Stochastic Vestibular Stimulation in Dopamine Related Disorders

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The world is full of magical things patiently waiting for our wits to grow sharper!

Bertrand Russell

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ABSTRACT

Dopamine related disorders usually respond to dopaminergic drugs, but not all symptoms are equally responsive. In Parkinson's disease (PD) in particular, axial symptoms resulting in impaired gait and postural control are difficult to treat. Stochastic vestibular stimulation (SVS) has been put forward as a method to improve CNS function in dopamine related disorders, but the mechanisms of action are not well understood.

This thesis aimed to investigate the effects of SVS on neuronal brain activity and to evaluate the possible enhancing effect of SVS on motor control in PD and on cognitive functions and motor learning in Attention deficit hyperactivity disorder (ADHD).

Behavioural tests were conducted in the 6-OHDA rat model of PD using the accelerating Rotarod and the Montoya skilled reach test to evaluate the effect of SVS on motor control. The effect of SVS on brain activity was assessed using in vivo microdialysis and immunohistochemistry. We evaluated the effect of SVS on postural control and Parkinsonism in patients with PD and the effect of SVS on cognitive function in people with ADHD.

The behavioural animal studies indicate that SVS may have an enhancing effect on locomotion, but not skilled forepaw function. SVS increased GABA transmission in the ipsilesional substantia nigra (SN) and may have a rebalancing effect on dysfunctional brain activity. SVS increased c-Fos activity more than levodopa and saline in the vestibular nucleus of all animals. c-Fos expression was also higher in this region in the 6-OHDA lesioned than in shamlesioned animals, supporting the theory that SVS may have larger effects in the dopamine depleted brain. SVS increased c-Fos expression in the habenula nucleus substantially more than levodopa did. Furthermore, SVS and levodopa had similar effects on many brain regions, including the striatum, where saline had no effect. The clinical studies revealed improvement of postural control in PD during SVS. There was a trend towards reduced Parkinsonism during SVS when off levodopa. No substantial effects were found on cognitive performance in ADHD.

In PD, SVS may improve motor control by inhibiting the overactive SN, possibly through a non-dopaminergic modulatory pathway involving increased neurotransmission in the habenula nucleus. SVS could be trialled in larger studies to evaluate long-term effects on treatment resistant axial symptoms associated with PD.

Keywords

Vestibular stimulation, Microdialysis, GABA, Substantia nigra, c-Fos, Habenula nucleus

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SAMMANFATTNING PÅ SVENSKA

Tillgängliga behandlingar vid Parkinsons sjukdom (PD) är vanligen mer effektiva för rörelsesymptom i extremiteter och mindre effektiva för axiella rörelsesymptom, såsom balanssvårigheter. Vidare är icke-motoriska och neuropsykiatriska symptom vid PD mer eller mindre resistenta mot de vanligaste behandlingarna, levodopa och djup hjärnstimulering (deep brain stimulation – DBS). Levodopa, en dopaminerg behandling, kan framkalla överrörlighet, dyskinesi, och framkalla eller försämra kognitiva funktionsnedsättningar.

Galvanisk stokastisk vestibulär stimulering (SVS) med strömstyrkor nära tröskeln för aktivering av balansreaktioner, aktiverar balansnerverna genom en elektrisk ström genom de bilaterala vestibulära perifera organen. Det finns tidigare rapporter att balans kan förbättras av SVS, och även förbättrad kognitiv funktion och förbättrade autonoma kardiovaskulära funktioner vid neurodegenerativa sjukdomar. Dessutom har man funnit att stimulering av hörselsystemet med stokastiskt ljud (vitt brus) kan förbättra den kognitiva förmågan hos personer med Attention Deficit Hyperactivity Disorder (ADHD). Det övergripande syftet med denna avhandling var att utvärdera effekterna av galvanisk SVS i förhållande till levodopa i både kliniska och prekliniska studier, och att undersöka de möjliga mekanismerna bakom dessa effekter. Dessutom var vi intresserade av huruvida SVS har samma positiva resultat på kognitiva funktioner som stokastiskt ljud.

I den första studien (delarbete I) undersökte vi effekten av SVS och levodopa på lokomotion och finmotorik i en råttmodell av PD där dopaminsystemet slagits ut i ena hjärnhalvan med toxinet 6-OHDA. Vidare studerade vi effekten av SVS på frisättning av signalämnen (särskilt dopamin och GABA) i intakta och i 6-OHDA hemilesionerade råttor. Effekterna av SVS jämfördes med de akuta effekterna av en dos levodopa. Vi fann att SVS förbättrade förmågan att hålla sig kvar på en roterande stav (lokomotion) jämfört med shamSVS (icke-aktiv stimulering) i hemilesionerade råttor. Finmotorik påverkades inte av SVS. Vi visade också en ökad

frisättning av GABA i substantia nigra pars reticulata i intakta råttor och en balansering av GABA-frisättning i samma kärnor i hemilesionerade råttor. Dopaminfrisättning förändrades dock inte av SVS i några djur, vilket tyder på att effekten av SVS inte medieras av dopaminfrisättning.

I den andra studien (delarbete II) analyserade vi effekten av SVS eller levodopa i olika hjärnregioner genom att kvantifiera uttrycket av proteinprodukten av c-Fos-genen, som är en markör för ökad nervcellsaktivitet. Vi upptäckte att SVS ledde till en ökad c-Fos-aktivitet i de vestibulära kärnorna i 6-OHDA djuren jämfört med sham-lesionerade djur. Ett intressant fynd var att SVS även ökade aktiviteten i laterala habenula-kärnan, både i 6-OHDA och sham-lesionerade djur, medan levodopa- och koksaltinjektioner hade minimala effekter. Dessa resultat tyder på att SVS kan har större effekt på det vestibulära systemet vid hypodopaminerga tillstånd, samt att habenula kärnan skulle kunna vara involverad.

I den tredje studien (delarbete III) undersökte vi om SVS och levodopa kan förbättra balanssvårigheter hos patienter med PD i en randomiserad cross-over pilotstudie. SVS förbättrade den tid det tog att återfå balansen efter en påtvingad rörelse bakåt. De olika testerna antydde även en trend till minskade Parkinsonssymptom under SVS när patienten var utan samtidig dopaminerg medicin.

Vi undersökte effekterna av SVS på kognitiv förmåga hos deltagare med ADHD i den sista studien (delarbete IV). I en pilotstudie med en randomiserad cross-over design fick forskningspersoner med ADHD genomgå tre tester (Rey Auditory Verbal Learning Test, Span-board och Flower trail test), under antingen SVS eller shamSVS. Vi kunde inte påvisa några positiva effekter av SVS på arbetsminne, handmotorik eller inlärning/minne.

Sammanfattningsvis verkar SVS ha olika effekter i den intakta hjärnan i jämförelse med en hypodopaminerg hjärna. Neurokemiska djurdata indikerar att SVS kan balansera aktiviteten i de basala ganglierna. Immunohistokemiska djurdata stöder hypotesen att SVS har större effekter i en hypodopaminerg hjärna, och indikerar att den aktiverar neuroner i många hjärnregioner (bland annat striatum) i likhet med levodopa, och slutligen att habenula-kärnan kan vara involverad i dess mechanism. Klinisk data pekar på små positiva effekter på postural balans vid PD, men inte på tydligt förbättrad kognitiv förmåga vid ADHD.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Ghazaleh Samoudi, Hans Nissbrandt, Mayank B. Dutia and Filip Bergquist. Noisy galvanic vestibular stimulation promotes GABA release in the substantia nigra and improves locomotion in hemiparkinsonian rats. 2012. *PLoS ONE, vol. 7, no. 1, e29308.*

II. Ghazaleh Samoudi, Andrea Nilsson, Thomas Carlsson and Filip Bergquist. Expression of c-Fos after stochastic vestibular stimulation and Levodopa in 6-OHDA hemilesioned rats. *Manuscript*

III. Ghazaleh Samoudi, Maria Jivegård, Ajitkumar P. Mulavara and Filip Bergquist. Effects of Stochastic Vestibular Galvanic Stimulation and LDOPA on Balance and Motor Symptoms in Patients with Parkinson's Disease. 2015. *Brain Stimulation, vol. 8, no. 3, pp. 474–80*

IV. Ghazaleh Samoudi*, Daniel Eckernäs*, Göran Söderlund and Filip Bergquist. Does stochastic vestibular galvanic stimulation improve cognitive performance in ADHD? A pilot study. *Manuscript*

*) Contributed equally

CONTENT

ABBREVIATIONS

Acb	Nucleus Accumbens
ADHD	Attention Deficit Hyperactivity Disorder
BIC	Brachium Inferior Colliculus
CnF	Cuneiform nucleus
CPu	CaudoPutamen (Dorsal striatum)
DBS	Deep Brain Stimulation
DP	Dorsal Peduncular
GABA	Gamma-Amino-Butyric Acid
GP(e/i)	Globus Pallidus (external/internal segment)
ILL	Intermediate nucleus of Lateral Lemniscus
LHb	Lateral Habenula nucleus
MVePC	Medial Vestibular nucleus - Parvocellular part
PD	Parkinson's Disease
PPN	Pedunculopontine nucleus
Rt	Reticular thalamic nucleus
RVLM	Ventrolateral Medullary Region
SN(c/r)	Substantia Nigra (compacta/reticulate)
STN	Subthalamic nucleus
SVS	Stochastic Vestibular Stimulation
VM	Ventromedial thalamus
VTA	Ventral Tegmental Area
6-OHDA	6-hydroxydopamine

INTRODUCTION

A defining feature of neurodegenerative disorders is the progressive death of nerve cells in central and/or peripheral structures of the nervous system. Common to several neurodegenerative disorders are difficulties in motor control as well as various degrees of cognitive impairment. Idiopathic Parkinson's disease (PD) is one of the most common neurodegenerative disorders. The primary neuropathological characteristic feature of PD is the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc). Attention deficit hyperactivity disorder (ADHD) is not a neurodegenerative disorder, but some of the symptoms in ADHD seem to be related to the dopamine pathways. The pathophysiology of ADHD is however not fully known.

In 1958 Carlsson and colleagues [1] discovered that dopamine is a neurotransmitter in its own right and not just the precursor to adrenaline and noradrenaline. Not long after this discovery, it was established that dopaminergic cell bodies are primarily found in particular midbrain areas, namely the ventral tegmental area (VTA) and the substantia nigra (SN) [2, 3]. Since then, research around the function and mechanism of neurotransmitters has boomed, contributing to a research field yet expanding. As dopamine is involved in an array of networks within the nervous system, the abnormal function of this neurotransmitter is the ground for symptom profiles ranging from mild cognitive impairment to severe motor dysfunction.

The most noted motor difficulties in PD include bradykinesia, rest tremor and rigidity. These normally respond well to levodopa, a precursor to dopamine which restores some of the dopamine loss in the hypodopaminergic brain. Many of these motor symptoms appear to be a direct consequence of dopaminergic loss in the central nervous system [4]. Other motor difficulties, such as postural instability, balance problems, falls and freezing of gait are assumed to be partially indirect consequences of dopaminergic loss. These respond less to levodopa medication and will typically develop in later stages of the disease [5]. Long-term use of levodopa medication can trigger other symptoms as well, such as dyskinesia and weaker impulse control [6, 7]. Furthermore, non-motor difficulties can follow due to neurotransmitter deficiencies in the central and

peripheral nervous system. These include mental problems such as cognitive decline, sleep disturbances and depression, as well as autonomic problems such as constipation, postural hypotension and sexual disturbances [5, 8, 9]. These symptoms often appear years before motor symptoms do, and are a challenge to treat effectively. Mild cognitive impairment in PD for instance has a prevalence of 15-40% at the time of diagnosis [10]. Many, but not all, of the cognitive levodopa non-responsive symptoms can be categorised as executive dysfunctions. In some respects, the cognitive problems of patients with PD resemble the cognitive impairments in ADHD. ADHD can be defined as a disorder which primarily affects the executive functions such as cognition, attention and motor learning as well as self-control [11].

In the late nineteenth century, the neurologist Charcot discovered that his PD patients experienced reduced resting tremor symptoms during train journeys. He proposed that the effect was induced by vibrations and therefore created a vibrating therapy chair for these patients and reported improvements in symptoms. Not long after, a vibrating helmet followed [12]. The principles of vibration for relief of motor symptoms have been tested in recent years with varying outcomes [13, 14]. One study found some improvement of PD symptomatology, however the improvements were generated equally by the relaxing auditory stimuli applied at the same time as vibration [15]. There is consequently some support for the idea that sensory stimuli can improve some aspects of PD symptoms.

It is possible that some of the dysfunctional executive functions in PD and other dopamine related disorders are in part an effect of inadequate integration of the sensorimotor and proprioceptive feedback system [16]. Executive dysfunction has been associated with balance and gait difficulties in the healthy elderly [17] and the chances of developing dementia is three times higher in persons with gait disorders [18]. Additionally, PD patients suffering from gait and balance difficulties also perform poorly on spatial working memory tasks [19] and show increased gait difficulties during attention demanding dual-tasking [20]. Despite great progress in relieving many of the symptoms caused by dopamine degeneration, or abnormalities in dopamine transmission function, many executive dysfunctions as well as balance and gait difficulties remain hard to treat. Hence, the main aim of this thesis was to assess the function and mechanism of an alternative or add-on therapeutic intervention in relieving hard to treat symptoms in dopamine related disorders.

Pathophysiology

Parkinson's disease

Studies during the last few decades have illuminated the clinical features of this multisystem, multifactorial disorder. The age at disease onset can range between 31-85 years of age. Furthermore, a vast range of motor and non-motor symptoms have been identified [4, 8], some of which are levodopa responsive and others not [5]. Four subgroups of PD have been suggested: a young disease onset group, a rapid-disease progression group, a tremor-dominant group and a non-tremor-dominant group [21, 22].

Neuronal cell death occurs not only in the central nervous system but also in the peripheral nervous system [23]. Neurodegeneration starts before dopaminergic cell death in the SNpc, and spreads across and past different areas of the basal ganglia circuitry. Indeed, Braak and colleagues [24, 25] have argued that the pathological progression of the disease may originate from the lower brainstem, including the anterior olfactory nucleus, medulla and pontine tegmentum. This supports the notion of a preclinical stage with non-motor indicators. They propose that dopaminergic cell loss in the substantia nigra (SN) occurs somewhat mid-stage in the disease development, and thus correlates with the motor related manifestations of the disease. Significant cognitive decline comes about at the latest stages when the cortical areas are affected, although mild cognitive impairment is often part of the early stage non-motor indicators. Suggesting dopaminergic degeneration is only part of the etiology of PD, this hypothesis further acknowledges the role of other neurotransmitters in the development of PD symptoms. Altered serotonergic neurotransmission has for instance been connected to PD symptomatology [26]. Serotonin receptors modulate the release and reuptake of dopamine as well as of GABA and glutamate. The dorsal and medial raphe nuclei are the main areas that send out serotonergic transmission to the striatum [27].

Dopaminergic cell degeneration has been associated with both genetic and environmental factors [28]. What initiates neurodegeneration in the first place however remains largely unidentified. A marker for the disease that eventually leads to neuronal death and is associated with the degenerative process is the presence of Lewy bodies in the nerve cells [25]. The presynaptic nerve terminal protein α -synuclein, a key component in

Lewy bodies, is a contributor to PD pathogenesis, where dopaminergic neurons accumulate aggregates of misfolded α-synuclein [29]. α-synuclein is not confined to the cell soma of involved cells in SNpc, but has been found in various brain structures in PD patients. In many cases it has also been found in other disorders such as Alzheimer's disease (AD) and Multiple system atrophy (MSA) [23, 30].

Recent research explains the role of autophagy on the development of mitochondrial dysfunction leading to increased Lewy-bodies [31]. The autophagy-lysosome pathway (ALP) is one of the most important mechanisms behind recycling abnormal protein structures. During the process of autophagy, parts of the cytoplasm gets engulfed by a double-membrane vesicle called an autophagosome, this in turn targets the lysosome in the cells and separates cytoplasmic compartments. This way, autophagosomes repair or even eliminate protein aggregates on their transportation path from the tip of the axon toward the cell soma [32, 33]. The overexpression of α-synuclein blocks autophagosome formation and inhibits the autophagy early in the process [31]. Thus, the aggregation of misfolded α-synuclein could cause disruption of the nervous system's normal ability to remove damaged proteins. Or vice versa, damaged protein accumulation which cannot get cleared out due to e.g. oxidative stress, may increase misfolded α-synuclein aggregates within the cell.

Attention deficit hyperactivity disorder

Known as a developmental neurobehavioural condition, generally expressed during preschool years, and often persisting into adulthood, ADHD is characterized by three dominant subtypes; hyperactive and impulsive behaviour, inattentive behaviour or a combined type [34].

Although the pathology of this disorder is unclear, the cortico-striato-thalamical circuits, including the prefrontal brain regions as well as the basal ganglia, appear to be involved [35]. Some studies suggest that non-fronto-striatal circuitries such as the cerebellum and the parietal lobes also play a role in ADHD manifestation [35]. A common pathophysiological theory is that the brain dysfunction in ADHD is caused, at least in part, by abnormalities in the release and reuptake of the neurotransmitters dopamine and noradrenaline. The theory is supported by the efficacy of psychostimulants, such as methylphenidate, that facilitate dopamine release in the treatment of ADHD [36].

It is possible that the different behavioural and neuropsychological characteristics of ADHD have different genetic or environmental etiology [34]. Although ADHD symptomatology is often associated with higher dopamine reuptake, in what can be defined as a hypo-dopaminergic state, a hyper-dopamine state is also a possibility [11, 37]. A dual-pathway model has been suggested, with a diverse influence of cortical and subcortical mechanisms in the different expression of ADHD [11]. Lower noradrenaline activity and its effect on dopamine transmission has been linked to a hyper-dopamine state and the interaction of dopamine and serotonin activity to a hypo-dopamine state [37].

In a descriptive matched control study it was found that dopaminergic transmission in the brain's reward pathway is less active in participants with ADHD [38]. Other researchers looked at the morphological characteristics in several nuclei in the basal ganglia using magnetic resonance imaging (MRI) scans [39]. They found a decreased volume of the putamen in ADHD youths as compared with control youths. They further discovered that the putamen, caudate and the globus pallidus (GP) were shaped differently in the ADHD youths, a finding that was not evident in ADHD youths treated with stimulants. Overall volume in the putamen was however not increased in the group treated with stimulants. There have been quite a few reports that the overall brain size of children and adolescents with ADHD is somewhat smaller than controls [40, 41]. The findings of a normalising effect of stimulants on brain size are however inconclusive, with some findings indicating a protective effect of stimulants on brain size [42] and others indicating no effect of stimulants on brain size [41].

The role of Basal Ganglia in movement and cognition

Voluntary movement occurs when circuits within the brain receive and project signals to and from different brain structures and the premotor cortex and cerebellum. The basal ganglia, a group of nuclei situated in the midbrain and forebrain, consist principally of the striatum, GP, subthalamic nucleus (STN), SN and the ventral tegmental area (VTA). The basal ganglia acts together with the cerebellum and spinal cord via the midbrain extrapyramidal area (MEA) and superior colliculus (SC) [43], as crucial subcortical structures that shape these signals before they reach their destination [44], Fig 1. Basal ganglia neurotransmission takes place primarily via two well-balanced pathways projecting from the striatum.

These are known as the direct and indirect pathways of the basal ganglia, where the striatum and the STN are the most prominent input nuclei and the SNr and globus pallidus internal segment (GPi) are the main output nuclei. The two pathways pose competing effects on movement and to some extent cognition. Facilitation in the basal ganglia nuclei with the inhibitory and excitatory function lead to the final selection of locomotor commands [45, 46].

Basal ganglia circuits can also be seen as part of two main networks, the striato-nigral-striatal network and the thalamo-cortical-thalamic network. Dopaminergic neurons receive direct and indirect input from the limbic system by means of the striatum. The mesolimbic dopaminergic pathways (responsible for the reward system as well as depressive and aggressive behaviour) and the nigrostriatal dopaminergic pathways (responsible for control of movement and motivated behaviours) are modulated by the reciprocal striato-nigral-striatal network [47]. Within the thalamo-cortical-thalamic network on the other hand, one-directional pathways relay information to the cortex, including the prefrontal and supplementary motor areas. This network has a regulatory influence on automatic and voluntary motor execution and motor responses, reinforcing wanted behaviour and suppressing unwanted motor and behaviour output [43], and has a similar function on attention and behavioural decision making [48].

In the direct pathway, inhibitory (GABAergic) projection neurons in the striatum, known as medium-sized spiny neurons (MSNs), express dopamine receptors D1 and project to the SNr and GPi nuclei. MSN neurons that project to the GPe nucleus are part of the direct loop and express D2 receptors on their dendrites and cell bodies in the striatum [49]. Degeneration of dopamine terminals in the striatum leads to less activity in the D1-expressing MSNs of the direct pathway and increased activity of the D2-expressing MSNs of the indirect pathway. This results in an increased inactivity in the STN and an increased activity in the inhibitory output nuclei (SNr and GPi) which in turn impedes the selection and maintenance of movements and probably also thought processes [50].

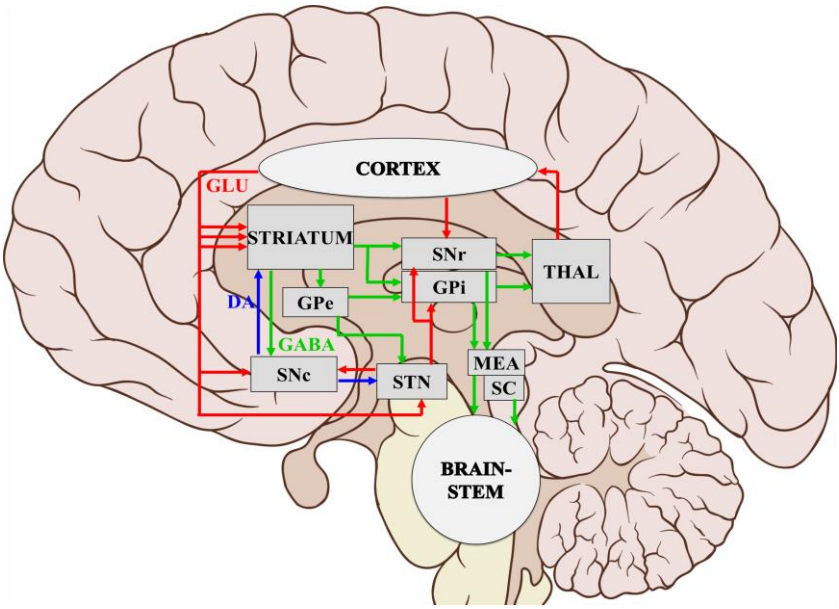

Figure 1 The normal circuitry of the basal ganglia. Located deep and central within the cerebral hemispheres, the basal ganglion connects to many areas of the brain. The main neurotransmitters are the inhibitory GABA (green), the excitatory Glutamate (GLU, red) as well as dopamine (DA, blue). Image adapted, original image by Patrick J. Lynch; https://creativecommons.org/licenses/by/2.5/legalcode

Newer findings suggest that the dopaminergic system is more diverse than previously assumed. Dopamine axons from the SN have the ability to release GABA by activating the vesicular monoamine transporter for dopamine, VMAT2, and cause inhibitory responses in the striatum [51]. Similarly, GABAergic cells appear to have the ability to release dopamine. A cell population in the intact mouse striatum have been found to release GABA as well as contain Tyrosine Hydroxylase (TH), the rate limiting enzyme necessary for dopamine production [52, 53] suggesting there could be dopamine producing interneurons in the striatum itself.

Subsets of dopamine neurons also have the ability to release glutamate through the vesicular glutamate transporter 2 (VGluT2). Glutamate releasing dopamine neurons are mainly found in the VTA, but the VGluT2 have also been found in the nucleus accumbens [54]. Stimulant drugs can

alter the locomotor response in knock-out mice lacking VGluT2 specifically in the dopamine neurons [55]. Thus, excitatory glutamate transmission from the VTA has a regulatory effect in physiological responses.

Basal ganglia dysfunction plays a critical role in the development of many PD motor symptoms as well as non-motor symptoms. How exactly the loss of midbrain dopamine neurons cause alterations in the basal ganglia pathways leading to such a diverse disease profile is less understood. There are complex interactions between the different circuits, via the different neurons and neurotransmitters. The cerebellum also plays a part in these interactions as the cerebello-thalamo-cortical circuit has proven to be involved in PD tremors and motor behaviour [56]. To what extent it does so, is less clear.

Non-invasive brain stimulation

Invasive stimulation of targeted brain areas via implanted electrodes, deep brain stimulation (DBS), results in significant improvements of motor symptoms in PD. However, the mechanisms behind these effects are still not fully understood. An early theory, which may still partly hold true, suggests that local activation of the presynaptic inhibitory afferents inhibits the overactive neurons [57]. Newer findings suggest that DBS may improve PD symptomatology by modulating ongoing brain activity, through altering the electric activity known as brain oscillations [58, 59]. In recent years there has been a surge in the interest for non-invasive brain stimulation using direct or indirect non-invasive brain stimulation methods. In the direct stimulation methods the simulation is directed directly to superficial or deeper parts of the brain, whereas the indirect methods act by stimulation of peripheral afferents to the brain or spinal cord. The premise is that non-invasive stimulation methods could also have positive effects on motor and/or non-motor symptoms in neurodegenerative disorders such as PD, but without the need for an invasive surgical procedure. Dysfunctional neurotransmission can affect normal brain oscillations, and the theory is that by externally altering the brain oscillations, the neurotransmission could normalise to some degree. This in turn may have a positive effect on the behaviour affected by disease. Motor cortical excitability is commonly assessed by measuring motor evoked potentials (MEP). MEPs are muscle contractions as a result of the

neuro-electrical signals that arise from the spinal cord due to single or repetitive pulse-stimulation of the brain, thus give information of the motor cortex physiology during stimulation [60]. They do not necessarily provide evidence of any effect on motor behaviour.

Direct methods

Repetitive transcranial magnetic stimulation (rTMS) is administered via an electromagnetic coil on the scalp. The coil turns the electrical currents into magnetic fields which enter the brain surface without affecting skin or bone. The magnetic pulses which are directed repeatedly over the target area promote activity by inducing an electrical current between the nerve cells [61]. The effect of rTMS in PD is still subject to debate. On one hand some studies have found motor improvement in PD after rTMS, with gradual improvement of gait and hand bradykinesia over a 4 week period [62], and an immediate improvement of cognitive processing on the Stroop test after rTMS [63]. On the other hand recent studies have shown that gait, bradykinesia, rigidity, tremor, axial symptoms and the Unified Parkinson's Disease Rating Scale (UPDRS) scores are not affected by rTMS after short but consecutive use, regardless of low (1 Hz) frequency [64] or high (50 Hz) frequencies [65].

Transcranial direct current stimulation (tDCS) is delivered through skin electrodes placed on the scalp over cortical target areas and directly stimulate or inhibit (depending on the polarity of the electrode) the underlying neuronal tissue. In a recent study, stimulation of the primary motor cortex in PD patients resulted in improvement in both number of and the duration of freezing of gait events [66]. In another study, tDCS through the motor and prefrontal cortices was evaluated to establish any effect on gait and bradykinesia as well as several other PD symptoms. The primary outcome was a slight improvement of gait, with increased walking speed off-medication, however this effect only lasted for a short while and did not occur while on medication [67]. When analysing cognition during a working memory task in a PD cohort off medication, tDCS delivered to the left dorsolateral prefrontal cortex was found to improve performance [68].

Transcranial alternating current stimulation (tACS) is applied by attaching two or more electrodes on the scalp. The alternating sinusoidal current is believed to synchronise neuronal networks, like an external

electrical oscillation that is interacting with ongoing oscillations in the cortex. Thereby it could retune unusual oscillatory patterns associated with PD symptomatology [69]. Resting tremor in PD could be reduced by almost 50% with tACS over the motor cortex [70]. Additionally, sinusoidal tACS at 20 Hz over the motor cortex has been found to slow down voluntary movement during a visuomotor task in healthy participants [71]. This suggests an inhibitory effect, which could alter underlying motor control by adjusting neuronal communication. EEG assessments of tACS oscillatory effects suggest that alpha band oscillations are elevated even after stimulation [72, 73].

Transcranial random noise stimulation (tRNS) is administered by placing a stimulation electrode over the target area and a reference electrode on the contralateral side. In a healthy participant group, tRNS over the motor cortex enhanced corticospinal excitability. This occurred specially during the higher frequency spectrum, and appeared to last for 60 min after the 10 min stimulation period [74]. The mechanism of how this excitability comes about is unclear. It is believed that tRNS interferes with the ongoing neural oscillations and thereby modulates cortical excitability. Carbamazepine (CBZ), a voltage-gated sodium channel blocker, has been found to significantly shorten the excitability effect of tRNS, suggesting that the application of repetitive tRNS may alter the repolarisation and depolarisation of the ion channels and thereby increase cortical excitability [75]. This is possible as CBZ has a cell membrane stabilising quality and has an effect only when the membrane potential is reduced. With repetitive high-frequency stimulation, which activates the sodium channels and increases depolarisation, CBZ binds to the sodium channels and slows down the depolarisation process [76]. When this process is repeated continuously, the sodium channels constantly repolarise and depolarise, thereby yielding a heightened effect of tRNS and increased excitability [74]. It could be argued that this repetitive effect increases neuro-plasticity and leads to enhanced cognitive performance.

Indirect methods

Vagus nerve stimulation (VNS) is designed to send regular, mild electrical pulses to the brain via the vagus nerve, a major component of the autonomic nervous system. The vagus nerve is part of the peripheral nervous system and makes its way from the medulla in the brainstem and directly out to the body. It appears that about 20% of the fibres in the

vagus nerve carry information from the brain to the body (efferent), while the rest of the fibres carry information from the body to the brain (afferent) [77]. Furthermore, it regulates cognitive functions through direct and indirect connections to the cortical-limbic-thalamic-striatal neural pathways [78]. VNS is currently used in epilepsy but could be an emerging technology for treating other neurological disorders too. In a study looking at skilled motor tasks in rats, VNS during 5 days of training was found to increase the area of the motor cortex [79], suggesting VNS could have an effect on plasticity within the motor system. In a clinical word recognition task, participants read a section with some highlighted words, after which they either underwent VNS or not [80]. The subjects' ability to remember highlighted words improved significantly after VNS. Therefore, it is possible that VNS may have the ability to enhance memory retention.

Transcutaneous electrical nerve stimulation (TENS) is applied via either one set or two sets of electrodes directly on the skin, emitting low-voltage electrical currents. These currents can be adjusted for pulse, frequency and intensity, classified as high frequency (>50 Hz), low frequency (<10 Hz) or in burst configuration where bursts of a high frequency is submitted intermittently during a low constant frequency [81] . It is widely used for treating acute and chronic pain, often following neurological disorders including musculoskeletal diseases and neuropathy [82]. In PD it is sometimes used as complimentary therapeutic aid in aim to reduce pain following muscle tension and rigidity, although very few clinical studies have been conducted to assess its benefits in PD. Some studies have looked at the effect of TENS on motor impairment. In patients with dystonia, TENS was found to improve handwriting [83] and it improved the abdominal dyskinesia dramatically in a case study [84].

Step-synchronised vibration therapy has been assessed for treating gait disturbances in PD. Short-term effects of this procedure appear to improve gait steadiness [85]. The method involves small vibration devices embedded at different pressure points in the soles of constructed shoes. These deliver supra-threshold (70 Hz) vibration pulses when pressed down during walking which stop when pressure is eased [85]. In a recent study the effects of this procedure was assessed during 1 week in a participant with freezing of gait difficulties and in a participant with implanted DBS. In both PD cases there was improvement in several gait indices [86].

Acoustic sensory noise is a non-invasive method which could indirectly stimulate different neurological pathways. This method entails adding high level (65-85 dB) white background noise. The noise is delivered binaurally using high quality headphones with a stochastic (randomly fluctuating) frequency during testing. Auditory processing allows the acoustic noise carrying waves to reach the auditory pathways, where they are turned into neuronal action potentials through transduction. After this, the sound stimuli is encoded and transmitted to subcortical structures for specific processing [87]. Therefore, higher cognitive function could indirectly be affected by acoustic noise. The effects of this kind of stimulation have been assessed mainly on cognitive function. Acoustic noise appears to improve cognitive performance in low-attentive children, while having the opposite effect in super-attentive children and has no significant effect in normal-attentive children [88], potentially counterbalancing episodic memory differences between low-attentive and normal-attentive children [89], irrespective of medication [90]. Acoustic stochastic noise also appears to prompt positive effects, similar to stimulants, on motor learning in the spontaneously hypertensive (SH) rat model of ADHD but not on control rats [91].

Stochastic Vestibular Stimulation

The vestibular system

Within the vestibular system, Fig 2, the sensory organ in the inner ear contains three semi-circular ducts (anterior, horizontal, posterior) bilaterally, which respond to rotational movements and head acceleration. The utricle and saccule, the two otolith organs connecting to the ducts, react to linear accelerations. A head movement or acceleration in one direction excites the receptor cells in the semi-circular ducts on one side while inhibiting them on the other side, as fluid moves the vestibular hair cells in opposing directions [92]. There is a constant discharge of vestibular afferent neurons and the vestibular system responds to very small head movements and changes in gravity (which is a form of linear acceleration). The vestibular system reflectively regulates muscular as well as autonomic responses to the body's spatial orientation, thereby maintaining postural balance and providing early cardiovascular responses to changes in gravitational direction when a person stands up e.g. One of the

best studied vestibular reflexes is the vestibulo-ocular reflex (VOR) which stabilizes gaze. The vestibular system also provides crucial information to the hippocampus which enables the spatial specificity of hippocampal place cells and thereby plays an important role in spatial orientation including the maintenance of an internal map of our environment [93-95].

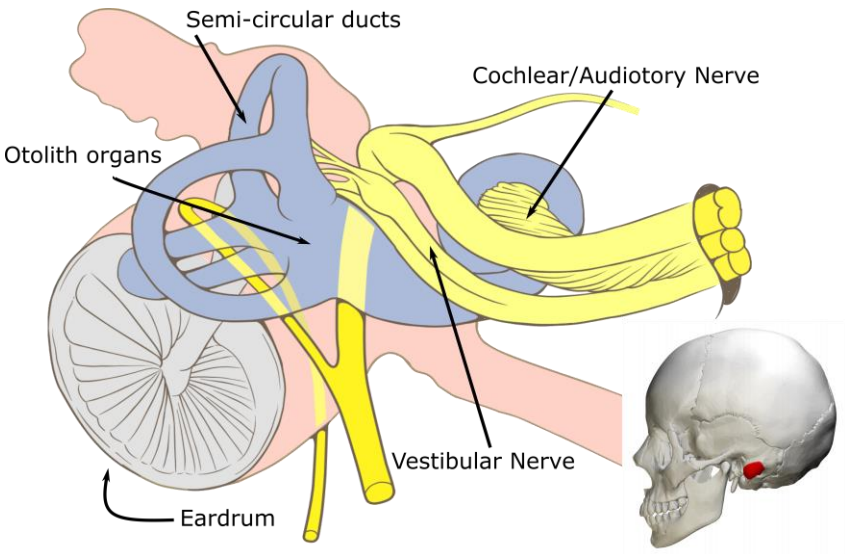

Figure 2 Vestibular system, the vestibular nerve connects to the semi-circular ducts and the auditory nerve connects to the cochlea in the inner ear. View from behind, parallel to the posterior part of the petrous bone right under the mastoid process, marked red on the skull image.
Image adapted, original images by Patrick J. Lynch and Database Center for Life Science https://creativecommons.org/licenses/by/2.5/legalcode

The inferior, medial, lateral and superior vestibular nuclei are located in the medulla and pons found in the brain stem, connecting to the mesencephalon. Projections from the peripheral end organs go through the vestibular afferent nerves in the internal auditory meatus to one of the four vestibular nuclei and onwards from there to cerebellum, cortex, and other brain structures.

The gaze stabilizing VOR generates an eye movement in response to a head movement, and allows the gaze to stay fixed in relation to the surrounding. The central vestibular system can distinguish tilts of the head and VOR takes place despite angular or linear head accelerations [96]. The vestibular afferents also evoke head-stabilisation in space during locomotion, the vestibulo-collic reflex (VCR). Sensory processing in the brain stem initiates the VCR. Vestibular neurons receive convergent input from the cerebellum as well as the semi-circular ducts and the otolith organs, these further descend to the spinal motor neurons [97]. Head rotation in a different direction than the body's direction is driven by this reflex as well [98].

If vestibular afferent signals are less than optimal, the appropriate balance response may be impaired. In PD, the loss of optimal dopaminergic regulation in the basal ganglia also produces difficulty in patients adaptation to postural disturbances [99]. Researchers have demonstrated impaired balance control in healthy participants after they received erroneous vestibular afferent signals [100]. Visual input and somatosensory inputs are also of importance for optimal balance control. One way to assess the part these factors play for postural control is by the Romberg test. By standing with feet close together and arms crossed over the chest, one can appreciate the difference in maintained balance with eyes open or eyes closed. For a more demanding version, this can be done on a compliant surface like a mattress. In PD, balance control is significantly reduced during this test compared to age matched controls [101, 102].

Basal ganglia receive vestibular information via a number of different pathways through various regions including the motor cortex and the hippocampus. Most recent studies of the vestibular-basal ganglia connection suggest that vestibular signals go through the dorsolateral striatum as a main input site and thereby modulate motor behaviour [92]. A recent finding in mice [103] was that both dopaminergic and GABAergic neurons in the SN are necessary for postural control and are specifically activated by head tilts. Both kinds of neurons receive input from the vestibular system mediated via the subthalamic nucleus (STN) and the pedunculopontine nucleus (PPN) [103].

Why SVS?

Transcutaneous galvanic stochastic vestibular stimulation (SVS) is an adaptation of galvanic vestibular stimulation (GVS), with the element of a noisy signal. It is therefore sometimes referred to as noisy GVS. Both procedures involve applying a cathodal current (negative) on one side and an anodal current (positive) on the opposite side [104, 105]. The difference between the two procedures lies in the applied waveforms. While studies with GVS employ structured square-waved, sinusoidal direct currents, the stochastic application employs a randomly fluctuating (usually imperceptible) current. When this current is applied at a near threshold amplitude, it is possible to affect vestibular afferents without unpleasant side-effects such as skin-irritation, nausea, vertigo or nystagmus [106]. In fact, it appears that near threshold currents particularly activates afferent neurons with irregular spontaneous firing rate [107] and could therefore target only certain vestibular afferents. By exciting the receptor cells, a response is initiated without engaging other sensory systems [105]. It has been hypothesised that the otolith, and not the semi-circular ducts, mainly responds to near threshold galvanic vestibular stimulation of the vestibular nerve [108]. In SVS, the amplitudes are usually set individually as different amplitudes are required to produce the same effect in different individuals. The sensory threshold here refers to the amplitude where an ordered (e.g. sinusoid or square wave) current leads to a noticeable activation of the vestibular system in the individual, with a gentle rocking of the head, or a sensation that the head is rocking. The frequency in most studies using stochastic currents is between 0-30 Hz, although in some studies frequencies can range up to 50 Hz or even higher.

In two clinical studies on healthy participants, low-intensity SVS was found to have the greatest effect in improving walking stability and balance performance in the range of 0.1-0.5 mA (amplitudes tested were between 0-1.5 mA) [109, 110]. Walking stability was assessed during a perturbed walking condition with a treadmill that moved from side to side [109]. The effect of SVS on balance performance was measured during a version of the Romberg balance task where participants stood on medium density foam [110]. Another study on healthy participants found that SVS evokes muscle responses in the lower limbs during regular stance, at a high intensity (±3 mA, 0-20 Hz) applied in a binaural bipolar arrangement. These effects were not found during other electrode placements (like the forehead), suggesting lower limb muscle responses as a specific consequence of modulated firing of the vestibular afferents [111].

The motor responsiveness, as measured by trunk activity, and heart rate dynamics of patients with PD or multisystem atrophy, was improved during the application of SVS (mean current = 0.33 mA) [112]. Improvement of trunk activity was also found in PD patients unresponsive to levodopa medication. The authors suggest that noisy vestibular stimulation can improve the function of the neurodegenerative brain in these disorders. Furthermore, balance function has also been assessed during noisy vestibular stimulation. A small decrease in sway was found in PD patients but not in healthy controls during low 0.1 mA intensity [113]. A recent study has found that SVS improves motor performance in a visuomotor tracking task [114], thus signifying that SVS may induce an effect also on sensorimotor processing.

As well as improved motor function, low-intensity SVS have been shown to improve cognitive performance in PD. An improvement of reaction time during cognitive assessments in the levodopa unresponsive PD patients has been demonstrated, suggestive of increased autonomic responsiveness [112]. Although studies on the role of SVS in cognitive performance are limited, the effects of GVS have been studied to some extent, suggesting a link between vestibular information processing and cognitive performance. Low-intensity GVS (0.7 mA) in hemi-spatial neglect was found to reduce deficits in a number of object-centred visuospatial tasks, including the line bisection task [115]. GVS in this configuration have also been found to improve a figure copying deficit in a case study of hemi-spatial neglect [116]. Furthermore, a large study found long lasting positive effects of GVS on the Behavioural inattention test (lasting for at least 1 month) [117]. Interestingly, GVS has been found to have an enhancing effect on the line bisection task in visuospatial neglect, but not in stroke patients without neglect [118]. Thus, it appears that vestibular stimulation may enhance neuronal interaction in patients with stroke, where spatial cognition is impaired, affecting bilateral integration. In view of that, supra-threshold GVS (2 mA) also improved postural asymmetry significantly in patients with left or right hemispheric lesion [119].

SVS – what actually happens?

While visual and proprioceptive information help to maintain the postural control system, vestibular information is critical for sustaining balance [104]. In disorders where balance is impaired, vestibular stimulation appears to increase the attentiveness to vestibular cues, instigating an

effect on motor problems [120, 121]. How sustainable this effect is in dopamine related disorders is still largely unknown.

One theory is that the stochastic sensory stimulation can improve the performance of neuronal systems by a phenomenon known as stochastic resonance (SR). This entails that near threshold noise can help carry a weak signal through a non-linear system to the detection threshold [122, 123]. SR can thereby affect physiological systems within the individual, in many instances improving less-than optimal function [124]. The moderate brain arousal (MBA) hypothesis introduced in 2007 [125] proposes that adding a moderate level of white noise to a low noise system will improve neuronal system function, but only if the neuronal system is not working optimally already (which is a general condition for SR). The MBA theory also assumes that low levels of dopamine transmission may be associated with insufficient neuronal noise, which in turn impairs the neuronal communication. Adding external noise would improve the function of neuronal systems in hypodopaminergic conditions, but would have no positive effects in an optimally working system with normal dopamine transmission [125, 126].

AIMS

Overall aim of thesis

The overall aim of this thesis was to assess the effects of galvanic SVS in relation to levodopa in both clinical and preclinical trials, and to evaluate the possible mechanisms behind these effects. Furthermore, we were interested in whether SVS has the same positive effect on cognitive performance in ADHD as auditory stochastic noise appears to have.

Specific objectives

1. How does SVS affect brain activity in the intact and the dopamine hemi-lesioned brain?

2. What are the similarities of SVS and levodopa in terms of brain activation patterns and neurotransmission?

3. Does SVS improve motor performance in an animal model of PD?

4. Is SVS tolerated in combination with levodopa in PD patients?

5. How do behavioural SVS effects compare with levodopa effects in patients with PD?

6. Does SVS induce similar improvements in cognitive performance in ADHD as acoustic noise?

MATERIAL & METHOD

The first three studies carried out for this thesis primarily assessed the effects of galvanic stochastic vestibular stimulation (SVS) on motor performance and the underlying brain activity which could explain these possible effects. The first two studies used the 6-hydroxydopamine hydrochloride (6-OHDA) hemilesioned rat model of PD. The third study assessed the effects of SVS and levodopa in a clinical cohort of participants with PD. Finally, the possible effect of stochastic vestibular stimulation on cognitive performance was trialled in a clinical cohort of subjects with attention deficit hyperactivity disorder (ADHD).

SVS protocol

Three different setups were used for the stimulation protocol during the four different studies. During the first preclinical study (paper I) the NeuroLog NL800 (Digitimer Ltd. Hertfordshire) and the analogue stimulus isolator 2200 A-M Systems (Sequim, Washington, USA) were used to apply sinusoidal and stochastic noise. For study III, the first clinical pilot study, a portable and programmable stimulation device [127] developed at Universities Space Research Association, Houston Tx, USA, was used. In paper II and IV a new portable device (Galvanic Stimulator, Ilves engineering, Gothenburg, Sweden) was used, specifically designed and developed for in house trials with galvanic stimulation, Fig 3.

The stimulator was programmed to deliver a sinusoid signal (1 Hz) at different amplitude levels, which was used to determine the individual threshold for stimulation induced perceptible sway. The lowest amplitude level where a gentle rocking of the head (from side to side) became noticeable was used as the maximum allowed amplitude of the SVS protocol. As a second step, the stimulator was reprogrammed to deliver bipolar stochastic vestibular signals, using a Gaussian white noise pattern generator filtered using a 10th order low-pass Butterworth filter with a cut-off frequency at 30 Hz.

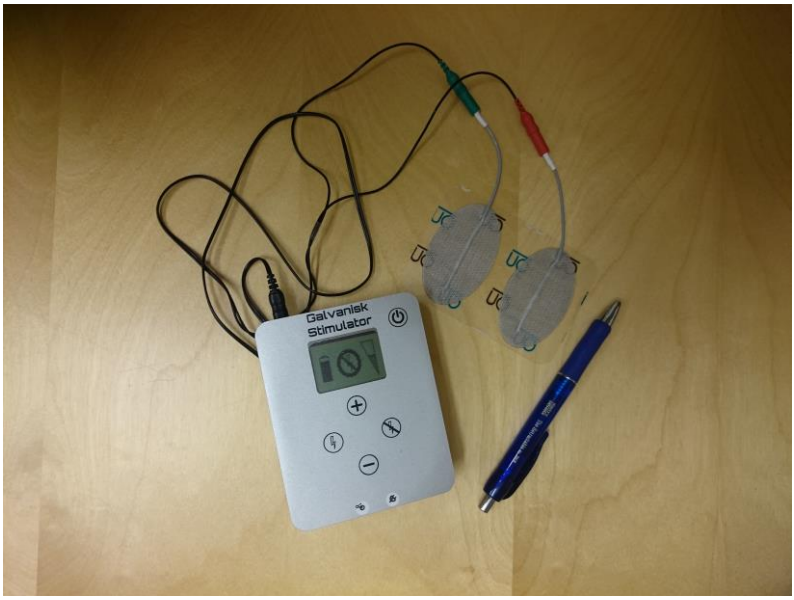

Figure 3 Programmable Galvanic stimulator in study II and IV. In study II, the electrode wires were connected to small crimp contact electrodes placed on the top of the rat skull. In study IV electrodes (as seen on image) were firmly placed over the mastoid process.

Preclinical studies (paper I & II)

Animals

The local ethical committee *Göteborgs djurförsöksetiska nämnd* and *UK Home Office* approved all surgical and experimental designs, in accordance with the European Communities Council Directive of November 24th, 1986.

Sprague Dawley (SD) rats were used for the experiments. In paper I, female rats were used due to their smaller weight gain over time as weight gain may distort the results in the motor performance tests. The normal unlesioned rats in the microdialysis trial were male, as they did not undergo behavioural testing. In Paper II, male rats were selected to avoid interference of the female cycle on brain activity, as the behavioural test

element was redundant. Animals were maintained in a conventional animal facility with a 12 h light/12 h dark cycle, in cages of four, with access to food and water. Before any behavioural training or test, animals were given the opportunity to acclimatise to new surroundings.

Surgical procedures

6-OHDA lesions are extensively studied in rat models of PD, where the neurotoxin is injected in distinct brain structures, promoting dopaminergic cell death. In our model, we injected this toxin hemi-laterally in the medial forebrain bundle, causing destruction of the nigro-striatal pathway.

The lesion procedure was performed under isoflurane anaesthesia. The skull was exposed, a hole was drilled over the medial forebrain bundle and 6-OHDA dissolved in 0.9% NaCl, 0.3% ascorbate, 5 µg/µl, was injected. The hole was covered with periost membrane and the wound was closed. Sham-treated animals received the saline ascorbate vehicle only.

Approximately 4-6 weeks after the lesion procedure, sterilised vestibular electrodes were implanted in a bilateral arrangement. The electrodes were constructed in our labs, using Teflon coated stainless steel wires (0.2 mm Ø) and small crimp contact electrodes. The animal was put under anaesthesia as described, the skull was exposed and two stainless steel jeweller's screws were fastened in the parietal bones, the electrodes were lowered gently and fastened with acrylic cement foundation. The surgical area over the horizontal canals of the two labyrinths was then exposed and the 1 mm peeled, and looped, end of the steel wire was secured by pushing it through the most ventral ends of the bilateral petrosal crests, Fig 4. The wounds were closed with the electrodes externalised. Some animals received microdialysis implants (paper I) in the same surgical session.

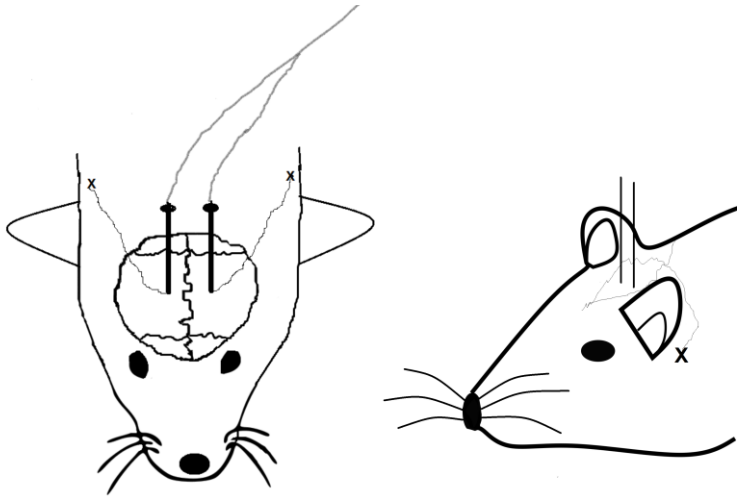

Figure 4 Illustration of the electrode placements. Two crimp contact electrodes were fastened on the top of the parietal bones with acrylic cement. Bilaterally, the petrous part of the temporal bone was identified and the peeled 1mm end of a 0.2mm Ø steel wire was secured through the most ventral end of the crest. Wounds were closed over the wires by stitching, and the electrodes were left externalised.

Microdialysis (paper I)

Microdialysis is a method used to sample and measure neurotransmitters and other soluble molecules in the extracellular tissue fluid [128]. We employed this technique to analyse different monoamines and their metabolites in selected brain areas affected by PD and directly related to the basal ganglia pathways or involved in brain stem afferent processing.

The microdialysis probe locations were determined with reference to the Paxinos and Watson rat brain atlas [129] and were implanted bilaterally in the striatum, substantia nigra (SN), pedunculopontine nucleus (PPN) and ventromedial thalamus (VM). Samples were collected every 30 minutes, after two baseline samples, stochastic vestibular stimulation/sham stimulation was conducted for 30 minutes. Microdialysis perfusion continued for another 60 minutes, providing totally 5 microdialysis samples from each probe. The following day the same animals received a single injection of levodopa and benserazide (6 mg/kg

and 12 mg/kg, respectively, i.p.) or saline instead of stochastic stimulation.

The collected samples were analysed for basal levels of amino acids and neurotransmitters, including dopamine, serotonin (5-HT), GABA and glutamate concentrations. The dialysate fractions were analysed for amines and amine metabolites by using a two-dimensional high performance liquid chromatography system with electrochemical detection (HPLC-ED), and amino acids were separated and detected by HPLC followed by fluorescence detection after pre-derivatization with o-phthaldialdehyde (OPA).

Immunohistochemistry (paper II)

c-Fos protein expression (the protein product of the immediate early gene *c-fos* mRNA) can be used to demonstrate neuronal activity in a subset of cells and can be viewed as markers that visualise neuronal interaction in functional pathways [130].

After recovering from the vestibular electrode implantation (3-5 days), the animals received either SVS or sham SVS for 30 minutes and underwent a transcardial perfusion 90 minutes after stimulation seized. Alternatively, they were perfused 120 minutes after a levodopa and benserazide (6 mg/kg and 12 mg/kg, respectively, i.p.) or saline injection.

Immediately after perfusion, the brain was removed and post-fixed in paraformaldehyde (4%, ph 7.4). The brain was sliced in serial coronal sections (35 μm) using a cryostat and went through a series of incubation procedures. As a final step they were stained with peroxidase DAB solution (25 mg/mL 3,3′-diaminobenzidine and 0.005% H_2O_2) to achieve a colour reaction which was analysed to assess expression of the c-Fos protein in different brain regions.

Assessments

For the behavioural assessments (paper I) we trained the animals for two tests, the Rotarod locomotion test and the Montoya staircase test, Fig 5.

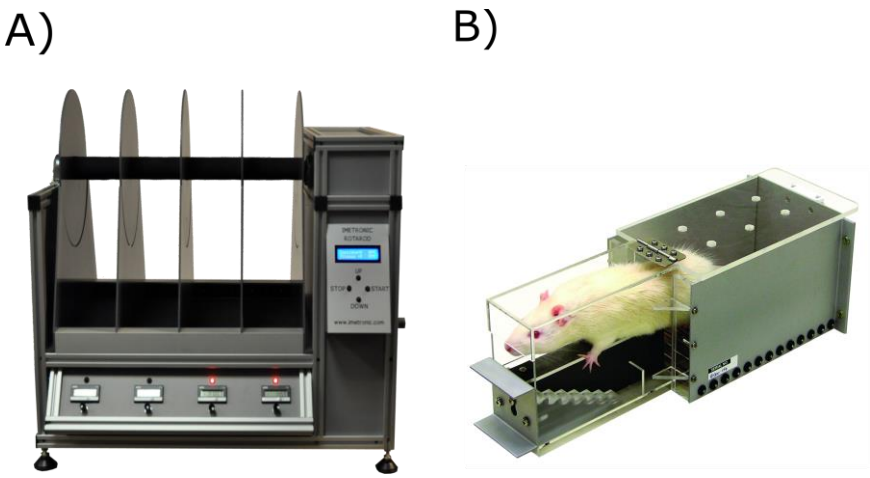

Figure 5 The Rotarod locomotion test and the Montoya staircase test. A) Accelerating 4-lane Rotarod for rats. When the rat stops running, it will glide down to the lever which will record the time. B) The Montoya staircase box. Sugar pellets are placed in little wells on each stair and the rat will have to reach out with the forelimb to retrieve them.
Images A with permission from https://creativecommons.org/licenses/by-sa/4.0/deed.sv
Image B has been reprinted with kind permission from C/O Lafayette Instrument Company, Inc.

Rotarod has previously been demonstrated as a highly sensitive measure for motor impairment after brain injury [131]. We tested the animals on the Rotarod in order to assess motor behaviour during the different conditions. Animals were trained on the accelerating rod before any surgical procedures took place. Three weeks after the lesion procedure, the animals were tested, to assess the time spend on the rod. Testing was further conducted three to five days after electrode implantation. Treatment or sham treatment was administered in a counterbalanced order, with animals receiving either treatment or sham treatment on one day and the opposite condition on the following day. The animals were stimulated/sham stimulated for 30 min prior and throughout the testing period. Alternatively levodopa or saline was injected 30 min prior to testing.

To measure fine motor skills we further tested the animals in the Montoya staircase test, an objective test of skilled reach and independent use of forelimbs [132]. Before the lesion procedure, rats were food restrained and trained to retrieve sugar pellets, having to reach out their forelimbs

from a small plexiglas box with a staircase on each side. One week after electrode implantation, the rats were food restrained once more and tested in a counterbalanced order. Due to technical reasons, the animals were stimulated for 30 minutes prior to the testing only.

For paper II, we first screened all brain sections visually to assess any emerging c-Fos expression and identify possible group-specific patterns in regions based on Paxinos and Watson rat brain atlas [129]. At the initial screening the examiner was blinded to groups and treatments. The regions which appeared to have group specific c-Fos expression were the dorsal peduncular (DP), nucleus accumbens (Acb), lateral habenula nucleus (LHb), reticular thalamic nucleus (Rt), intermediate nucleus of lateral lemniscus (ILL), brachium inferior colliculus (BIC) and the cuneiform nucleus (CnF). Beside this unbiased selection of brain regions, we had some predetermined regions of interest based on previous research that we also chose to assess further. This selection included the substantia nigra (SN), pedunculopontine nucleus (PPN), ventromedial thalamus (VM), caudoputamen (CPu), subthalamic nucleus (STN) and the vestibular nuclei including the medial vestibular nucleus (MVePC) and the ventrolateral medullary region (RVLM). Full cell quantification was performed using ImageJ (U.S. National Institutes of Health, Bethesda, Maryland, USA).

Clinical studies (paper III & IV)

Participants

Approval of the clinical studies was obtained from the regional ethical review board in Gothenburg, and written informed consent was obtained before any testing commenced. Participants were encouraged to report any discomfort throughout the entire trial, and any adverse reactions were noted. At the end of the entire trial, participants were debriefed using a structured interview protocol.

Paper III was a pilot study with the main aim to investigate the feasibility of use of an SVS device in PD. Thus our sample, recruited from the Neurology Clinic at Sahlgrenska University Hospital in Gothenburg, Sweden, was quite small (n=10). The study followed a randomised crossover design, which took place during two different days, generally one-two weeks

apart. The effects of SVS or sham SVS were evaluated after 12 h of medication abstinence as well as after a single dose of levodopa, Madopar Quick, 200 mg. The stimulation procedure was double-blinded, due to obvious reasons the medication was not. As the effect of medication was of importance in the study, responsiveness to levodopa was part of the inclusion criteria, as was a Hoehn & Yahr disease stage of ≤3. Excluded were participants with implanted electronic devices or diagnosed vestibular diseases.

The final study in this thesis (paper IV) was carried out in collaboration with the Gillberg Neuropsychiatry Centre in Gothenburg, Sweden and the Child and Adolescent Psychiatric unit, Lund, Sweden.

Table 1
Baseline characteristics of study participants in Paper IV, ADHD-I = Inattentive type, ADHD-H = Hyperactive-Impulsive type, ADHD-C= Combined type, ADD = Attention Deficit Disorder.

Subject	Age	Sex	Medication	Subtype	mA
1	8	M	None	ADHD-I	300
2	10	M	None	ADHD-C	300
3	11	F	None	ADHD-C	300
4	12	M	Concerta	ADHD-C	450
5	13	F	None	ADHD-C	450
6	14	M	None	ADHD-C	400
7	14	M	Concerta	ADHD-C	600
8	14	M	Concerta	ADHD-C	600
9	17	M	None	ADHD-I	300
10	18	M	None	ADHD-I	400
11	19	F	None	ADHD-I	300
12	20	M	None	ADHD-I	400
13	22	M	None	ADHD-C	450
14	23	M	None	ADHD-I	400
15	37	M	Ritalin	ADD	350
16	42	M	None	Data missing	600

Included in the study were participants (n=16, Table 1) with definitive ADHD diagnosis, irrespective of subtype at this stage, as this pilot study aimed to investigate the effects of SVS on cognitive ability in typical ADHD. The exclusion criteria were implanted electronic devices, comorbid autism, epilepsy or Tourette's syndrome. Similar to the study in paper II a double-blinded crossover design was followed. All participants functioned as their own control and underwent testing with SVS and sham SVS in different trials with at least one week interval to minimise any carry-over effects. Evaluations were conducted after a minimum 12h washout of any ADHD medication.

Behavioural assessments

Dynamic balance response test (paper III): The participant wore a harness, connected to a thin rope which pulled with a force corresponding to 3% of the participant's weight, creating a slight and steady pull. Suddenly releasing the rope with an electromagnetic switch, a spontaneous backward sway was produced until the subject reacted, stopped and reversed the backward sway. Sway movements in anterioposterior (Y) and mediolateral (X) directions, as well as the perturbation correction time(s) were recorded using a Kistler force plate (Kistler Nordic AB, Sweden).

Static balance tests (paper III): The participant stood on the force plate, barefoot, eyes closed and arms folded over chest. The same procedure was conducted while the participant stood on a 10 x 5 x 50 cm pad of medium density foam, decreasing the proprioceptive input.

Unified Parkinson's disease rating scale, UPDRS (paper III): A trained examiner performed UPDRS part III, while recording the examination with a full HD camcorder. The evaluation was done twice by the same rater, once immediately after session and once on a later occasion. When ratings differed between the two assessments a second trained rater was consulted for arbitration.

Posturo-Locomotor-Manual test, PLM (paper III): An optoelectronic measuring system (Qbtech/PDMonitor, Qbtech AB, Sweden), recorded a repeated movement where the participant picked up an object and transferred it to a chin-levelled platform 2 m ahead.

The Rey Auditory Verbal Learning Test, RAVLT (paper IV): This word-recall test evaluated short-term verbal memory. Participants listened to a list of 15 unrelated words and were asked to repeat the words they remembered, in five repeated trials. This was followed by a distractor list with 15 other unrelated words which subjects were asked to repeat. Finally they were asked to retrieve as many words as they remembered from the initial list.

Span-board task (paper IV): To assess visuo-spatial working memory, participants were sat in front of a screen where stimulus sequences were presented, starting with a short sequence which became longer and more difficult with each trial. Participants were asked to repeat each sequence on the screen immediately after it was shown. The test continued until the participant made an error two sequences in a row.

Flower trail test (paper IV): A trailing/tracking test was used to evaluate visually aided learning of a new motor skill. Participants drew a line between two lines shaping a large flower pattern, without lifting the pen and while avoiding to transect the lines. The completion of each identical flower pattern was timed, and 15 patterns were completed.

Statistical analysis

All statistical analyses were performed using SPSS (PASW Statistics 18), and the significance threshold was set at 0.05.

Paper I

Repeated measure two-way ANOVA was used to evaluate the treatment effect on neurotransmitter concentrations, with treatment and time as independent factors. Subsequently, one-sample t-tests were conducted where appropriate. Paired t-tests evaluated effects of SVS on change in locomotion time (s) on rod. In the Montoya skilled reach test, the overall number of sugar pellets consumed, total number of pellets on each side as well as the ratio between number of pellets eaten from impaired (contralesional) side and non-impaired (ipsilesional) side after SVS or sham SVS were evaluated by paired t-tests.

Paper II

Each investigated region was analysed separately with a fixed-effect, unstructured linear mixed model to analyse c-Fos protein positive cell count. Ipsi- and contralesional side was used as repeated measures, and the treatment (SVS/levodopa/saline) and condition (6-OHDA hemilesion/sham hemilesion) was used as independent factors.

Paper III

Data distributions were normalised with logarithmic transformations and all data except maximum sway passed normal distribution tests. Non-parametric Friedman test, with Wilcoxon's paired test as a post-hoc measure was used to analyse maximum sway. Other variables were analysed with repeated measures linear mixed model analyses (fixed-effect, unstructured) to assess the main effects of SVS and levodopa treatment as well as interaction between the two. In the static posturography, reduced proprioceptive input was used as a third main factor.

Paper IV

The different variables from span-board test were analysed using linear mixed model analysis (fixed-effects, unstructured), with trial day and trial number as repeated measures and treatment (SVS/sham SVS) as a factor. For the Rey AVLT, repeated measures two-way ANOVA was performed, assessing the mean difference of the first trial to trial 2-5, as well as the treatment effect on trial 1-5. Treatment effect on the final recall trial was computed with a paired t-test. For the Flower trail test, two-way ANOVAs were carried out for drawing time and number of errors, with the treatment group as a factor. A Pearson product-moment correlation coefficient was computed to assess the relationship between the different parameters.

RESULTS & DISCUSSION

Based on previous research, we assumed stimulation of the vestibular pathways could have some potential as a therapeutic aid in dopamine related disorders. The physiological effects of SVS on brain function, specifically in comparison to levodopa have not been elucidated. Just as importantly, there is a lack of knowledge about the clinical effects of SVS on motor function in PD and cognitive performance in ADHD, with or without the combination of specific medication. This thesis was aimed at investigating some of these aspects, and to add clues to how SVS may affect symptoms mainly in PD, but also in ADHD.

What are the mechanisms of SVS?

To assess how the activity pattern and neurotransmission in the brain may change during SVS, microdialysis was carried out in rats in four key brain regions connected to the basal ganglia circuitry. Extracellular concentrations of dopamine, dopamine metabolites and amino acids were collected from the striatum, SN, PPN and VM before, during and after SVS and levodopa treatment.

In unlesioned animals GABA concentrations increased in the SN after SVS, while remaining unchanged after sham SVS. The results suggested that the increase started early in the SVS procedure and remained for at least 30 min after stimulation ceased. Dopamine levels remained unchanged in the SN and the striatum. SVS also affected the glycine and glutamate concentration level in the SN, but the changes in concentration were not significant at group level. No substantial differences were found in any of the other investigated regions.

SVS only affected GABA concentrations in the SN. The GABA reuptake inhibitor, NNC 711, was included in the perfusion fluid to detect rapid increases of GABA. There is a possibility that the addition of this reuptake inhibitor could prolong the increases of GABA concentrations and cause secondary changes in network activity. However, significant increase of GABA was not found in any of the other investigated regions, and was

selective to the SN. The increased mean values of glutamate and glycine in the SN suggest that SVS can affect other neurotransmitters in the investigated regions and this possibility cannot be disregarded as re-uptake inhibition may be needed to detect physiological changes in amino acid neurotransmitter release. Furthermore, microdialysis during SVS in other related regions such as the STN, might give a broader understanding of its effects in dopamine related disorders. The STN is important for parkinsonian symptoms as demonstrated by STN-DBS, and high frequency stimulation of STN has demonstrated increased release of nigral GABA and glutamate in the rat brain [133].

In 6-OHDA hemilesioned animals there was a steady increase of GABA concentration in both ipsilesional and contralesional SN after levodopa injection. Similar concentrations of GABA could be measured in the ipsilesional SN after SVS, Fig 6A.

An increase of GABA in the SN counteracts parkinsonian symptoms by disinhibiting the activation of specific regions in the basal ganglia. An interesting outcome is that while levodopa increased dopamine concentrations in the SN (the striatum was not investigated), SVS did not alter dopamine concentrations in any of the regions investigated, Fig 6B. As SVS also increased nigral GABA, it seems that SVS may share some features with levodopa. However the lack of increased dopamine levels after SVS leads to the assumption that SVS mediates nigral GABA release through a different mechanism than the dopaminergic system. Another finding was that SVS appeared to alter GABA concentrations differently in the ipsi- and contralesional SN in the 6-OHDA lesioned animals. So, despite the bilateral nature of the stimulation, GABA concentrations increased on the ipsilesional side while they decreased on the contralesional side. The result being that SVS appeared to balance out the absolute levels of GABA in the SN. In contrast, levodopa induced a parallel increase of GABA on both sides. Similarly, there was an increase of dopamine levels on both sides, although higher levels were observed on the ipsilesional side.

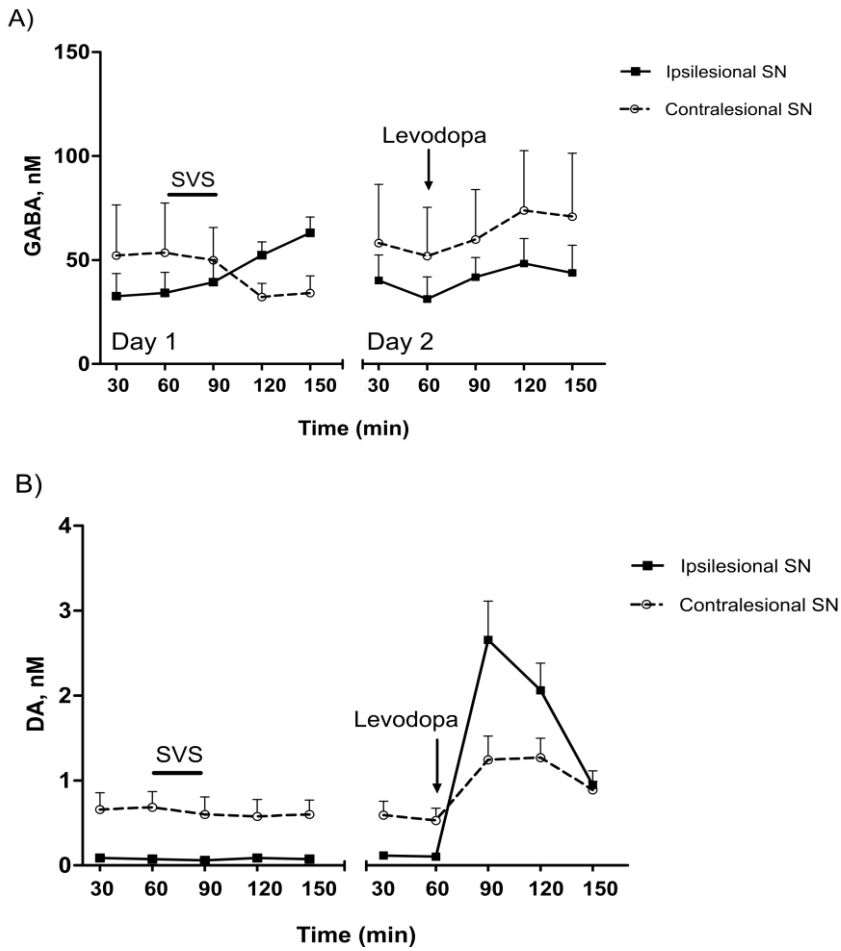

Figure 6 GABA and dopamine (DA) concentrations (nM, mean±SEM) in the ipsi- and contralesional SN of hemilesioned 6-OHDA animals following SVS and Levodopa treatment. Panel A shows the GABA concentrations and panel B the simultaneous DA concentrations, during day 1 and day 2. NNC 711 (30 μM) was present throughout the experiment and left in the microdialysis tube that was re-sealed over night. SVS treatment is indicated by a horisontal bar, and the levodopa injection by an arrow.

Symptoms in PD that do not respond well to levodopa could stem from dysfunctional activity involving neurotransmitters such as GABA, noradrenaline, serotonin and glutamate [134]. Efferent GABAergic neurons and glutamate are not only found in the striatum, but interneurons are also expressed widely throughout the entire brain [135]. Locus ceruleus,

part of the brain stem, is a main area mediating noradrenergic neurons which project to widespread areas of the brain and spinal cord [136]. One of the major serotonergic nuclei is the raphe nuclei, projecting to many parts of the brain but appear to mainly involve the sensory, motor and limbic systems [137]. SVS could affect motor control through altered activity in the SN by involving a non-dopaminergic pathway, perhaps involving the cerebello-thalamo-cortical circuit.

In paper II, we failed to observe any c-Fos expression in the SN after SVS or levodopa. Previous studies have also demonstrated very little to no c-Fos expression in the SN of non-dyskinetic hemilesioned rats [138-140]. This is more or less expected as treatments that increase GABA release in the SNr will inhibit the activity in this region. The region which projects directly to the SNr is the CPu, and SVS induced a similar level of c-Fos activation in this region, in both shamlesioned and 6-OHDA hemilesioned animals. Saline did not induce any activation in the CPu. In the 6-OHDA hemilesioned animals, levodopa appeared to induce a higher level of c-Fos activation in the ipsilesional side than did SVS. However, there were no significant differences. Consistent with the findings of paper I, we did not find any significant c-Fos activation in the PPN or the VM either. In the STN, some c-Fos expression could be observed, although no group-specific differences were found. Overall, SVS activated a number of regions in the brain aside from the CPu, which overlapped with a single dose of levodopa but not saline. These regions were: the intermediate nucleus of lateral lemniscus (ILL) and ventrolateral medullary region (RVLM). Two regions that did differ in c-Fos expression as an effect of SVS or levodopa were the MVePC and the LHb. Activation in the MVePC following SVS was expected as it is the primary recipient of vestibular stimulation.

Levodopa did not induce any substantial c-Fos expression in the MVePC of either 6-OHDA hemilesioned or shamlesioned animals. SVS on the other hand induced significantly more c-Fos expression in the 6-OHDA animals than levodopa did. Interestingly, SVS also increased c-Fos expression significantly more in 6-OHDA animals than it did in shamlesioned animals. This is in agreement with the theory that vestibular activity is downregulated by dopamine depletion. It also supports the evidence which indicate that the activity or responsiveness of MVePC neurons is decreased in PD [141-144]. A possible explanation to improved balance responses in PD during SVS is therefore that SVS ameliorate the decreased responsiveness.

One region that we did not anticipate any activity in was the LHb. However, in both 6-OHDA hemilesioned and shamlesioned animals we found significant c-Fos expression here after SVS, but very little after levodopa and not at all after saline. The habenula nucleus is part of the epithalamus and located close to the midline. It consists of two subnuclei, the medial and the lateral habenula [145]. In recent years, research has pointed to neurocommunication between the LHb and the pre-frontal cortex, parts of the basal ganglia and the hypothalamus. It has been suggested that the LHb receives both GABA and glutamate inputs, and have a modulating effect not only on dopaminergic system, but also the serotonergic and noradrenergic systems [146]. Activation in this region plays a big part in reward behaviour and response to aversive stimuli. It is likely to be involved in modulation of symptoms including depression, sleep disturbances, spatial awareness and autonomic decision-making as well as failure in associative learning [145, 147-149]. Furthermore, LHb may be involved in inhibiting dopamine neurons directly and have an excitatory effect through projections to the VTA [149].

Many of the symptoms linked with LHb regulation are associated with PD and ADHD. Interestingly, behaviours that appear to be modulated by the LHb are some of the symptoms in PD which do not respond aptly to levodopa medication [5]. Albeit some of these symptoms, such as sleep disturbances, are hypothesised to have a dopaminergic component [150], they may not be solitarily dopamine regulated. It is known that the basal ganglia circuitry plays a crucial part in parkinsonian and dyskinetic disorders. The roles of other subcortical regions and their interplay of excitatory and inhibitory neurotransmission to/from the basal ganglia are less established in regard to these disorders. Newer findings have shown an increased level of activity in the LHb during levodopa-induced dyskinesia, and inhibition of this region reduced these abnormal involuntary movements in 6-OHDA rats [151]. Furthermore, a 6-OHDA hemilesion in the SN of rats was found to change GABA transmission in the LHb, and regulation of GABA receptors through injection of a GABA agonist in the LHb produced anti-depressive effects [152]. Also, high-frequency stimulation of the STN have been found to alter activity in the LHb in rats, and it has been proposed that this region may mediate some of the neuropsychiatric symptoms in PD, such as depression [153].

We propose that the mechanism behind SVS involves inhibition of the overactive SNr though an increase of GABA transmission in the nigrostriatal circuit. This process appears to be mainly non-dopaminergic, alt-

hough some of the brain regions induced by SVS activation overlap with the activity observed after levodopa. Given the influence SVS has on the habenula, it is possible that SVS through its mediation on the non-dopaminergic pathway could have positive effects on the neuropsychiatric symptoms of PD. Consequently, future studies could explore the effects of SVS on such dysfunctions in PD, including depression, anxiety, apathy, fatigue and psychotic symptoms.

An interesting aspect to consider for future studies could be to look at the activation pattern in the medial and lateral part of the habenula separately. In our findings there appeared to be differences in the pattern activated, with c-Fos expression across the entire LHb in some animals and only in the medial or lateral part in other animals.

The main limitation of paper II was the lack of a sham-stimulated control group with implanted electrodes. Thus, it is not possible to discount possible effects of wearing the electrodes. Furthermore, there may have been some carry-over effects of the threshold determination, although this would have affected both the 6-OHDA and shamlesioned animals.

Effects of SVS on motor functions

In paper I, the overall time spent on the rotarod during treatment (SVS/sham SVS or levodopa/saline) was compared to a baseline measurement during that same day, to avoid day to day performance bias. There was no effect of the treatment order/treatment day, as an ANOVA with treatment and trial day as independent factors revealed. During the SVS treatment 6-OHDA hemilesioned animals improved their performance on the rod compared to the sham SVS treatment. The levodopa treatment did not improve the overall performance on the rod compared to saline. However, when looking more closely at the performance of individual animals, levodopa had an improving effect similar to the SVS outcome in half of the animals, while it impaired performance in the other half. Thus, there were positive and negative responders to levodopa treatment. Perhaps the bilateral increase of nigral GABA after levodopa explains this inconsistent rotarod performance. As levodopa increases nigral GABA in a parallel fashion, it may not reduce the imbalance in locomotor functions. SVS, however, improved overall locomotion on the

rotarod in all lesioned animals. This supports the theory of a rebalancing effect in locomotion, consistent with the more balanced GABA release between SN on the ipsi- and contralesioned side. In the sham hemilesioned animals, no significant effect of SVS could be found as animals performed no better or worse during SVS than during sham SVS. Consequently, the impaired brain (6-OHDA hemilesion) appears to improve more after noisy stimulation than does a normally working brain (sham hemilesion).

On the Montoya staircase test, we evaluated the effects of SVS on fine motor skills by assessing number of pellets picked up on either lesioned or intact side. The procedure was similar to the rotarod testing, however while animals received 30 minutes of SVS/sham SVS, testing continued without stimulation. The reason for this procedure was that the electrode wires did not allow free movement in the test-box and therefore hindered the performance.

Animals with a 6-OHDA-hemilesion had poorer performance and picked up fewer pellets with the contralesional forelimb compared to the ipsilesional forelimb. This was expected as a 6-OHDA hemilesion leads to poorer ability to use the contralesional side, something that is visible mainly on forelimb use. There were no effects of SVS on forelimb use in either the 6-OHDA or the sham hemilesioned animals.

One of the primary results of paper I was that SVS has an enhancing effect on locomotion, but this enhancing effect does not appear to affect fine motor skills. As the vestibular pathways have a modulating effect on axial motor systems, it is possible that SVS mainly, or at least initially, affects posture, balance and locomotion rather than the appendicular motor system. In a subgroup of PD patients and in advanced PD (Hoehn and Yahr >3), balance and other modalities which are affected by axial function often do not respond satisfactory to levodopa medication [5]. Additionally, STN-DBS is limited in improving these particular symptoms [154], possibly supporting the theory of a non-dopaminergic pathology behind these postural difficulties [155]. STN-DBS in combination with high-frequency DBS of the SNr have been found to improve axial symptoms and gait, something that could not be achieved with STN-DBS alone [156]. Given that both DBS-SNr and SVS inhibit the overactive SNr, evaluating SVS as an add-on therapy in PD patients with a predominance of axial symptoms is an attractive possibility.

In paper III, we attempted to evaluate the effect of short-term SVS on posture and locomotion in a clinical trial. Participants were treated with SVS or sham SVS on separate days for a maximum of 3h in a cross-over design. On each occasion an acute levodopa (200 mg) challenge was performed after initial assessments in their worst OFF-condition after an overnight pause of medication.

SVS improved the time it took for participants to correct a forced pull/release motion in the dynamic balance test. Off medication, SVS further reduced the centre of pressure (COP) deviations in mediolateral correction and in backward correction. In the static balance test, SVS reduced the centre of pressure sway-path compared to sham SVS. Sway-path was also reduced with SVS when participants stood on a foam mattress. This effect was only found off medication. Medication had no significant effect on postural responses. It did however have a main effect on UPDRS-III scores and on PLM times. In several patients, the PLM times improved after levodopa treatment during SVS compared to sham SVS, although the improvements were not statistically significant. Furthermore, there was an interaction between the effects of medication and SVS on the UPDRS-III scores, with lower scores during the off medication state. Albeit small improvements were observed, these finding suggest that the recovery after a postural perturbation as well as overall postural control improved with SVS. Interestingly, the enhancing effect of SVS was found primarily during the off medication state. This was true for most assessments, even where SVS did not significantly improve function there were interactions with medication, suggesting larger effect off medication.

A reduced vestibulocollic reflex has previously been found in PD patients, and levodopa was shown to normalise this impairment [142]. The decreased sway-path found during SVS was greatest when standing on a foam mattress blindfolded. This condition reduces the proprioceptive input from the limbs and without visual input the subject has to rely more on the vestibular system. Near threshold SVS could by increasing the responsiveness of a supressed vestibular system enhance the impaired vestibulospinal responses in PD.

Our observation that levodopa medication had little substantial effect on both dynamic and static balance control is consistent with previous findings [157]. While dopaminergic medications successfully decrease stiffness, they can often lead to dyskinesia, which could have negative effect

on overall postural balance [158]. Dopaminergic medication primarily enhances appendicular motor symptoms, leaving axial motor dysfunctions less improved. It is possible that SVS could be used to target these symptoms, in particular balance and gait difficulties.

Noisy vestibular stimulation has the potential to increase or decrease cortical excitability depending on the frequency used [159]. This may enhance or disrupt atypical cortical as well as subcortical oscillatory activity, thereby possibly improving motor behaviour [160, 161]. We used a near-threshold stimulation paradigm, where the maximum current was determined based on subjective and/or objective observation of rhythmic stimulation. The stimulation currents applied (mean amplitude = 0.4 - 0.5 mA) were similar to currents in other clinical trials (mean amplitude = 0.1 – 0.4 mA) where improvements in balance and motor function were found [112, 127, 162]. As the clinical evaluations were based on a small pilot study (n=10), the outcomes should be interpreted with caution. Larger studies are required for assessing the clinical relevance, and the long term effects of near threshold SVS on motor improvement.

SVS in relation to levodopa

The interactions and differences between SVS and levodopa on motor functions and neurotransmission have already been reported in previous chapters of this thesis. Some adverse events were however reported in study III.

There was one report of mild headache and nausea during active SVS and similarly one report of slight vertigo during sham SVS (but not active SVS). In combination with levodopa, there was one report of slight nausea. One participant experienced slight nausea during sham SVS after levodopa, but had more severe nausea during SVS in combination with levodopa with two incidents of vomiting. The single dose of levodopa was the same for all participants (200mg) and was higher than the standard dose this participant was used to.

None of the participants was able to distinguish whether SVS was active or not. Furthermore, none of the participants in either study reported any significant discomfort during SVS alone. A potential for worsening of medication evoked nausea with SVS is however noted, and may be taken

into consideration in future clinical trials. In conclusion, we found short term treatment with SVS was safe in an adult population with PD and in a mixed population with ADHD off medication.

Effect of SVS on cognitive performance in ADHD

It has been reported that galvanic vestibular stimulation can activate multisensory cortical areas, including temporoparietal cortex, basal ganglia, and anterior cingulated gyrus [163], as well as the hippocampus [164], indicating a relationship between the vestibular system and memory. The role of SVS in spatial memory and cognitive performance in ADHD has not been tested before.

Although we found a trend toward better spatial memory in ADHD, we did not find support for improved cognitive performance in paper IV. Treatment improved the number of correct series carried out as well as number of correctly indicated markers within a series in the span-board task. The primary outcome of overall correctly indicated markers was however not affected by SVS treatment. Furthermore, no treatment effects were found in the word-recall test or the flower trail tests.

An important reason for trying out SVS in ADHD are the recent observations that acoustic white noise improves performance in the word-recall test and the Span-board task in children with ADHD, where noise benefit was greater without the combination of medicine on word recall, but equally beneficial with or without medication in the Span-board task [90]. The findings in our pilot study indicate that SVS, without the combination of medication does not induce similar positive effects on cognitive performance as auditory noise. As we have found changes in neurochemistry as well as improvement in motor function (paper I) and postural control (paper III) during this particular configuration, a more prominent outcome was expected if indeed any effects were present. Noise modality could be of importance, suggesting that any kind of stochastic noise will not have effect. It is also possible that the near threshold amplitude of the stimulus applied was not appropriate for assessing short-term verbal memory or visuo-spatial working memory.

Noisy vestibular stimulation has improved cognitive ability in a number of previous studies. In an animal study, rats with an induced cognitive

impairment performed better on the Morris Water Maze task after noisy vestibular stimulation [165]. However, cognitive improvements were not apparent after 1 session, but only observed after repetitive stimulation (5 sessions). In stroke patients with hemi-spatial neglect, repetitive noisy galvanic vestibular stimulation induced long-term improvements on lateral attention. The participants performance increased after 1, 5 or 10 sessions of noisy stimulation [117]. However, the mean amplitude (1 mA) was higher than the near threshold protocol used in our study, and could be one reason for the contrasting outcome. Furthermore persons with ADHD do not have a neglect problem.

One difference between our study and the mentioned previous findings of SVS on cognitive performance was that we only applied the stimulation for a short period of time (approximately 1h) and all testing was done during this time. A larger study population, specifically with ADHD subtypes in separate study groups, could provide a more representative outcome of the role of SVS on cognitive performance. It is also possible that other indices of higher function could benefit from noise.

CONCLUSION

We suggest that short-term application of SVS influences motor control by a dopamine independent disinhibition of the basal ganglia output. The effects are thus far modest in a clinical setting. Short-term application of SVS has, in the configurations used, failed to improve cognitive performance significantly in ADHD, the cognitive effects in PD have not been assessed in this thesis. Long-term application may affect behavioural outcomes differently. We have found that near threshold SVS mainly leads to activity in specific areas of the vestibular nuclei and the LHb, areas where levodopa leads to a slight activation and saline fails to activate all together. Furthermore, SVS and levodopa induced similar activity patterns in some key regions where the negative saline control did not, including the striatum, the ILL and RVLM. These similarities in activation pattern during SVS and levodopa treatment may explain some of the motor enhancements observed in both preclinical and clinical studies. However, while levodopa mainly affects the nigrostriatal pathway, SVS could have a larger influence on a non-dopaminergic pathway, possibly including an increased transmission through the LHb. In PD, enhancement of vestibular function could also indirectly improve motor symptoms important in balance and gait.

Further studies may add more specific clues to how vestibular stimulation functions and perhaps illuminate the pathway it activates. This could give indications to which behaviours that are mainly affected by its application. The available data suggest that PD patients with axial symptoms that respond less to levodopa medication and/or report falls may be more likely to benefit from SVS and it would therefore be of interest to study long-term use of SVS in this population.

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