissue, as a function of the stimulation parameter settings (McIntyre et al., 2014). The results are then embedded into a 3D graphical user interface which visualizes DBS in each patient (McIntyre et al., 2014).

1.7.1.5. Development of different DBS models

Deep brain stimulation programming, regardless of its type (either traditional or computational) is based on a subjective evaluation of clinical benefits (Miocinovic et al., 2013). Closed-loop DBS is a more objective approach and is defined as an active adjustment of stimulation parameters based on patient's clinical response such as kinematics of movement, neural firing, or neurotransmitter levels in a target nucleus (Miocinovic et al., 2013). The Electrophysiological signals from the regularized activity of neural populations surrounding the recording electrodes summate into a local field potential (LFP) and can be analyzed as time series data. In a closed-loop control system, these data are transformed into the frequency domain and the power is

monitored in a specific frequency band. When that power exceeds a certain threshold, the stimulation is triggered (McIntyre et al., 2014). Given that PD has various symptoms with possibly different pathological signs, logic dictates that different PD manifestations may respond to different stimulation settings. In addition, various symptoms could be influenced by stimulation of distinct regions within the target area (Miocinovic et al., 2013). Due to these shortcomings, and side effects or ineffectiveness of DBS in some patients, different patterns, and models of stimulation, were introduced and increased the complexity of stimulation systems. As an example, the idea of pulse interleaving was put forward. This model has provided the possibility of rapid switching between two sets of parameters and electrode contacts. This approach is substantially advantageous, especially in cases where stimulation of one contact cannot alleviate all symptoms while simultaneous stimulation with similar amplitudes at multiple contacts is followed by clinical side effects (Miocinovic et al., 2013). Moreover, novel electrode designs such as segmented electrode arrays can be used to direct the current, and preferentially activate the desired region of a target area (Miocinovic et al., 2013). Another complex model for DBS is desynchronizing stimulation which can be applied in three different methods (Nowak et al., 2011). Multiple coordinated reset stimulations at different contacts within the target region is a methods which desynchronizes the stimulation and seems to prolong the therapeutic benefits of DBS (Detorakis et al., 2015; Gunduz et al., 2015; Hauptmann & Tass, 2007; McIntyre et al., 2014; Miocinovic et al., 2013; Nowak et al., 2011; Tass et al., 2012). The concept of coordinated reset stimulations was developed using computational models based on non-linear dynamics and their obtained result has been shown to be relevant for selecting appropriate locations for DBS electrodes (Hauptmann & Tass, 2007; Miocinovic et al., 2013). Nonlinear delayed feedback stimulation, and multisite coordinated delayed feedback stimulation has been proposed as

another method of desynchronizing stimulation (Nowak et al., 2011). Moreover, in the recent years different patterns for DBS have been suggested, including rapidly cycling DBS on and off (Dorval et al., 2010; Hescham et al., 2013; Hess et al., 2013; Kuncel et al., 2012), DBS with periodic pulses such as absence and presence patterns (Dorval et al., 2010; Hess et al., 2013; Brocker et al., 2013), and DBS with non-periodic pulses such as uniform, unipeak, and bimodal pulse trains (Dorval et al., 2010; Hess et al., 2013; Birdno et al., 2012; Brocker et al., 2013).

1.8. Aim of the present work and our experimental scheme

The purpose of this detailed introduction is to provide an appropriate foundation and conceptual framework to explain how deep brain stimulation influences the brain pathways and exerts its therapeutic effects in Parkinson's disease. As it was mentioned in before, many fundamental questions regarding the etiology of Parkinson's disease and underlying mechanism for therapeutic effects of DBS have remained unresolved. The efficacy of DBS in treating Parkinson's disease has been well documented (Benabid, 2003; Ashkan et al., 2013; Krack et al., 2010; Hohlefeld et al., 2012). However, the effect of different parameters of DBS on its therapeutic efficacy still needs to be further investigated. It has been demonstrated that frequency and amplitude intensity influences DBS effects in reducing motor abnormalities in a hemiparkinsonian rodent model (So et al., 2012). Studies have suggested that only high frequency stimulation results in desired effect in hemiparkinsonian rats (So et al., 2012; Herrington et al., 2016), while some other features such as polarity of the pulses has been found to be ineffective in DBS effects in hemiparkinsonian rats (So et al., 2012). The effect of other DBS parameters, like pulse regularity, however, is still a matter of intense debate. The role of synchronization and brain oscillations in the therapeutic effects of DBS has brought much

attention to the regularity issue in DBS studies. On the other hand, the pulse regularity is important from the economical point of view. Since applying random pulses in different intervals would need lesser energy consumption compared to the regular pulse DBS, this strategy (applying random pulses) would increase the pulse generator battery life. This would accordingly reduce the need for replacing the pulse generator, leading to a reduced after surgery side effect and increases the cost-benefit ratio in treatment with DBS. To test the effect of stochastic pulses, we developed our simple irregular pattern of DBS, by dropping 10, 20 and 30 percent of the pulses. We examined our stochastic pattern on a robust animal model for Parkinson's disease (6-OHDA rat model). The aim of our study was to investigate whether the random dropping of the pulses would change the DBS efficacy in a hemiparkinsonian rodent model. Since DBS has been reported to be effective in retrieving the main parkinsonian symptom, dyskinesia, we selected the most compatible behavior test in the rodents which represent dyskinesia in the human patients. Therefore, we designed our experiments to test the effects of the random dropping of pulses on the hemiparkinsonian rats performing the amphetamine-induced rotation behavior. With these experiments, we have addressed such questions: what is the effect of the random pulse-DBS on the amphetamine-induced turning behavior? Are these effects similar or different, compared to the classic regular pulse-DBS? We have also investigated the correlation between the current intensity and DBS effects on the amphetamine-induced turning behavior in hemiparkinsonian rats.

2. Chapter two: Materials and methods

2.1. Animals

A total number of 57 male Wistar rats weighing 280-290 g at the beginning of each experiment were used in these experiments. The animals were housed under standard laboratory conditions (12h light/dark cycle, and lights on at 7:00 A.M.). The animals were kept in individual standard cages for rats with food and water provided ad libitum. Each individual animal just used for one experiment and all the experiments were performed according to guidelines on animal ethics (reference number: USTR-3804R).

2.2. Drugs

The following drugs were used in this experiment:

6-Hydroxydopamine (6-OHDA; Sigma-Aldrich, Germany) was dissolved in Ascorbate Saline (normal saline containing 1% Ascorbate acid) and injected directly into the median forebrain bundle. Desipramine (3-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-N-methylpropan-1-amine; Sigma-Aldrich, Germany), as a noradrenergic neurons protector, was dissolved in distilled water and injected intra-peritoneally. Amphetamine (Sigma-Aldrich, Germany) and apomorphine (Sigma-Aldrich, Germany) were dissolved in normal saline and injected intra-peritoneally or subcutaneously, respectively.

2.3. 6-OH Dopamine Model and Electrode Implantation

The hemiparkinsonian rat model was induced by unilateral injection of 6-hydroxydopamine (6-OHDA) into median forebrain bundle (MFB). The animals were deeply anesthetized with an intra-peretoneal injection of Ketamine (100 mg/kg) and Xylazine (5 mg/kg) and then secured in the stereotaxic frame. An incision was made and the skull completely cleaned and dried. Using a scalpel blade some scratches were made on the skull to make the cement attach to the skull more tightly. Bone adhesive glue was applied on the scratched skull to provide a base for the cement. To protect noradrenergic neurons from lesion induced by 6-OHDA, Desipramine (25 mg/kg; subcutaneous (s.c.)) was administered between 30 minutes to one hour before 6-OHDA injection. A total amount of 4 µl of 6-OHDA (4 µg/µl) was delivered into the MFB via a 10µl Hamilton microsyringe attached to a 30 g needle. All the injections were performed by injector pump with the rate of 1µl/min. The control rats received saline containing 1% ascorbic acid (4 µl) instead of the toxin. The stereotaxic coordinates of the MFB relative to the Bregma were as follows: -4.4 posterior to the Bregma, 1.2-1.7 lateral to the midline and -7.8 dorsoventral from the dura (Fig.1.a, b), according to Paxinos & Watson Atlas of rat brain (Paxinos & Watson, 2005). The injector cannula was left in the injection site for 30 and 15 minutes before and after injection, respectively. The injector cannula was slowly retracted and the wound was cleaned. Chronic bipolar electrodes were implanted to the STN ipsilateral to the lesion side (AP −3.5, ML ±2.5, DV −7.3 mm) according to the stereotaxic coordinates of Paxinos & Watson Atlas of rat brain (Fig.1.a,c) (Paxinos & Watson, 2005). Two types of the electrodes were used: a classic electrode and the concentric electrode. The classic electrodes were constructed from Tungsten wire 120 µm in diameter. The Tungsten wires were connected to the head plug via female pin connector. The connection was soldered and covered with adhesive glue. Concentric electrodes

were made using glass covered Tungsten. The Tungsten is shielded with a 30G injector needle, except for the 5mm tip of it. From the exposed tip, 1 mm is unshielded. Copper wires (0.1 mm in diameter) were respectively connected cathode and anode part of the head plug to the injector needle and Tungsten wire, via silver paste. The connections were later soldered and protected by adhesive glue. After implanting the electrode to our desired target, the head plug was attached to the skull with dental cement and the attachment was secured with five stainless steel screws (Fig.1.a). After the completion of the operation, the rats received antibiotic powder around the incision. Before performing any experiment, the rats were recovered from surgery for at least two weeks.

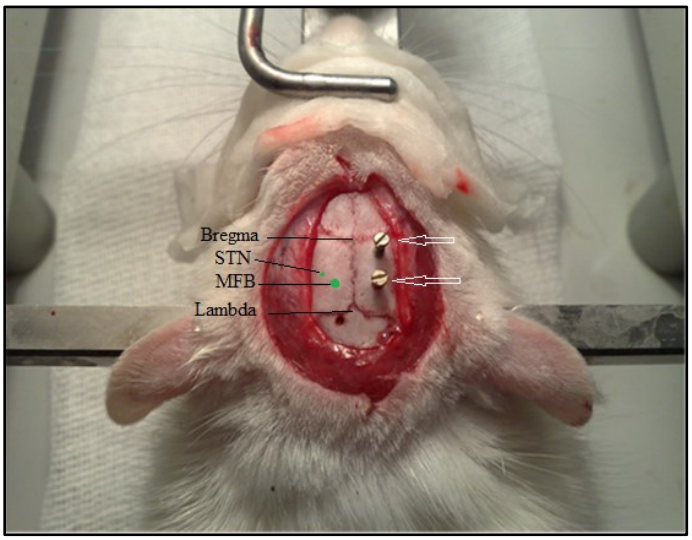


Fig.1. Location of injector needle, electrode, and screws on a rat head fixed in a stereotaxic frame. Bregma and lambda points are marked. The green dots represent the surface area of MFB (bigger dot) and STN (smaller dot). The white arrows show two of the small screws on a rat skull.

2.4. Rotation Assay

The animals were allowed to recover from surgery for three weeks. Then, apomorphine/amphetamine-induced turning behavior was tested to confirm whether the hemiparkinsonian rat model was successfully induced. Before beginning the experiment the rats were marked with two different color spots, on their back, in order to be tracked for offline video analysis. After testing several colors, the fluorescent pink and green were selected for marking the rats. Each rat was injected with apomorphine (0.05 mg/kg, s.c.) or amphetamine (2-3.5 mg/kg), put in a costume made rotation set up (40 cm diameter; Fig.3.a) and recorded over a period of 1 hour. A recording camera was mounted above the set up in a way to cover the whole experimental field. The rotation set up was illuminated with white/blue LED lights in order to enhance the color intensity of the fluorescent markers. The assay was done once per week and repeated for several continuous weeks. The first week amphetamine was used for induction of rotation, and the second week the apomorphine was administered. All the videos were analyzed offline using image analysis with Mathematica. The number of rotations was counted from the recorded video and only those rats exhibiting rotation speed (regardless of the direction of the rotation) exceeding 6 turns /min were considered successful. The dose of the amphetamine was increased over the different trials of the experiment, due to the systemic tolerance to the amphetamines, so that in order to keep the same rotation rate in all the sessions, when the rat was showing tolerance to the injected dose of amphetamine (in average every 4 sessions), the dose was boosted by 25 percent.

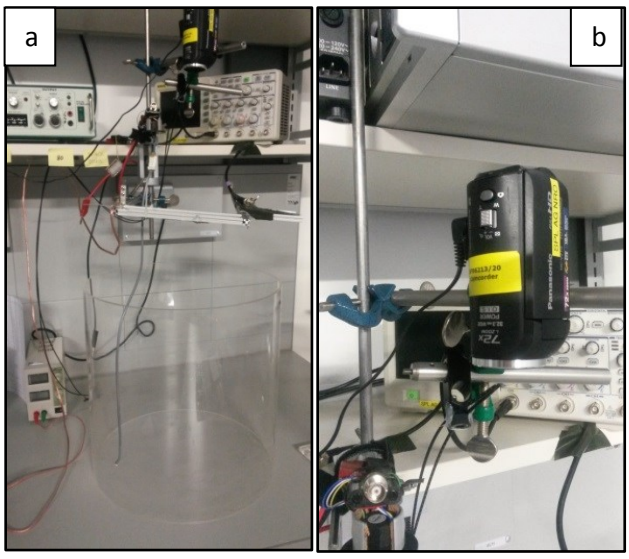


Fig.3. Rotation cylinder (a), bucket (b), and the mounted camera, on a rotation system.

2.5. Tracking and Image Analysis

Two spots in different colors (one pink and the other one green) were painted on the rats' back, before performing the rotation assay. The rats were recorded via a camera which was mounted above the set up, and offline Image analysis was done on the video files by running scripts in Mathematica (Wolfram Research, Inc.). The analysis was based on the color intensities so that two color spots gave an orientation vector per timeframe. The angle between every two vectors and subsequently the cumulative angles per minute were calculated. Since every 360 degree equals to a complete turn, the cumulative angles were used to compute the number of rotations per minute.

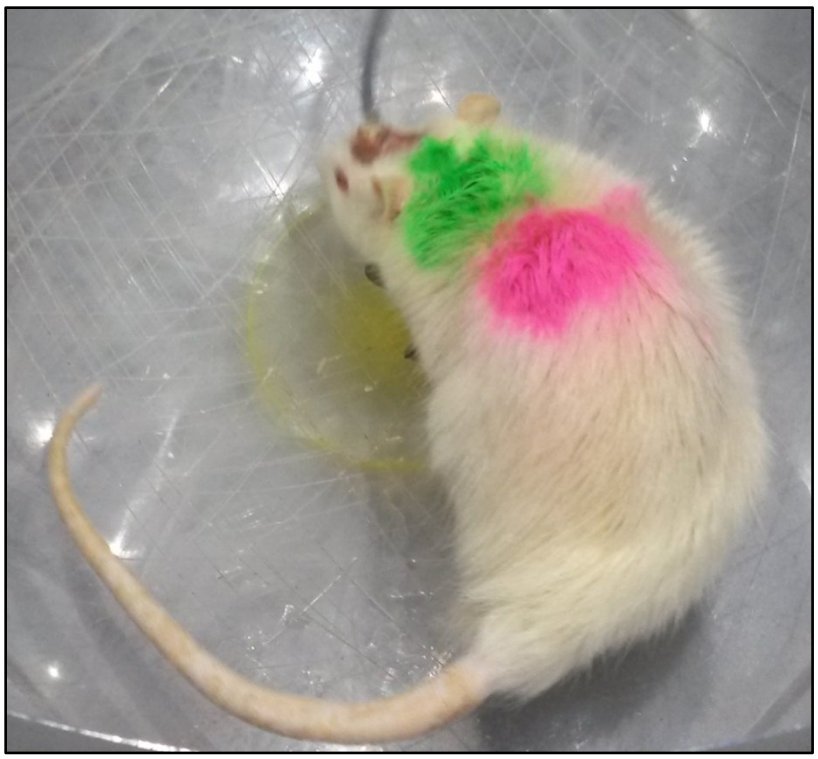


Fig.4. A rotating rat with pink and green spots on its back. The head plug is connected to the stimulator via a swivel.

2.6. Deep Brain Stimulation

High-frequency bipolar stimulation with rectangular biphasic pulses (with negative phase leading), 60 μs pulse interval, and frequency of 130 Hz, was applied via isolated stimulator (Isolated pulse stimulator model 2100; fig.5.a) to the subthalamic nucleus. The current was applied by a stimulator and delivered through a cable which was connected to the electrode cap on the head of the animal. A swivel made it possible to apply Deep brain Stimulation (DBS) while the animal is performing the behavioral test.

Stimulation intensity was separately set for each rat, in a way to be lower than the amplitude that induced side effects in the correspondent rat. To assign the threshold, the delivered amplitude was steadily increased from 0 to 450 μA until contralateral turning or muscle contractions

became apparent. Selected stimulation intensities were the values below the threshold. In the regular DBS, the normal regular pulses were delivered without any specific changes in the pattern. In order to apply irregular stochastic DBS, 10, 20, or 30 percent of the pulses were randomly dropped. The dropping was done via arbitrary waveform generator (Agilent model 33220A; fig.5.b) which can be manually set to create arbitrary pulses. The stimulation was applied when the number of rotations increased stably beyond a criterion value (more than 6 rotations/minute), which was generally the case after half an hour of amphetamine injection.

Fig.5. Isolated stimulator (a), and arbitrary pulse generator (b).

2.7. Perfusion, brain freezing, and slicing

After completion of the experiments, the animals were deeply anesthetized with ketamine and xylazine. Then, they were transcardially perfused with Phosphate Buffered Saline (PBS) and 4% Paraformaldehyde (PFA) solution. The animals were sacrificed and the brains were removed. They were kept in 25% sucrose in PBS for cryoprotection overnight and in order to prevent the

crystal formation in the brain. Ultimately, after 2 or 3 days, the brains were frozen. To freeze the brains, they were transferred to a small isopentane container. The container was kept in the nitrogen liquid till the temperature reaches -50 ºC. The brains were post-fixed in -18 for at least half an hour. Then, the brains were blocked and cut coronally into 50-µm sections. The sections were used for Nissl staining and immunohistochemistry.

2.8. Tyrosin Hydroxylase and Nissl Staining

The tyrosine hydroxylase (TH) staining was done to die the dopaminergic neurons and visualize the neuro-depigmentation in the MFB and SNc. For TH immunostaining, sections were rinsed in potassium phosphate buffered saline (KPBS) to block aldehyde group for 20 minute. Following that, the sections were kept for another 20 minute in methanol PBS and hydrogen peroxide (30%) to block the endogenous peroxidase. After two rinsing steps in KPBS, the sections were incubated in a blocking solution containing 5% BSA in PBS and 0.1-0.3% Triton, to block unspecific antibody binding sites. After two times rinsing with PBS, the sections were incubated overnight in the block solution which contains the primary antibody mouse anti-TH (1:1000). The next day, the sections were washed two times in phosphate buffer. In the next step, the sections were incubated for two hours in blocking solution at a 1:200 dilution of the secondary antibody, biotinylated anti-mouse IgG. The biotin complex in KPBS was applied for two hours before being rinsed again. The sections then were rinsed twice with PBS and once with 0.05M Tris (hydroxymethyl) aminoethane-HCI buffer. The DAB reaction was developed by incubation in 5 mg diamminobenzidine in 10 ml Tris plus 100 µl imidazole solution and 5 and µl hydrogen peroxide. After one time rinsing with Tris and twice with PBS, the sections were mounted on gelatin microscope slides and dried. The dehydration was done with application of isopropanol

for two minutes and three times of Roticlear (ROTH) for 5 min (Tronci et al., 2012). The microscope slides then were covered with Merck glass (MERCK) coverslips and let dry for two days. For each TH section, an adjacent section was stained with cresyl violet for Nissl staining. Cresyl violet staining can dye the Nissl substance (rough endoplasmic reticulum) found in the neurons. Using this method the cell bodies can be detected and therefore, the location of the electrode will be more visible.

2.9. Microscopy

Serial sections were examined under a light microscope. The neuro-depigmentation was checked under light microscopy, in order to confirm the dopamine depletion in the toxin injected hemisphere. Since TH staining dies the dopaminergic neurons, the intact hemisphere is dark, while the toxin injected hemisphere does not receive any color (due to the degeneration of dopaminergic neurons). Moreover, the location of the electrode tip was checked using Paxinos & Watson rat brain atlas. Only the rats with clearly observable depigmentation and correct electrode placement were included for the analysis. Cases where the lesion was not established (without dopamine degeneration in one hemisphere) or electrode placements were found to miss the STN target were excluded from the final statistics.

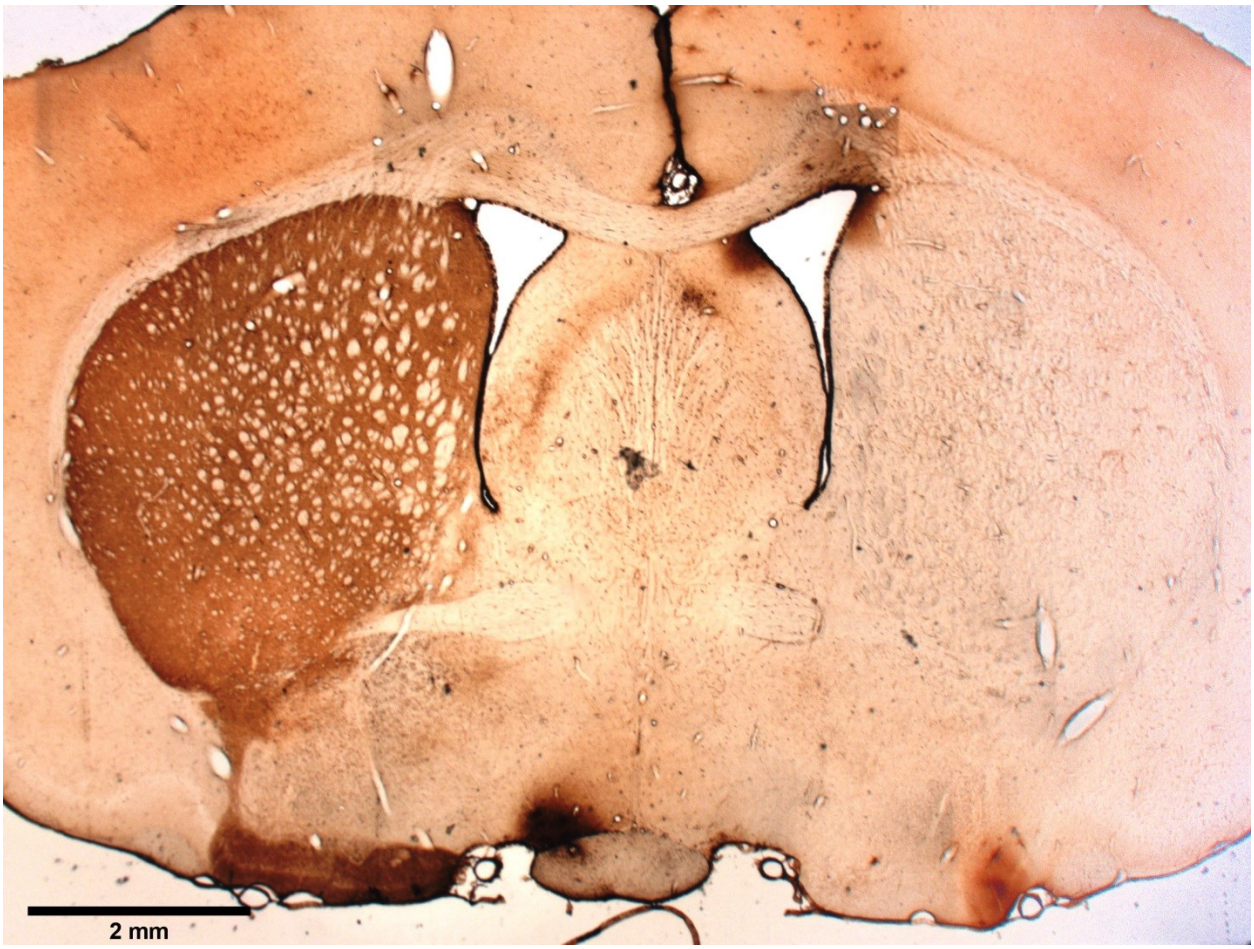


Fig.6. Histological verification of lesion induction, tyrosine hydroxylase (TH) immuno-staining.

The dopaminergic degeneration in the site of toxin injection (right side) is visible, which confirms that the model has been successfully established

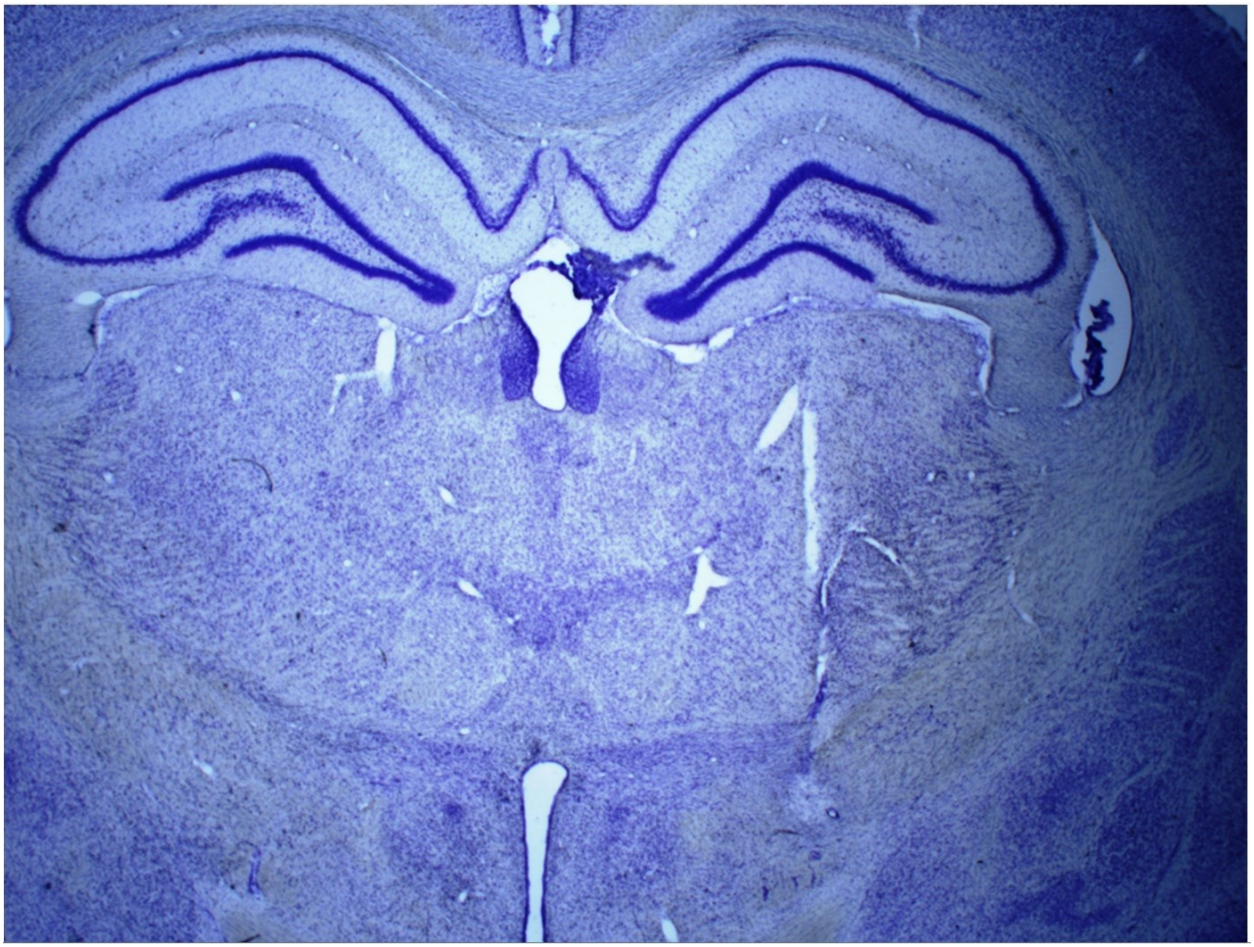


Fig.7. The electrode location, Nissl staining.

The tip of the electrode is located in the STN.

2.10. Statistics

Rotation index represents the ratio between the number of rotation during the stimulation and the average number of rotations before and after stimulation. Data were processed by commercially available software Graph Pad Prism® 5.0 and MatLab. In order to compare the number of rotations and rotation index obtained in all groups (amphetamine-induced rotation test without stimulation, apomorphine-induced rotation test without stimulation, amphetamine-induced rotation test plus classic regular stimulation, amphetamine-induced rotation test plus non-regular stimulation) non-parametric analysis of variance followed by post-hoc analysis (Dunn's multiple comparisons tests), Kolmogorow Smirnow (KS), and wilcoxon signed rank test were used, as appropriated. Furthermore, linear regression and correlation between rotation index and amplitude were also examined. In all the statistical analysis processes, *P*-values lesser than 0.05 ($P < 0.05$) were considered to be statistically significant.

3. Chapter three: Results

In experiment 1, effects of amphetamine/apomorphine (as dopamine agonists) on the rotation test, without applying any current, was investigated. This experiment was performed in order to confirm that the lesion has been successfully induced and hemiparkinsonian rat model has been established. In experiment 2, the effect of classic DBS on amphetamine-induced rotation test was examined in order to demonstrate the effects of different current intensities on the turning behavior in a hemiparkinsonian rat model. The stimulation features were as described in the method section. The stimulation was applied to the subthalamic nucleus through a bipolar, unilateral Tungsten electrode. In experiment 3, a new pattern of DBS was used to investigate the differences between classic regular pulse DBS and non-regular DBS. To this aim, a different type of current with the random dropping of 10, 20 and 30 percent of the pulses was applied.

3.1. Experiment1. Amphetamine/Apomorphine-induced turning behavior

In this experiment, animals were injected with amphetamine or apomorphine and put in the rotation test set up. Our data confirmed that both amphetamine and apomorphine cause an increase in the number of complete rotations after injection compared to saline control group (Fig. 1a,b). A total number of 13 rats were used in these experiments. Kruskal-Wallis analysis followed by Dunn's multiple comparisons test showed that the increase was significant only in the animals which had received amphetamine [$F(3,194)= 52.38$, $P<0.0001$; $F(3,179)= 11.15$, $P<0.05$]. In order to compare the timing of the effect of the amphetamine and apomorphine, the number of rotations in three different time epochs including the first ten minutes (0-10), the middle ten minutes (20-30), and the last ten minutes (40-50) after injection, was calculated. Our analysis illustrated that amphetamine results in higher number of rotations compared to

apomorphine. Moreover, the direction of the rotation behavior is different between amphetamine and apomorphine, which is ipsilateral and contralateral, respectively. The ipsilateral rotation is shown with positive numbers (Fig.1.a) while the contralateral rotation is represented by negative numbers (Fig.1.b). In addition, amphetamine's effect is increasing over the time (Fig.1.a). On the other hand, apomorphine causes the highest rotation rate in the first 10 minute after injection followed by a decrease in the effect on the rotation behavior (Fig. 1.b).

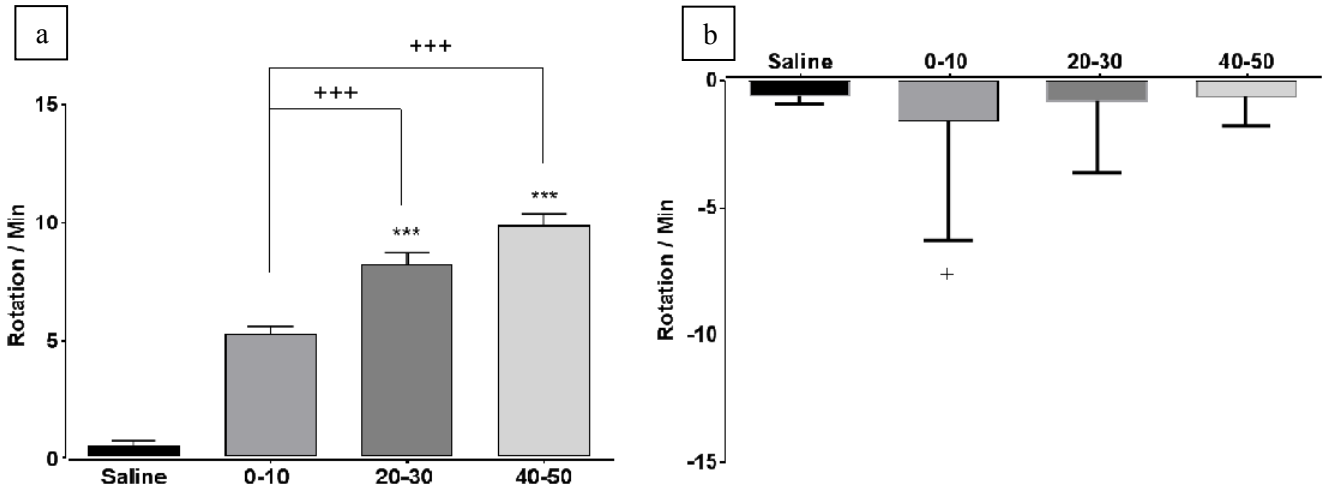


Fig.1. Effects of amphetamine and apomorphine administration on the rotation behavior.

The figure shows that both amphetamine and apomorphine cause an increase in the number of rotations after injection compared to saline control group. The time after the injection has divided to three different periods, including the first 10 minute after injection, the middle time after injection (20-30 minute after injection), and the last period after injection (40-50 minute after injection).

a. As indicated in the figure, the number of rotations after amphetamine injection is significantly higher compared to the saline control group. Moreover, the number of rotation in the first 10 minute after injection is significantly lower compared to the middle and last period. Each point shows mean ± SEM.

Number of rats = 13; Number of observations = 58

***$P < 0.001$ different from the saline control group

+++$P < 0.001$ compared to the first 10 minute after injection

b. Non-parametric ANOVA revealed that the number of turns after apomorphine injection is higher compared to the saline control group. Moreover, the number of rotation in the first 10 minute after injection is significantly higher compared to the middle period. Each point shows mean ± SEM.
Number of rats = 13; number of observations = 63
+$P < 0.05$ compared to the first 10 minute after injection

3.2. Experiment2. Effects of STN-regular pulse DBS on the amphetamine-induced rotation behavior

In these set of experiments, the animals were injected with amphetamine. After 30 minutes, when the number of rotations increased stably beyond a criterion value (more than 6 rotations/minute), different current amplitudes (0, 50, 75,100, 125,150, 200, 250, 300, and 350μA) were randomly applied were applied at inter-stimulus-intervals of four min. Each session consisted of nine stimulation blocks. The stimulation frequency and pulse width were kept at 130 Hz and 60 μsec, respectively. The pulses were delivered with a regular pattern and without any drop. To compute the rotation index the positive and negative numbers represent the ipsilateral and contralateral turning respectively. The rotation index was calculated by the ratio between the number of rotations observed during one-minute stimulation (A) and the average number of rotations one minute before (B) and after (C) stimulation, due to the following equation:

$$Rotation\ index = \frac{Mean(A)}{\frac{Mean(B) + Mean(C)}{2}}$$

Fig.3 shows an example of the rotation graph per time frame for one session of an animal. The absolute number of rotations during one session of the experiment in one rat is shown. The drop of the number of rotations after applying 250, 200, and 300 μA is marked with the red arrows.

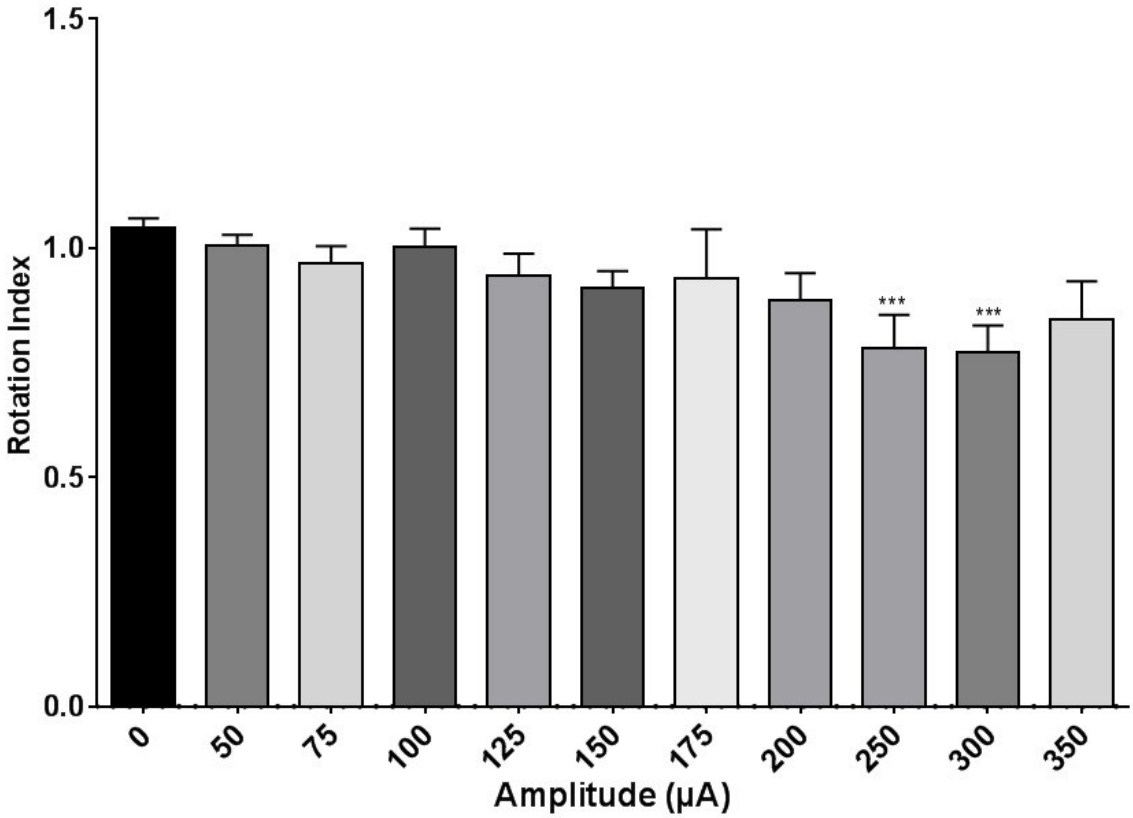
Our obtained data revealed that applying bipolar stimulation at the above-mentioned settings to the STN reduces the rotation index in an amplitude dependent manner. Kruskal-Wallis non-parametric analysis displayed significant drop of the rotation index after applying current [K (10, 382) = 28.48, *P*-value = 0.0008; Fig.4.a]. In order to check the existence of a correlation between DBS amplitude and the rotation index, Spearman non-parametric correlation test was conducted. A significant correlation was shown between amplitude and rotation index [R (16) = -0.8118, *P*-value < 0.001; Fig.4.b]. Moreover, a linear regression was calculated to predict rotation index based on amplitude. A significant regression equation was found [F (1, 14) = 19.71, *P*-value = 0.0006; Fig.4.b), with an r^2 of 0.5848, and the slope of -0.0004673 ± 0.0001052. Furthermore, in order to examine the effects of electrode location on the effectivity of STN-DBS, the slope of linear regression in the rats with correct electrode position (the tip of electrode inside STN, n=9; Fig.5.a) was compared to those with mislocated electrode (the tip of electrode outside STN, n=4; Fig.5.b). Kolmogorov-Smirnov (KS) test revealed that the probability distribution of the group with the correct electrode placement is significantly different from the probability distribution of the other group with wrong electrode location [h=1, k= 0.7778, *P*-value =0.0318; Fig.6).

Due to the observed high variance in the response to STN-DBS, between different individuals, each rat's behavioral response (quantified by rotation index) was examined individually. The Wilcoxon Signed-rank test illustrated a significant difference between the slopes of linear fit in different rats. Our results demonstrated a significant decrease from theoretical median (median = 0) to actual median (median = -0.0011) with the *P*-value of 0.0195 (Fig.7).

Fig.3. A sample rotation graph representing a number of rotations in the time bins for one session rotation test of one rat.

This graph illustrates a sample of row data analysis representing the number of rotations over the time frame. The reducing effect of DBS at the time of 30, 40, and 45 minutes (1 minute was considered 300 bins, in our analysis) can be seen. The frame rate is 5 per second.

a



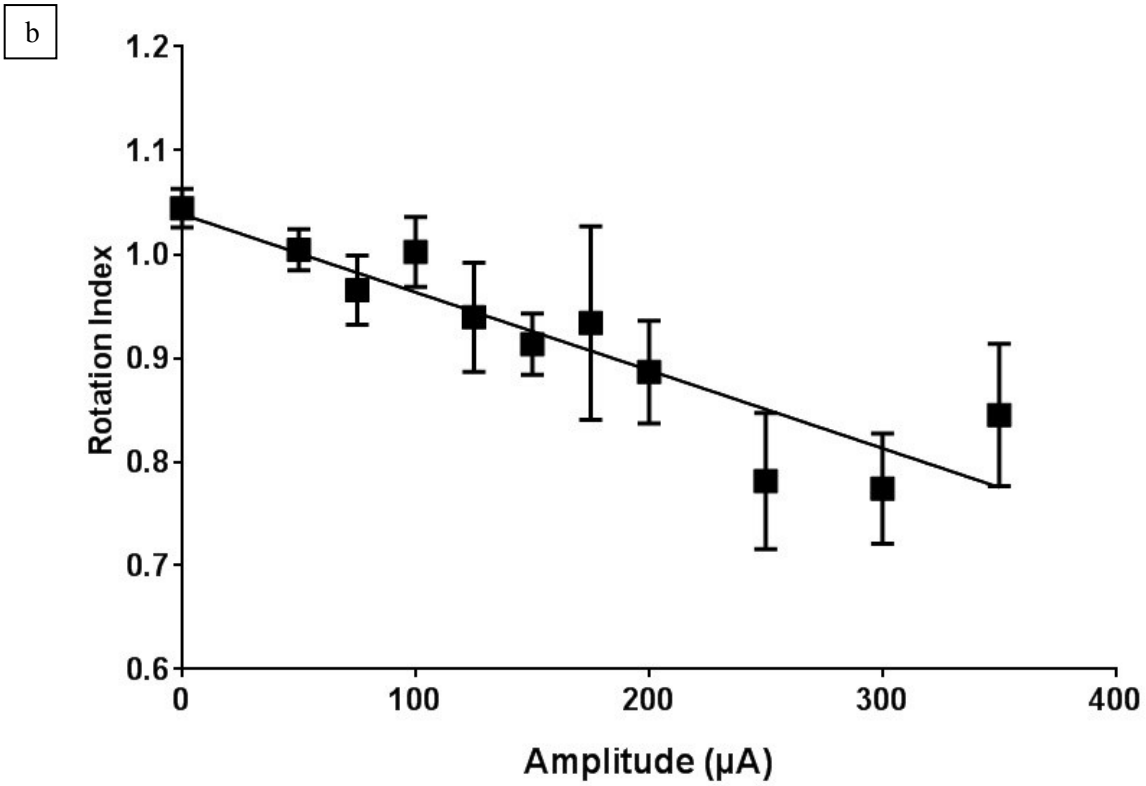


Fig.4. Effects of different current intensities in classic bipolar DBS (fixed regular pulses) with 130 Hz frequency and 60 µm pulse width, on the number of the rotations in hemiparkinsonian rats (a) and the correspondent linear fit (b).

a. Non-parametric ANOVA (Kruskal-Wallis analysis) indicated that application of 250 and 300 µA to the STN of the hemiparkinsonian rats significantly reduces the number of rotations induced by amphetamine. Each point shows mean ± SEM.

Number of rats = 9, Number of sessions = 5

***P<0. 001 compared to 0 µA

b. The Linear fit for the same experiment indicated a linear regression with the slope of -0.0004673 ± 0.0001052 between rotation index and the applied amplitude. Furthermore, our analysis revealed a Spearman correlation with r of -0.8118 between rotation index and DBS amplitude.

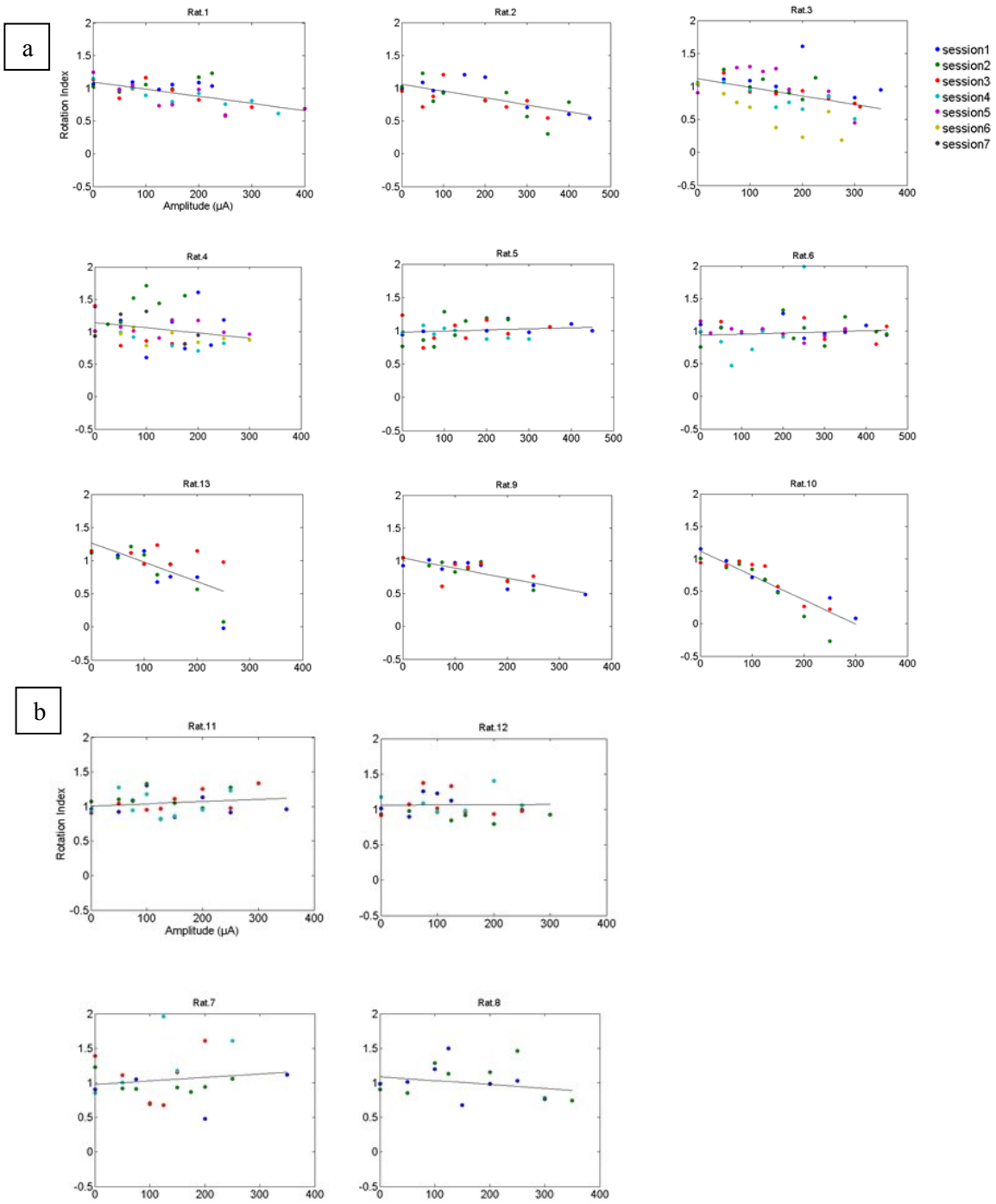


Fig.5. The correlation and the linear regression between rotation index and amplitude for the individual animals.

The different colors show various experimental sessions.

a. The graph represents the linear regression for nine rats in which the electrode was correctly implanted in the STN.

b. The linear regression graph for the animals (n=4) in which the electrode missed the desired target (STN).

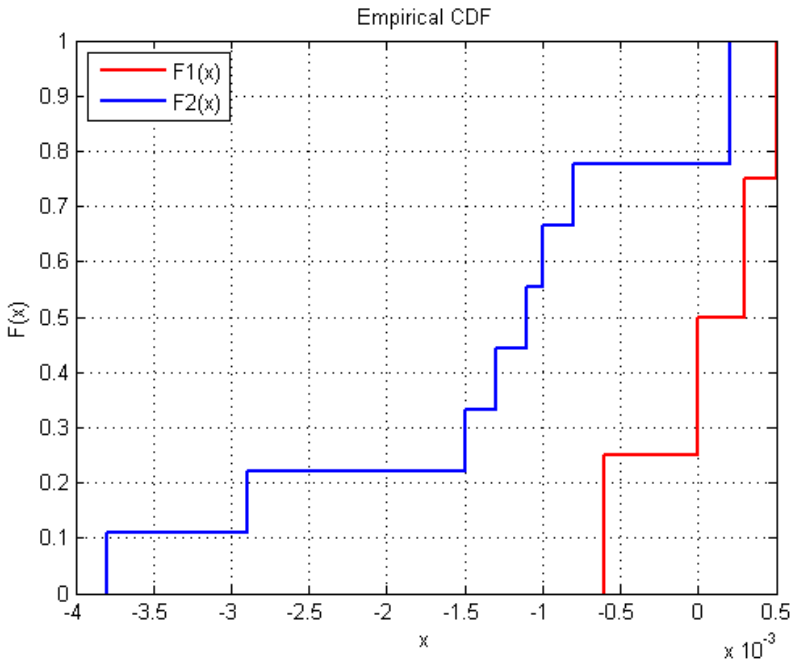


Fig.6. Kolmogorov Smirnov test of the probability distribution of the slopes of linear regression for two groups of the parkinsonian rats.

This graph demonstrates that the slopes of the linear regression of the group with the correct electrode placement (F2, blue line) do not follow the same distribution as the ones with wrong electrode position (F1, red line).

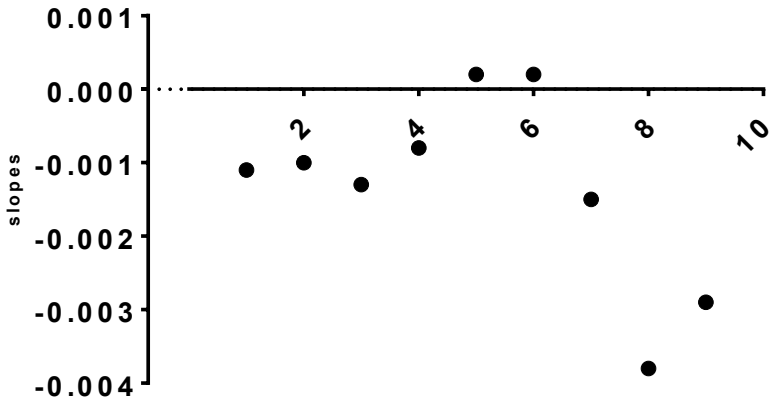


Fig.6. The Wilcoxon Signed-rank test for the slopes from the linear fit of individual rat (n=9).

The graph demonstrates a significant decrease from theoretical median (median = 0) to actual median (median = -0.0011) with the P-value of 0.0195.

3.3. Experiment3. Effects of STN-irregular pulse DBS on the amphetamine-induced rotation behavior

In these set of experiments, the animals were injected with amphetamine. After 30 minutes, when the number of rotations increased stably beyond a criterion value (more than 6 rotations/minute), four different effective current amplitudes which were chosen from the last experiments (200, 250, 300, 350 μA) were applied with a different pattern. The selected pattern was to randomly drop 10, 20, and 30 percent of the pulses. The stimulation duration was one minute with fure minute intervals. The stimulation frequency and pulse width were kept at 130 Hz and 60 μsec, respectively. Statistical analysis demonstrated that the amplitude had an effect on the STN-DBS while the pattern (changing the regularity of the pulses by dropping 10, 20, and 30 percent of the pulses) did not have any significant influence on the rotation behavior in response to the STN-DBS. (Amplitude effect: $F(3, 64) = 6.519$; Pattern effect: $F(3, 64) = 0.4686$; Interaction: $F(9, 64) = 1.011$; Fig.6). Our data reveals that there is no significant difference between STN-DBS without any drop and STN-DBS in the condition of 10, 20, and 30 percent drop of the pulses.

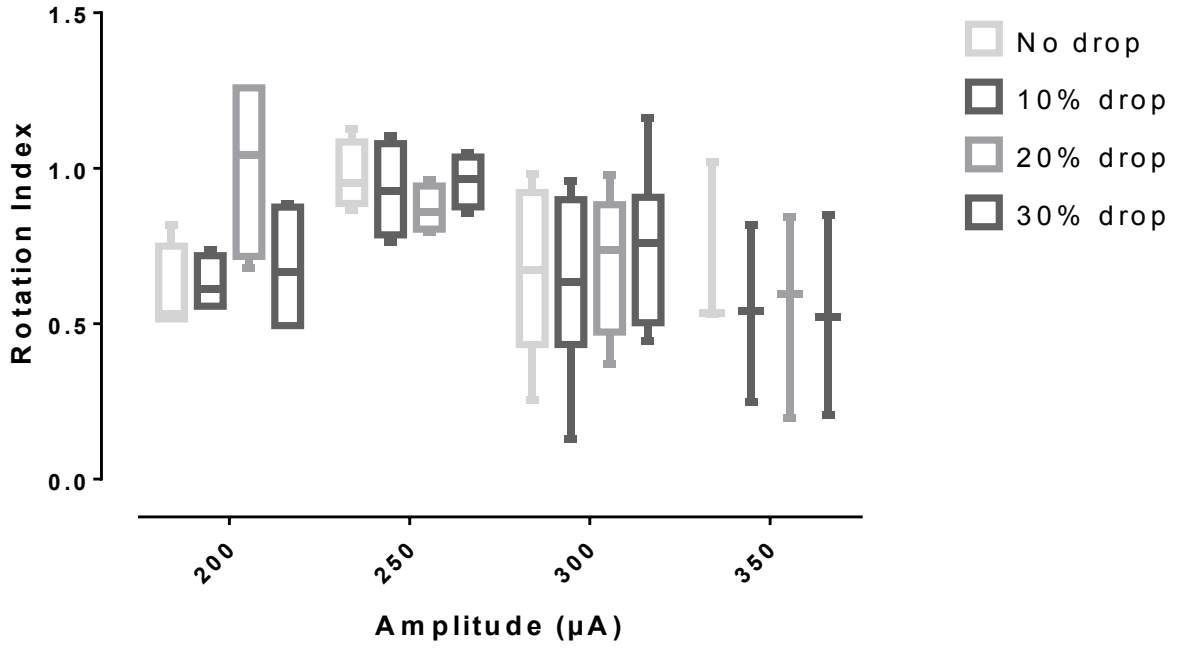


Fig.6. Effects of different current intensities with the stochastic pattern, on the number of the rotations in hemiparkinsonian rats.

Two-way ANOVA did not show any significant difference between normal DBS (without any drop) and random dropping of 10, 20, and 30 percent of the pulses. Each point shows median ± ranges (Max and Min).

Number of rats = 5; Number of sessions = 3

4. Chapter four: Discussion

In this thesis, we have examined the effects of changes in the stimulation parameters for DBS of the subthalamic nucleus in a hemiparkinsonian rodent model. The effects of the changes in the regularity of the stimulation pulses on one of the robust behavioral experiments mimicking parkinsonian symptom equivalent to dyskinesia were studied. The present study indicated that electrical stimulation of subthalamic nucleus reduced amphetamine-induced rotation behavior in hemiparkinsonian rats, in an amplitude-dependent manner. It was demonstrated that this reduction was positively correlated with the stimulation intensity. In addition, our data confirmed that systemic injection of both amphetamine and apomorphine in hemiparkinsonian rats caused ipsilateral or contralateral rotation behavior, respectively. Moreover, our experiments illustrated that changing the stimulation pattern, with dropping 10, 20, and 30 percent of the electrical pulses, does not reduce the therapeutic efficiency of STN-DBS in a hemiparkinsonian rat model.

The induction of rotation behavior as a sign of Parkinsonism in the rodent models of PD has been well documented (Jerussi & Glick, 1975; Smith et al., 2012; Pienaar et al., 2012; Torres et al., 2008). Our data confirmed the induction of turning behavior in hemiparkinsonian rat model when treated with amphetamine or apomorphine. Bilateral imbalance of the nigrostriatal dopamine receptors, ipsilateral or contralateral to the lesion, is likely to be the reason of such rotation behavior (Jerussi & Glick, 1975). Amphetamine, as a dopamine stimulant, increases the dopamine release and blocks its reuptake in the intact dopamine terminals; causing the rat to robustly rotate ipsilateral to the side of injection (Torres et al., 2008; Jerussi & Glick, 1975). Apomorphine (as a dopamine agonist), on the other hand, induces rotation in the opposite, contralateral direction, which is likely to be due to the postsynaptic development of the supersensitive dopamine receptors on striatal neurons of the lesion side (Torres et al., 2008).This supersensitivity has been proposed to be the consequence of upregulation of D2 dopamine

receptors (Narang & Wamsley, 1995; Robinet & Bardo, 2001). It has been reported that the reduction of the D3 receptors is also involved in the rotation behavior (Robinet & Bardo, 2001). Although, the initial observation had proposed that the non-dopaminergic mechanisms are only involved in the conditioned rotation, not in the drug-induced rotation (Carey, 1990), a critical involvement of the serotonergic (5-HT) and glutamatergic systems has recently been put forward (Smith et al., 2012; Smith, 2010).

Several studies have remarked on the correlation between the amounts of the nigrostriatal dopamine depletion and the rotation behavior. Henderson et al. have found that the apomorphine-induced rotation only occurs in the rats with more than 70% nigral DA loss and more than 80% striatal dopaminergic cell death (Henderson et al., 2003). Moreover, results from earlier studies have suggested that maximal lesions of the striatum and substantia nigra are fundamental for induction of rotation with low doses of apomorphine, but not with amphetamine (Hudson et al., 1993). This lends support to the part of our result in which the rotation induced by apomorphine, is not significantly higher compared to the saline control group. We hypothesize that in some animals the lesion was probably not extensive enough to cause the upregulation of DA receptors and expression of the supersensitive receptors which respond to the low doses of dopamine agonist. Interestingly, some observations have demonstrated that rats with maximal striatal lesions fail to rotate after apomorphine injection (Hudson et al., 1993), presumably because of the predominance of the substatia nigra over the striatum which might be a consequence of the possible modulatory action of the nigral dopaminergic neurons over the striatal afferents (Hudson et al., 1993). Moreover, the amount of the injected toxin, the site of injection and the spread of the toxin over multiple injection sites, also, play a pivotal role in the magnitude of the lesion (Dowd & Dunnett, 2005; Kirik et al., 1998; Truong et al., 2006).

Injection of the toxin into the median forebrain bundle leads to near-complete dopamine degeneration in the substantia nigra, ventral tegmental area, and to some extent striatum and nucleus accumbens (Yuan et al., 2005; Dowd & Dunnett, 2005). On the other hand, injection of the toxin into the striatum, or substantia nigra causes a partial (Yuan et al., 2005; Dowd & Dunnett, 2005) or more moderate (Smith et al., 2012) lesion, respectively. Therefore, the MFB model is more compatible with late-stage Parkinson's disease, which makes it a better target in order to get robust behavioral patterns similar to those which can be seen in advanced PD. Nevertheless, the moderate lesion caused by an injection into the striatum mimics the early stage PD and is hence more suitable for testing neuroprotective and neurotrophic drugs (Yuan et al., 2005). On the other hand, the drug-induced rotation in the rodent hemiparkinsonian model has been proposed to be related to the dyskinesia in the human patients (Smith et al., 2012; Tronci et al., 2012). This reason makes the rotation test a reliable behavior experiment which can reproduce robust Parkinsonian symptoms, similar to the human manifestation, in the rodent models. In agreement with the above-mentioned studies, in our model we have used the MFB animal model (as a model for advanced PD) and rotation test (which is correlated to dyskinesia in human patients).This combination is, indeed, one of the most accurate behavioral simulations in the basic PD studies for the late stage of the Parkinson's disease.

It is notable that, the major aim of our study was to investigate the efficiency of deep brain stimulation in hemiparkinsonian rats. This part of our experiments has confirmed that the application of regular electrical pulses to the subthalamic nucleus of the hemiparkinsonian rats reduced the amphetamine-induced rotation behavior. A substantial body of the literature suggests that deep brain stimulation is likely to be the most effective treatment for Parkinson's disease, especially for its advanced stage (Benabid et al., 1987; Coelho & Ferreira, 2012; Kocabicak et

al., 2012). Previous studies have addressed the inhibitory effect of STN-DBS on rotation behavior in hemiparkinsonian rats (McConnell et al., 2012; Li et al., 2012; Lehmkuhle et al., 2009; So et al., 2012; Fang et al., 2006; Fang et al., 2010) and our finding is in line with these recent observations. So et al. demonstrated that STN-DBS does not result in decreased turning rate following high doses of methamphetamine. However, when a lower dose of methamphetamine is administered to the same rat, STN-DBS reduces the rotation rate (So et al., 2012). The findings of this study support parts of our finding in which STN-DBS was unable to reduce the overrated contralateral rotation (after the injection of a high dose of amphetamine). On the other hand, in agreement with Meissner et al., STN-DBS in some cases caused rotation when the rat was not turning, most likely, as a consequence of low-dose of the amphetamine (Meissner et al., 2002). In addition, it has been found that frequency and intensity of the STN-DBS influence the rotation behavior in hemiparkinsonian rats (So et al., 2012; Moro et al., 2002; McConnell et al., 2012). Our data is in good agreement with So et al. finding which revealed that the amplitude of stimulation affects the results of STN-DBS in amphetamine-induced turning behavior, and the amplitude has to be individually set for each rat (So et al., 2012). In the contrary, we found relatively higher effective amplitude with respect to So et al., which could be because of the differences in experimental procedures and methodologies such as the type of the electrode, the number of stimulation blocks in each trial, and even the rat strain. Several recent studies have demonstrated that the material and the geometry of the electrodes have a considerable effect on the efficacy of the STN-DBS (Harnack et al., 2004; Gimsa et al., 2005; Gimsa et al., 2006). The increased impedance resulting from gliosis can also impact the effective intensity (So et al., 2012). The exact underlying mechanism of the STN-DBS, however, still remains to be elucidated. Strikingly, a great many explanations have been put forward for the

possible mechanism of action of DBS. Initial studies suggested that in the PD brain, the basal ganglia are hyperactive. The lack of the dopamine release to the striatal neurons, on one hand, leads to the decreased GABA release on the GP neurons (via the direct pathway), making them more active. On the other hand, the absence of dopamine in the basal ganglia network (via the indirect pathway) leads to an increased neural firing of the STN and striatal neurons _to compensate for the missing dopamine_ and a modified burst pattern (Chang et al., 2008). Subsequently, the SNc neurons are excited as a result of these changes in the BG network (Chang et al., 2008). The hyperactive STN reciprocally activates the GABAergic neurons of the BG output nuclei (SNc and GP), therefore inhibiting their thalamic and brain stem targets (Degos et al., 2008). Nevertheless, the mounting evidence argues that the problem in PD is not simply that the GP and SN are hyperactive, but, in fact, the pattern of GP and SN discharge is disrupted (Surmeier & Bevan, 2003). Therefore, GP and SN neurons fire synchronously in rhythmic bursts (Surmeier & Bevan, 2003). The recent studies propose that DBS not only reduces the STN neural activity but also alters the pattern of its neural activity (Herrington et al., 2016). Nevertheless, the recent studies have controversial results, regarding the effects of STN-DBS on the STN neural activity, which has been reported to be inhibitory (Tai et al., 2003; Meissner et al., 2005) or excitatory (Hashimoto et al., 2003; Xu et al., 2008). Although, the replacement of the pathologic pattern with disruptive rhythmic neural activity has been generally accepted (Hashimoto et al., 2003; Benabid, 2003). The antidromic evoked potentials with short latency have been proposed to be involved in the therapeutic effects of STN-DBS, by changing the cortical activities (Devergnas & Wichmann, 2011). On the other hand, it is strongly suspected that beta synchronization throughout the basal ganglia triggers Parkinsonian symptoms (Jenkinson & Brown, 2011; Johnson et al., 2008; McConnell et al., 2012; Schwab et al., 2013;

Dostrovsky & Lozano, 2002; Özkurt et al., 2011; Yang et al., 2014; Detorakis et al., 2015) and STN-DBS alleviates the symptoms by suppression of those pathologic beta oscillations (McConnell et al., 2012; Li et al., 2012; Anderson et al., 2015; Herrington et al., 2016). Failure of the GPe to desynchronize correlated inputs has been proposed as a possible explanation for synchronization in Parkinsonian BG (Schwab et al., 2013). Anderson et al. hypothesized that the disruptive beta oscillation is presumably the consequence of the increased information transmission between SNr and ventral anterior (VA) thalamus, and these changes are ceased by STN-DBS (Anderson et al., 2015). The suppression of the pathologic beta dominance has been suggested to be due to the regularization of the neural firing pattern in the basal ganglia (McConnell et al., 2012), or direct modification of the corticofugal neural firing probability via the cortical antidromic spikes originating from the STN (Li et al., 2012).

Despite the dominance of the classic model of DBS in clinics and basic research, recent years have witnessed the development of innovative methods for DBS. Nevertheless, some aspects of DBS such as pulse regularity or the temporal pattern of the stimulation have remained a neglected area and have not been dealt in depth. The single most striking observation to emerge from our data has addressed the impact of the pulse regularity on the effectiveness of STN-DBS in hemiparkinsonian rats. Our findings have demonstrated that irregular pattern of the stimulation pulses to the STN, which was obtained by the random dropping of 10, 20, or 30 percent of the pulses, does not influence its reductionary effect on the amphetamine-induced rotation behavior in hemiparkinsonian rats. Our data appear to correlate fairly well with Backer et al. investigation which has illustrated that the temporally irregular DBS did not worsen the motor performance compare to the regular DBS in the MPTP primate model of Parkinson (Baker et al., 2011). Moreover, Birdno et al reported that there is no significant difference in the degree

of tremor suppression, between two types of an irregular pattern of DBS (uniform and unipeack) or periodic pulse DBS, and normal regular DBD (Birdno et al., 2012). However, Brocker et al. stated that there is no significant difference between regular and irregular stimulation at a fixed frequency, and both regular and irregular DBS at 80 Hz did not differ from regular stimulation at 135 Hz, which to some extent supports our data (Brocker et al., 2013; Hess et al., 2013). However, their main finding revealed that some non-regular patterns were actually more effective in reducing bradykinesia in human patients (Brocker et al., 2013; Hess et al., 2013). Similarly, in a considerable amount of the computational, clinical and basic investigations, temporal pattern regularity has been proved to be an important factor in the therapeutic efficacy of the DBS (Brocker et al., 2013; Hess et al., 2013; Dorval et al., 2010; Summerson et al., 2015). It has been demonstrated that irregular patterns of the stimulation relieve motor symptoms more effectively than the regular DBS (Brocker et al., 2013; Hess et al., 2013; Dorval et al., 2010; Summerson et al., 2015). Previous findings have hypothesized that an irregular stimulation signal causes a more variable response to the change in firing rates of the BG neurons that were simulated, compared to the regular DBS (Summerson et al., 2015). In the Parkinsonian brain, cortical cells have more burst activity, which leads to a higher entropy. The irregular spacing between stimulus pulses is likely to reduce entropic noise in cortical neurons in consequence of increasing the diversification of response of BG neural activity (Summerson et al., 2015). The reduced entropy is believed to correlate with the attenuation or suppression of the beta band power, which is associated with improvement in motor symptoms of PD (Summerson et al., 2015). Therefore, a non-regular pattern of deep brain stimulation may suppress pathological rhythmic activity in the basal ganglia more effectively than regular stimulation (Summerson et al., 2015).

There are several possible reasons why such effects were not seen in our approach using dropped pulses. First, it is plausible that our randomly dropped pulse pattern would not fulfill the criteria to fit in the category of irregular DBS, and it could be considered as a simple semi-regular DBS. We suggest that, although dropping pulses make a new type of DBS which is different from regular standard DBS, its irregularity is not sufficient to make a considerable difference in reducing the BG entropy. Moreover, to our knowledge, it is not yet clear whether irregular DBS effects are specific to the symptoms (e.g. tremor, bradykinesia, and so an), (Hess et al., 2013). Therefore, it is probable that different results are obtained when experiments test different Parkinsonian manifestations. In addition, the DBS target in the brain, the type of the electrode and rat strain can substantially affect the final outcome. Moreover, the experimenter unintended bias can never be ruled out. Despite these pitfalls and shortcomings, we believe that our data could add insights to the ongoing research on DBS application. To the best of our knowledge, this is the first study which has addressed the effects of the random dropping of the pulses on STN-DBS efficiency. We assumed that our randomly dropped-pulse DBS would create a new stochastic pattern, which its effect on rodents' behavior has not yet been investigated. Given that the DBS underlying mechanism has still remained vague, every single investigation in this regard would be substantially valued. Importantly, our finding could be beneficial from the translational approach. Our result that dropping up to 30 percent of the pulses is equally effective as the standard DBS validates the lesser energy consumption for providing the therapeutic effect of DBS. Consequently, the decreased energy utilization would result in longer battery life and, therefore, lesser demand for changing the pulse generator battery, which in turn will influence patient comfort and could benefit the commercial aspect of DBS application.

Nonetheless, given the fact that our findings are based on a limited number of animals and DBS patterns, the final interpretation should thus be treated with the utmost caution, and there is certainly room for improvement. One downside factor regarding our methodology is the lack of electrophysiological proof of our behavioral observations. Further data collection would, therefore, be needed to fill this gap. On the other hand, the effects of different irregular patterns (such as absence and presence periodic pulses, or uniform and unipeak non-periodic pulses) as well as cycling STN-DBS on the amphetamine-induced rotation behavior, would certainly help to determine the exact involvement of regularity issue in STN-DBS efficacy. In addition, the relation between beta oscillations and motor improvement remains presently unclear. As a next step, this relationship could be examined in other experiments (e.g. open field, stepping test, corridor test, and so an) in which no chemical stimulation (e.g. agonist injection) is needed.

We hope that our research will serve as a base for future studies addressing the DBS mechanism of action and its clinical usefulness, although the design and development of a perfectly efficient DBS system with the least side effects may remain a challenge.

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Selbstständigkeitserklärung

Hiermit erkläre ich, dass ich die von mir eingereichte Dissertation zum Thema:

"A new paradigm for Deep Brain Stimulation in hemiparkinsonian rat model"

selbstständig verfasst, nicht schon als Dissertation verwendet habe und die benutzen Hilfsmittel und Quellen vollständig angegeben wurden. Weiterhin erkläre ich, dass ich weder diese, noch eine andere Arbeit zur Erlangung des akademischen Grades doctor rerum naturalium (Dr. rer. nat.) an anderen Einrichtungen eingereicht habe.

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Konferenzen

2009 32nd annual meeting of Japan Neuroscience Society (JNSS), Nagoya, Japan; Pegah Azizi, Mahsa Moaddab, Majid Hassanpour-Ezatti, Abbas Haghparast. Effects of CB1 receptor antagonist within the nucleus accumbens on the expression of morphine-induced conditioned place preference in morphine-sensitized rats.

2009 19th Iranian Congress of Physiology and Pharmacology, Tehran, Iran; Pegah Azizi, Mahsa Moaddab, Majid Hassanpour-Ezatti, Abbas Haghparast. The CB1 receptor antagonist within the nucleus accumbens reduced the acquisition of morphine-induced conditioned place preference in morphine-sensitized rats.

2010 40th neuroscience annual meeting (SfN), San Diego, USA; Pegah Azizi, Zahra Taslimi, Abbas Haghparast. CB1 receptor antagonist, AM251, reduced the firing rate of nucleus accumbens neurons in morphine-sensitized rats.

2011 8th world congress of IBRO, Florence, Italy; Pegah Azizi, Mojtaba Kermani, Abbas Haghparast, Fruit essential oil of cumin reduced the raising effect of L-Arginine on morphine-induced conditioned place preference in mice.

2012 2nd addiction congress, Tehran, Iran; Pegah Azizi, Saeid Yazdi-Ravandi, Sara Karimi, Soghra Hesam, Abbas Haghparast. Forced swim stress but not corticosterone induced the extinguished morphine-induced conditioned place preference.

2012 2nd Basic and Clinical Neuroscience Congress, Tehran, Iran; Pegah Azizi, Sara Karimi, Soghra Hesam, Saeid Yazdi, Abbas Haghparast, Effects of corticosterone and forced swim stress on morphine reward and reinstatement of extinguished conditioned place preference.

2015 11th Göttingen Meeting of the German Neuroscience Society, Göttingen, Germany; Pegah Azizi, Maria Mensch, Michael Lippert, Frank Ohl, Kentaroh Takagaki, Effects of STN-DBS on turning behavior induced by amphetamine injection in a hemiparkinsonian rat model.

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Publikation

2009 **Pegah Azizi**, Abbas Haghparast, Majid Hassanpour-Ezatti. Effects of CB1 receptor antagonist within the nucleus accumbens on the acquisition and expression of morphine-induced conditioned place preference in morphine-sensitized rats, Behavioral Brain Research, 197 (2009) 119–124

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